EXPRESSION PROFILING IN RESPONSE TO ASCOCHYTA RABIEI INOCULATIONS IN CHICKPEA

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EXPRESSION PROFILING IN RESPONSE TO ASCOCHYTA RABIEI INOCULATIONS IN CHICKPEA

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ABSTRACT

EXPRESSION PROFILING IN RESPONSE TO ASCOCHYTA RABIEI INOCULATIONS IN CHICKPEA

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In this study, it was aimed to identify chickpea (*Cicer arietinum*) genes or gene fragments expressed upon *Ascochyta rabiei* infection using a tolerant chickpea cultivar ILC195 and fungal isolates with varying level of pathogenicity. PCR amplification of resistance gene analogs (RGA) and disease related genes, and mRNA differential display reverse transcription (DDRT) were used to get these expressed gene fragments in chickpea. The constitutively or differentially expressed PCR product fragments were cloned and sequenced. Out of nearly 300 clones, 160 sequences (expressed sequence tags, ESTs) could be analyzed and these sequences were disclosed in this study. About 100 of these ESTs were classified according to predicted "molecular function", "biological process" and "cellular component". The most common ppredicted functions of the products coded by these ESTs were "Protein Fate", "Metabolism", "Cell Rescue, Defense and Virulence", "Transcription", "Transport", "Energy", and "Cell Fate". Six ESTs were subjected to Real-Time quantitative RT-PCR analysis to compare the response of ILC195 infected by one *A.rabiei* isolate with another resistant chickpea genotype (FLIP84-92C)/*A.rabiei* pathotype system. Some of these genes were differentially expressed among different chickpea/*A.rabiei* isolate combinations. Highly upregulated ESTs in all these combinations were a formate dehydrogenase (metabolism and detoxification), a serine carboxypeptidase

(protein fate and communication) and a hypothetical protein probably similar to acyl-CoA synthetases. A genetic mapping study was carried out with EST specific primers and two EST markers were assigned in the current chickpea genetic map. However, no genetic linkage of them was detected with known chickpea quantitative trait loci for *A.rabiei* resistance.

Key words: Chickpea, *Ascochyta rabiei*, Differential Display, Resistance Gene Analogs, Genetic Mapping.

ÖZ

ASCOCHYTA RABIEI İNOKULASYONLARINA KARŞI NOHUTTA OLUŞAN EKSPRESYON PROFİLİ

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Eylül 2008, 256 sayfa

Bu tezde, tolerant nohut (*Cicer arietinum*) çeşidi ILC195 ve çeşitli düzeyde patojeniteye sahip fungus izolatları kullanılarak, *Ascochyta rabiei* enfeksiyonuna karşı ifade gösteren nohut gen veya gen parçalarının tanımlanması amaçlanmıştır. Nohutta ifade olan gen parçalarının elde edilmesi için dayanıklılık geni analoglarının (RGA) ve hastalıkla ilişkili genlerin PCR yöntemiyle çoğaltılması ve mRNA farklılık gösterimi ters transkripsiyonu (DDRT) kullanılmıştır. Sabit veya farklı şekilde ifade olan PCR ürün parçaları klonlanarak dizi analizleri yapılmıştır. Yaklaşık 300 klon içinden, 160 dizi (ifade olan dizi etiketleri, ETS) analiz edilebilmiş ve bu diziler çalışmada verilmiştir. Bu EST'lerden yaklaşık 100 adedi tahmini "moleküler işlev", "biyolojik işlem" ve "hücre bileşeni"ne göre sınıflandırılmıştır. Bu EST'lerin kodladığı ürünlere ait en çok görülen tahmini işlevler "Protein Yazgısı", "Metabolizma", "Hücre Kurtarma, Savunma ve Virulans", "Transkripsiyon", "Taşıma", "Enerji" ve "Hücre Yazgısı"dır. Bir izolatla enfekte edilmiş ILC195'nin tepkisinin, başka bir dirençli nohut genotipi (FLIP84-92C)/*A.rabiei* patotipi sistemiyle karşılaştırılması amacıyla, altı EST gerçek zamanlı kantitatif RT-PCR analizine tabi tutulmuştur. Bu genlerden bazıları değişik nohut/*A.rabiei* izolatı kombinasyonlarında farklı şekilde ifade olmuştur. Tüm bu kombinasyonlarda yüksek derecede ifade olan EST'ler bir format dehidrogenaz (metabolizma ve detoksifikasyon), bir serin karboksipeptidaz (protein yazgısı ve haberleşme) ve alkil-CoA sentetaz olabilecek bir

varsayımsal proteindir. EST'lere özgü primerlerle bir genetik haritalama çalışması yapılmış ve iki EST etiketi güncel nohut genetik haritasına yerleştirilmiştir. Ancak, bunların *A.rabiei* dayanıklığına ilişkin bilinen nohut kantatif karakter lokuslarıyla genetik bağlantısı olmadığı görülmüştür.

Anahtar kelimeler: Nohut, *Ascochyta rabiei*, Farklılık Gösterimi, Dirençlik Geni Analogları, Genetik Haritalama

To Nameless People Who Have Been Slogging Away Scientific Research for Years

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ABBREVIATIONS

4CL	4-coumarate-coenzyme-A ligase
[α^{32}P]-dATP	[α^{32}P]-deoxyadenosinetriphosphate
[α^{33}P]-dATP	[α^{33}P]-deoxyadenosinetriphosphate
AAP7	Amino acid permease 7
AAT	Amino acid transporters
ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylic acid
ADP	Adenosine diphosphate
AFLP	Amplified Fragment Length Polymorphism
AGO	Argonaute
APS	Ammoniumpersulfate
APXs	Ascorbate peroxidases
avr	Avirulance
B1	bulk comprising total RNA of plant samples separately infected with aggressive isolates and mild isolates (Table 2.10), page 50.
B2	bulk comprising of total RNA of plant samples infected with one mild isolate (Table 2.10), page 50.
B3	bulk comprising of total RNA of uninfected plant samples (Table 2.10), page 50.
BAC	Bacterial Artificial Chromosome
BCB	Blue copper binding protein
BR	Brassinosteroids
BSA	Bulk segregant analysis
BTH	Benzo-(1,2,3)-thio-diazole-7-carbothionic acid S-methyl ester
C1	bulk of total RNA of uninfected plant samples taken at 10 hpi of the second infection experiment, Figure 3.3, page 78
C2	bulk of total RNA of uninfected plant samples taken at 24 hpi of the second infection experiment, Figure 3.3, page 78
C3	bulk of total RNA of uninfected plant samples taken at 3 dpi of the first infection experiment, Figure 3.3, page 78

C4H	Cinnamic acid 4-hydroxylase
CAB proteins	Chlorophyll a/b binding proteins
CAD/CAD1	Cinnamyl alcohol dehydrogenase
CAPS	Cleaved Amplified Polymorphic Sequences
CATs	Catalases
cb	cDNA from bulk of uninfected total RNA samples of ILC195; page 95
CBL	Calcineurin B–like
CC	Coiled-coil
CCF	Crude culture filtrate
CCOM	Caffeoyl-coA-methyltransferase
CDPKs	Calcium dependent protein kinases
CHS	Chalcone synthase
CIMS	Cobalamine independent methionine synthase
CLPTM1	Cleft lip and palate transmembrane protein 1
COMT	Caffeic acid O-methyl transferase
con.	concentration
CRR9	Cisplatin resistance related gene 9
CSMDA	Chickpea seed meal dextrose agar
CuAO	Copper amine oxidase
DCL	Dicer like protein
DD	Differential Display
DEPC	Diethylpyrocarbonate
DHOase	Dihydroorotase
DNA	Deoxyribonucleic acid
dNTP	Deoxy-nucleotide-triphosphate
dpi	Days post inoculation
DSMR	Double-stranded-RNA-binding motif
DXR	1-deoxy-D-xylulose-5-phosphate reductoisomerase
E	Ethylene
E2	Ubiquitin conjugating enzyme
EBF	EIN3-binding F-box
EDR1	Enhanced disease resistance 1
EDS1/EDS5	Enhanced disease susceptibility 1/5
EDTA	Ethylene diamine tetraacetic acid
eLRR	Extracellular LRR

ERD 4	Early responsive to dehydration 4
EREBPs	Ethylene response binding protein
ERF	Ethylene responsive factor
ESP4	Enhanced silencing phenotype 4
EST	Expressed sequence tag
EtBr	Ethidium Bromide
F3H	Flavanone 3-hydroxylase
Fc3	FLIP 8492C(2), CRIL-3 parent
Fc7	FLIP 8492C(3), CRIL-7 parent
FDH	Formate dehydrogenase
FKBPs	FK506 binding proteins
FL	FLIP94-508C
FPIP	Fungal pathogen-induced protein
GB	Gibberellins
GPT	Glucose 6-phosphate/phosphate translocator
GRP	Glycine rich proteins
GSP	Gene Specific Primers
GST	Glutathione S-transferase
h	Hour
H1	bulk of total RNA of infected plant samples taken at 10 hpi of the second infection experiment, Figure 3.3, page 78
H2	bulk of total RNA of infected plant samples taken at 24 hpi of the second infection experiment, Figure 3.3, page 78
H3	bulk of total RNA of infected plant samples taken at 3 dpi of the first infection experiment, Figure 3.3, page 78
hpi	Hours post infection
hpt	Hours post treatment
HR	Hypersensitive response
HRP	Hypersensitive response protein
HSP90	Heat Shock Protein 90
I	bulk of total RNA of infected plant samples described in page 80
ib	cDNA from bulk of infected total RNA samples of ILC195; page 95
IC	ICC3996
ICARDA	International Center for Agricultural Research in The Dry Areas
ICRISAT	International Crops Research Institute for The Semi-Arid Tropics

IL	*C. echinospermum* L. ILWC245
IPTG	Isopropyl β-D-1-thiogalactopyranoside
JA	Jasmonic acid
KAPP	Kinase-associated protein phosphatase
kb	Kilobase
LA	Lasseter
LB	Luria Bertani
LG	Linkage groups
LHC	Light harvesting protein complex
LiCl	Lithium Chloride
LOX	Lipoxygenases
LRR	Leucine-rich repeat
LRR-TM	Leucin rich repeat transmembrane domain
LTR	Long terminal repeats
LZ	Leucine zipper
M	Molar
μM	Micromolar
MAPK	Mitogen activating protein kinase
MAS	Marker assisted selection
MAT	Mating type locus
MJ	Methyl-JA
μg	Microgram
mg	Miligram
MIPS	Munich Information Center for Protein Sequences
min	Minute
μL	Microliter
mL	Milliliter
mM	Millimolar
MS	Methionine synthase
NAC	Polypeptide associated complex
NB	Nucleotide binding
NB-LRR	Nucleotide binding leucine rich repeat
NBS	Nucleotide binding site
NBS-LRR	Nucleotide binding site leucine rich repeat
NCBI	National Center for Biotechnology Information

NDR1	Non-race specific disease resistance 1
NER	Nucleotide excision repair
ng	Nanogram
NHO1	Non-Host1
nm	Nanometer
NO	Nitric oxide
NPR1	Non expressor of PR1
PI	Pathotype I
PII	Pathotype II
P450	Cytochromo P450 monoxygenase
PAD4	Phytoalexin deficient 4
PAGE	Poly-Acrylamide Gel Electrophoresis
PAL	Phenyl alanine lyase
PAMP	Pathogen Associated Molecular Pattern
PC	Plastocyanins
Pc3	PI 359075, CRIL-3 parent
Pc7	PI 599072, CRIL-7 parent
PCD	Programmed cell death
PCR	Polymerase chain reaction
PE	Pectin esterase
PK	Protein kinase domain
pmol	Picomole
PPIase	Peptidyl prolyl isomerase
PR	Pathogenesis related
PRPs	Proline rich proteins
PSI	Photosystem I
PSII	Photosystem II
QR	Quinone oxidoreductase
qRT-PCR	Quantitative Reverse Transcriptase PCR
QTL	Quantitative Trait Loci
R	Resistance
RAR1	Required for Mla-dependent resistance1
RE	Restriction enzyme
Real-Time	Real-Time Quantitative Reverse Transcriptase PCR
Reference map	Chickpea genetic map described by Winter *et al.* (2000).

RGA	Resistance gene analogs
RIL	Recombinant inbreed line
RISC	RNA Induced Silencing Complex
RLK	Receptor-like kinase
RNA	Ribonucleic acid
RNAi	RNA interference
ROS	Reactive oxygen species
RT-PCR	Reverse transcriptase polymerase chain reaction
SA	Salicylic acid
SABPs	SA-binding proteins
SAMMtases	S-adenosyl-L-methionine-dependent methyltransferases
SAR	Systemic Acquired Resistance
SCF	Standard Chromatogram Format
sec	second
SGT1	Suppressor of G2 allele of SKPI
SID2	Salicyclic acid induction deficient 2
SNP	Single nucleotide polymorphism
SOD	Superoxide dismutase
SSH	Suppression subtractive hybridization
SSI2	Suppressor of salicylate insensitivity of NPR1-5
SSR	Simple sequence repeat
STMS	Sequence tagged microsatellite Sites
Taq	*Thermus aquaticus*
TBE	Tris-Borate-EDTA
TEMED	N, N, N',N'-Tetramethyl ethylene diamine
TF	Transcription factor
TIR	Toll-interleukin-1 receptor domain
TLP	Thaumatin like protein
TM	Transmembrane domain
U	Unit
u	bulk of total RNA of uninfected plant samples described in page 80.
UFC1	ubiquitin-fold modifier-conjugating enzyme 1
UROD	Uroporphyrinogen decarboxylase
USDA-ARS	United States Department of Agriculture-Agricultural Research Service
UV	Ultraviolet

v/v	Volume per volume
VDAC	Voltage dependent gated anion channels
WIPK	Wound inducible protein kinase
X-gal	5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside
Xoo	*Xanthomonas oryzae* pv. *oryzae*

CHAPTER 1

INTRODUCTION

1.1 The Chickpea

The cultivated chickpea, *Cicer arietinum* L., is one of the most important grain legumes in the world (ICRISAT, 2008a). It is evolved from *Cicer reticulatum* in a region covering the southeastern of Turkey and northeastern of Syria (Ladizinsky and Adler, 1976; Singh, 1997). There are two main types of chickpea, namely, Desi (small and dark seeds) which is grown mainly in India, East Africa and Central Asia; and Kabuli (large and cream colored seeds) which is grown in the Mediterranean and Central Asia (Iruela *et al.*, 2002). Besides its importance in human diet, chickpea improves soil by symbiotic relationship with nitrogen fixing Rhizobium (ICRISAT, 2008a). India, Turkey and Pakistan are the major producing countries (ICRISAT, 2008a). In Turkey, chickpea is the major grain legume crop with an annual production nearly 630,000 tones and its yield has been stable about 1,000 kg ha^{-1} throughout 1987 to 2006 (Figure 1.1). It is grown without irrigation in arid and semi-arid areas in Turkey (Dusunceli *et al.*, 2007).

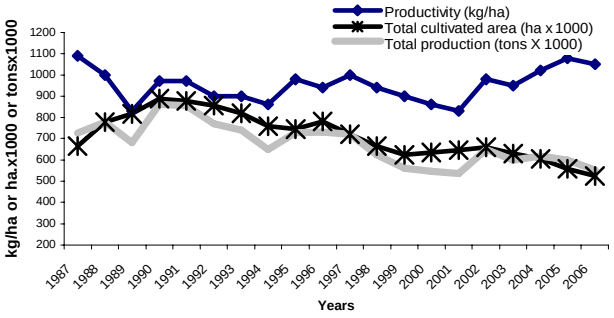

Figure 1.1 Chickpea production of Turkey in between 1987-2006. Data from Türkiye İstatistik Kurumu (Appendix A).

Chickpea is a self-pollinated diploid (2n=2x=16) with a genome size of 750 Mbp (Arumuganathan and Earle, 1991; Winter *et al.*, 2000). It has 8 chromosomes of approximately $1X10^9$ base pairs DNA (Bennett and Smith, 1976; Croser *et al.*, 2003). Rajesh *et al.* (2007) reported the gene density in chickpea as one per 9.2kb, with an average gene length of 2500 bp. The genus *Cicer* comprises 43 species distributed into two subgenera: Pseudononis (annual Monocicer and annual/perennial Chamaecicer) and *Viciastrum* (perennial Polycicer and Acanthocicer) (ICRISAT, 2008b). Based on morphological features, isozyme profiles (Kazan and Muehlbauer, 1991; Tayyar and Waines, 1995; Singh, 1997) and amplified fragment length polymorphism (AFLP) (Nguyen *et al.*, 2004); *C. arietinum* (chickpea), *C. reticulatum* and *C. echinospermum* were grouped in the Monocicer section. Due to low genetic distance of *C.echinospermum*, it was proposed to have also a role in the evolution of the chickpea (Iruela *et al.*, 2002; Nguyen *et al.*, 2004). As compared to wild Cicer species, the genetic variation of chickpea is very low (Croser *et al.*, 2003) which may resulted from a series of four evolutionary bottlenecks proposed by Abbo *et al.* (2003), namely; limited distribution of the wild progenitor (*C. reticulatum*), the founder effect associated with domestication, spring sowing due to Ascochyta blight and replacement of local landraces by modern cultivars. Consequently, *C. reticulatum* and *C. echinospermum* can be crossed with chickpea for genetic improvement in breeding (Croser *et al.*, 2003). By using tagged microsatellite sites (STMS) markers, which are very efficient and reliable for synteny studies in chickpea, Choumane *et al.* (2000) found that the perennial *C. anatolicum* is also phylogeneticaly close to be crossable with chickpea to introduce genes such as *Ascochyta* blight resistance.

1.2 Pathogens of Chickpea

Pathogens have three main strategies to attack plants: necrotropy, biotrophy and hemibiotrophy. Biotrophs are obligate pathogens feeding on living host tissues, whereas necrotrophs make use of nutrients by killing host tissue (Prell and Day, 2001). Pathogens, which show both biotrophic (early stages) and necrotrophic character depending on the conditions or stages of growth, are called as hemi-biotrophs (Glazebrook, 2005). The interaction of Arabidopsis and its pathogens is used as an informative model system. Compatible biotrophic fungal infection begins with germination of conidia, followed by hyphal penetration in between epidermal cells and formation of a feeding structure called haustoria, and finally sporulation without causing host cell death (Koch and Slusarenko, 1990; Glazebrook, 2005). The strategy of bacterial pathogen *P. syringae* is controversial

but proposed as hemi-biotrophic, since it may kill the host tissue later (Thaler *et al.*, 2004; Glazebrook, 2005). The fungal pathogens *Botrytis cinerea* and *Alternaria brassicicola* are Arabidopsis necrotroph pathogens which produce a variety of phytotoxins to kill host cells (Colmenares *et al.*, 2002; Otani *et al*., 1998; Glazebrook, 2005). Main necrotrophic pathogens of cool season food legumes are *Ascochyta* sp. (Ascochyta blight), *Botrytis* sp. (chocolate spot, grey mould), *Colletotrichum* sp. (anthracnose), Phomopsis blight and *Fusarium* sp. (Fusarium wilt) (Tivoli *et al.*, 2006). Tivoli *et al.* (2006) described the main features of necrotrophs as follows: germinated spores of necrotrophs form a structure called apressoria to penetrate the host through the cuticle, stomata or wounded tissues. These pathogens kill the host cells and infection develops on dead tissues until sporulation. Environmental conditions, inoculum and plant growth stages are important factors for the development and spread of these diseases.

The most important disease of chickpea is Ascochyta blight (*Ascochyta rabiei* (Pass) Labrousse). The second one is Fusarium wilt affecting the roots and caused by necrotrophic fungus *Fusarium oxysporum* f.sp. *ciceris* (Nene and Reddy, 1987; Millan *et al.*, 2006). Some other foliar diseases of chickpea are rust (*Uromices-ciceris-arietini*), Botrytis grey mold and Alternaria blight (ICRISAT, 2008c). Some root and stem fungal diseases are as follows: Dry root rot *(Macrophomina phaseolina),* black root rot (*Fusarium solani*), collar rot (*Sclerotium rolfsii*), root rot (*Fusarium* sp.). (ICRISAT, 2008d; Nene and Sheila, 1996). Chickpea is also affected by viruses such as stunt (ICRISAT, 2008e) and a variety of pests such as pod borer (*Helicoverpa armigera*), (Smithson *et al.*, 1985; Rajesh, 2001), root-knot nematode, *Meloidogyne javanica* (Treub) (Ansari *et al.*, 2004) leaf miner (Liriomyza *cicerina* Rond.), seed beetle (*Callosobruchus chinensis*L.), and cyst nematode (*Heterodera ciceri*) (Singh, 1997). The main parasitic weed of chickpea is crenate broomrape (*Orobanche crenata*) (Rubiales *et al.*, 2003).

1.3 Ascochyta Blight in Chickpea

Ascochyta blight, caused by the ascomycete *Ascochyta rabiei* (Pass) Labrousse (teleomorph: *Didymella rabiei* (Kovachevski) v. Arx. Syn. *Mycosphaerella rabiei* Kovachevski), is the main foliar fungal disease of chickpea. In suitable conditions it may cause economic losses up to 100% yield loss (Nene and Reddy, 1987; Dita *et al.*, 2006) *Didymella rabiei,* the telemorph (sexual stage), is heterothallic having bipolar, biallelic mating system (Wilson and Kaiser, 1995; Vail, 2005) and sexual reproduction is controlled

by a single regulatory locus called as mating type (MAT) (Coppin *et al.*, 1997; Nelson, 1996; Turgeon, 1993; Vail, 2005). Although Ascochyta blight fungi maybe proposed as hemibiotrophs, *A. rabiei* is determined as a necrotrophic pathogen due to its phytotoxins (solanapyrones A, B and C) (Höhl *et al.*, 1991; Tivoli and Banniza, 2007). Besides toxins, cell-wall degrading enzymes are also important for necrotrophs; such as pectic enzymes of *A. rabiei* (Tenhaken and Barz 1991; White and Chen, 2007).

The teleomorph develops only on dead infested chickpea after harvest and has fruiting structures called pseudothecia which discard ascospores; whereas the anamorph develops only on the living host and has pycnidium for discarding pycnidiospores (Kaiser, 1992; Kaiser, 1994). Besides leading to survival of the pathogen from one growing season to another (Kaiser and Kusmenoglu, 1997), the teleomorph is the reason for genetic diversity and varying virulence *via* sexual recombination (Kaiser, 1997). Spread of *A.rabiei* occurs by pycnidiospores to short distances *via* water drops, by ascospores to long distances *via* wind or by infected seed which introduce virulent and compatible mating types into new areas (Tivoli and Banniza, 2007). The disease cycle was shown in Figure 1.2.

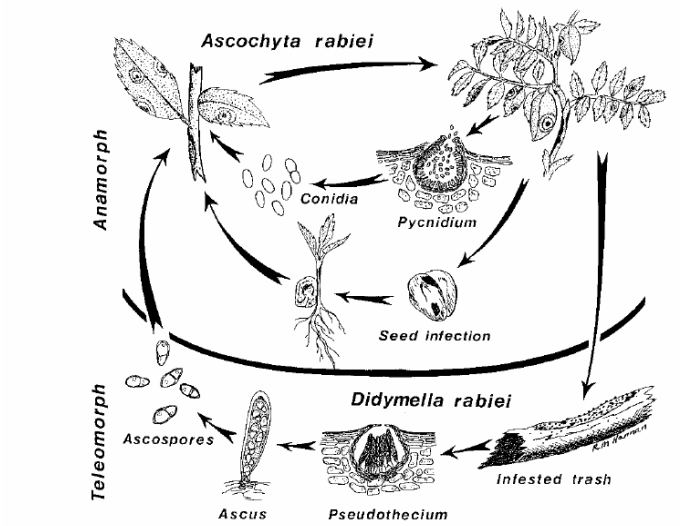

Figure 1.2 Disease cycle of Ascochyta blight (*Didymella rabiei*) on chickpea. Drawing by R.M.Hannan (Kaiser, 1997).

Hohl *et al.* (1990) proposed that fungal colonization, secretion of exudates and appressoria formation are identical on both resistant and susceptible chickpea cultivars. Pandey *et al.* (1987) explained the invasion process of a susceptible chickpea by *A.rabiei*: Germination of the spores occurred 12 h after inoculation followed by direct penetration of hypae after 24 h post inoculation (hpi) through the cuticule. After that, *A. rabiei* spreads in the apoplast, colonizes in the intercellular space and invades cells (Kohler *et al.*, 1995) by damaging the cortex about 3 days post inoculation (dpi) (Pandey *et al.*, 1987). Although time course of the further steps were reported slightly different by other authors, symptoms appear briefly as follows: macroscopic symptoms appear as yellow specks at about 4 dpi, then epidermal cells become necrotic, the hyphae in cortical tissue differentiates into pycnidia which matures about 6 dpi as black dots on the surface (Pandey *et al.*, 1987). Necrotic lesions develop firstly on leaflets and stems, then on pods and seeds (Tivoli and Banniza, 2007). Finally, pycnidiae cover leaflets and stems leading to plant death (Pandey *et al.*, 1987; Kohler *et al.*, 1995).

Tivoli *et al.* (2006) and Tivoli and Banniza (2007) indicated that phytoalexins of hosts, pathogen toxins, polycyclic character of the disease, environmental factors, inoculum pressure, form of inoculum, plant physiological and growth stage and variability of pathogenicity of fungi are common features of Ascochyta blights on grain legumes, including chickpea. For example, resistance of chickpea to *A. rabiei* decreases with increasing plant age and in the podding plants (Chongo and Gossen, 2001).

1.4 Management of Ascochyta Blight in Chickpea

Several strategies have been implied to manage Ascochyta blight in chickpea: traditional spring-sowing (Singh, 1997; Tivoli *et al.*, 2006), clean seed combined with foliar fungicides and crop rotation (Davidson and Kimber, 2007) and biological control agents to suppress *A. rabiei* growth (Dugan *et al.*, 2005; Davison and Kimber, 2007). Resistant cultivars are the main element of integrated management strategy for this disease. Desi germplasm is reported to have higher resistance than kabuli germplasm (Reddy *et al.*, 1992; Vail, 2005). International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, India) and International Center for Agricultural Research in the Dry Areas (ICARDA, Syria), the main institutions for chickpea research and breeding, have been studying on resistant chickpea cultivars (Tivoli *et al.*, 2006) and maintain collectively 20,000 germplasm accessions with a limited number of wild relatives (Choumane *et al.*,

2000). Due to the low genetic diversity of chickpea (Croser *et al.*, 2003) and complex host–pathogen relationship, no cultivars to date have been shown to be completely resistant to Ascochyta blight (Davidson and Kimber, 2007).

1.5 Understanding Molecular Basis of Ascochyta Blight Resistance in Chickpea

As in all host-pathogen relationships, the resistance mechanism should be well understood to develop *A.rabiei* resistant chickpea cultivars. For that purpose, general plant disease resistance mechanisms will be presented before presenting the special aspects for chickpea.

1.5.1 Resistance Mechanisms of Plants Against Pathogens

Plant defense responses consist of two main types, namely passive (constitutive) which are mechanisms already exist or constitutively expressed such as morphological characters and defense compounds; and active (inducible) responses which become active upon pathogen attack (Prell and Day, 2001). Race is defined as a sub-group of pathogens specialized on a specific cultivar of the host (Johnson and Booth, 1983; Mmbaga, 1997). Hammond-Kosack and Parker (2003) defined types of plant resistance against pathogens as follows: i) non-host resistance is effective against all known isolates of the pathogen resulting "no disease", ii) "race non-specific resistance" is "Resistance (R)-protein-mediated" and effective against all known isolates of the pathogen in resistant plant genotypes; iii) "race-specific resistance" is effective only in plants with R proteins corresponding to elicitors of specific isolates of the pathogen and each plant genotype exhibits resistance or susceptibility to this single isolate; iv) "basal defense" activated in susceptible genotypes of a host plant species resulting in variability of disease severity in susceptible plant genotypes. "Race-specific resistance" or "vertical resistance" or "complete resistance" which is defined as a Mendelian or qualitative trait is introduced as "gene-for-gene" resistance by Flor (1971) (Prell and Day, 2001). On the other hand, "race non-specific resistance" or "horizontal resistance" - also called "quantitative genetic resistance" or "partial resistance" - is a multigenic trait with a continuous resistance pattern assessed on a quantitative scale (Parlevliet, 1979; Tivoli *et al.*, 2006; Cho and Muehlbauer, 2004). According to Flor's "gene-for-gene" model for race-specific response, the product of a single dominant plant R gene recognizes the product of single dominant pathogen gene, *avirulance* or *avr*, directly or indirectly (guard hypothesis) and signal transduction is initiated and plant defense responses are activated (Flor, 1971 in Hammond-Kosack and

Jones, 1997; Mackey *et al.*, 2002; Hammond-Kosack and Parker, 2003). This situation is called “incompatibility” and the plant is “resistant” whereas the pathogen is “avirulent” and can not cause a disease. On the other hand, in the absence of “gene-for-gene” recognition - absence or mutation of the *avr* gene and/or of the *R* gene - the pathogen becomes virulent whereas the host is susceptible, and the interaction is “compatible” (Flor, 1971; Glazebrook, 2005).

Plants recognize pathogens through chemical substances called elicitors (Ebel and Mithöfer, 1998). Within this respect, Jones and Dangl (2006) collected responses of plants to pathogens into two groups: recognizing and responding to common molecules of many pathogens including non-pathogens; and recognizing and responding to pathogen virulence factors, either directly or through their effects on host targets. The first group, non-specific elicitors, may trigger non-host resistance, whereas the second group, specific elicitors, *avr* proteins, most of which have virulence effector functions, trigger race-specific defenses against pathogens that overcome non-host resistance mechanisms (Jones and Takemoto, 2004). Besides pathogen cellular components (PAMPs- pathogen-associated molecular patterns- like fungal cell wall peptidoglycans, lipopolysaccharides or bacterial flagellin) plants also recognize their own components (such as cell wall oligogalacturonides) released or modified by pathogen (Shibuya and Minami, 2001; Jones and Takemoto, 2004).

In plant defense response, these events occur sequentially: recognition of elicitors by R-proteins or PAMP receptors, signaling and expression of resistance (Jones and Takemoto, 2004). Immediately after elicitor perception, ion fluxes across the plasma membrane are enhanced, reactive oxygen intermediates are evolved and changes in protein phosphorylation and lipid oxidation are triggered. Expression of resistance includes induction of oxidative burst, hypersensitive response (HR), structural changes in the cell wall, release of antimicrobial compounds (such as pathogenesis-related (PR) proteins and phytoalexins) and release of systemic signals to form systemic acquired resistance (SAR) (Ebel and Mithöfer, 1998; Jones and Takemoto, 2004).

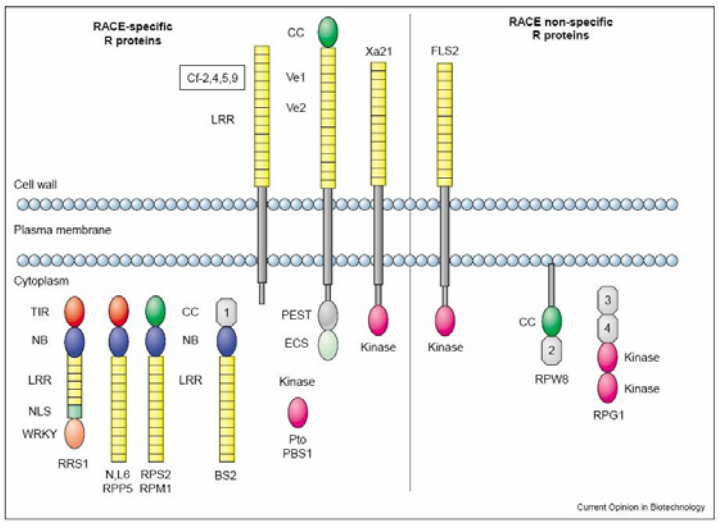

Figure 1. 3 Schematic representation of the predicted domains of R proteins which confer either race-specific or race non-specific resistance. R genes: RRS1 (*Arabidopsis* against *Ralstonia solanacearum* 1;), N (Tobacco against Mosaic virus), L6 (flax rust resistance 6), RPP5 (Arabidopsis against *Peronospora parasitica;*), RPS2 (*Arabidopsis* against *Pseudomonas syringae* p.v. *maculicola* - avrRpt2), RPM1 (*Arabidopsis* against *P. syringae* pv. *maculicola* - avrRPM1), BS2 (Pepper against *Xanthomonas campestris* pv. *vesicatoria* - avrBs2), Cf-2,4,5,9 (Tomato against *Cladosporium fulvum* races 2, 4, 5 and 9), Ve (Tomato against *Verticillium albo-atrum*), *Xa21* (Rice against *Xanthomonas oryzae* p.v. *oryzae*), Pto (Tomato against *P. syringae* p.v. *tomato* - avrPto), PBS1 (*Arabidopsis* against *P. syringae* p.v. *phaseolicola* - avrPphB), FLS2 (*Arabidopsis* against multiple bacteria having flagellin), RPW8 (*Arabidopsis* against multiple powdery mildew), RPG1 (Barley against *Puccinia graminis* f.sp. *tritici*). Other domains: NLS, nuclear localization sequences; zinc-finger transcription factor (WRKY) cytoplasm Pro-Glu-Ser-Thr-like sequence, PEST; endocytosis signal, ECS; 1–4, unconserved domains. From (Hammond-Kosack and Parker, 2003).

The classes of R genes are shown in the Figure 1.3. R genes rapidly evolve (Hammond-Kosack and Jones, 1997). The domains generally shared by R proteins are nucleotide-binding (NB) site (NBS), leucine-rich repeat (LRR), Toll-interleukin-1 receptor domain (TIR), coiled-coil (CC) or leucine zipper (LZ) structure, protein kinase domain (PK) and transmembrane domain (TM). Most R genes are classified into four main classes, namely, NB-LRR (or NBS-LRR), Receptor-Like Kinase (RLK), LRR-TM and TM-CC (Liu *et al.*, 2007). Yet, more classes are recognized by as shown in Figure 1.3. Few R genes code serine/threonine (Ser/Thr) protein kinases such as Pto of tomato (Martin *et al.* 1993; Hammond-Kosack and Jones, 1997) and extracellular LRR (eLRR) proteins (Hammond-Kosack and Parker, 2003). LRR domains manage specificity in pathogen recognition (Dangl and Jones, 2001; Hammond-Kosack and Parker, 2003). NB domains are involved in signal transduction by binding and hydrolyzing ATP (Tameling *et al.*, 2002; Hammond-Kosack and Parker, 2003). TIR domain, homologous to cytoplasmic domains of the *Drosophila* Toll protein and the mammalian interleukin-1 receptor, and the CC domain, important in protein-protein interactions, are other elements of signal transduction (Hammond-Kosack and Jones, 1997; Burkhard *et al.*, 2001; Liu *et al.*, 2007). Plant RLKs (transmembrane proteins) also transmit signals from pathogens such as the RLK *Xa21* of rice (Song *et al.*, 1995; Dievart and Clark, 2004; Liu *et al.*, 2007). Although R-*avr* type of resistance is usually common for biotrophic and hemibiotrophic pathogens, few examples of R genes recognizing necrotrophic pathogens are found: Hm1 of maize against fungal necrotroph *Helminthosporium maydis* (race 1) is a race-specific HC toxin reductase and can not be classified into above mentioned R gene classes (Hammond-Kosack and Parker, 2003). Another one is TM domain having Asc-1 of tomato against necrotroph *Alternaria alternata* f.sp. *lycopersici* which produces AAL toxin (Brandwagt *et al.*, 2000; Hammond-Kosack and Parker, 2003). Unlike the fragile race-specific resistance, non-host resistance is durable due to "*overlapping mechanisms*" (Hammond-Kosack and Parker, 2003). An example for genes related to non-host resistance is NHO1 (Non-Host1) against *Pseudomonas syringae pv. phaseolicola* (Lu *et al.*, 2001).

Upon recognition of the elicitor, the most rapid response in the plant cell are ion fluxes and changes in membrane permeability, mainly Ca+ and H+ influx, and K+ and Cl− efflux (Kombrink *et al.*, 1995; Atkinson, 1993; Wojtasek, 1997; Ward *et al.*, 1995; Ebel and Mithöfer, 1998). These result in activation of membrane-bound kinases, phosphatases, phospholipases and G proteins (Prell and Day, 2001). GTP-binding proteins are also shown to participate in the induction of ion fluxes, oxidative burst, and early signaling

events during plant defense response (Yang *et al.*, 1997). Kinases activate the NADPH-oxidase complex which transforms O_2 into reactive oxygen species (ROS: O_2-, superoxide; H_2O_2, hydrogen peroxide; $^{\cdot}HO_2$, perhydroxyl radical and $^{\cdot}OH$, hydroxyl radical) which lead to oxidative burst (Viard *et. al.,* 1994; Wojtasek, 1997; Prell and Day, 2001; Hammond-Kosack and Jones, 1996; Ebel and Mithöfer, 1998). ROS is also produced by generation of H_2O_2 catalyzed by a cell wall peroxidase activated *via* ion fluxes and germin-oxalate oxidase system (Bolwell *et al.*, 1995; Zhang *et al.*, 1995; Wojtaszek 1997). Rapid generation of ROS leads to a type of early defense response called "hypersentive response, HR", a type of programmed cell death (Lamb and Dixon, 1997). Influx of calcium ions occurs not only in the upstream but also in the downstream of (induced by) oxidative burst leading to HR (Levine *et al.*, 1996; Hancock *et al.*, 2002). Superoxide dismutase (SOD) catalyzes conversion of O_2-/$^{\cdot}HO_2$ to H_2O_2. In the presence of metals, H_2O_2, which is also toxic to pathogens, is converted to $^{\cdot}OH$ which initiates radical chain reactions leading lipid peroxidation, enzyme inactivation, and nucleic acid degradation (Peng and Kuc, 1992; Lamb and Dixon, 1997). It strengthens plant cell wall and stimulates benzoic acid-2 hydroxylase (BA_2-H) activity for salicylic acid (SA) biosynthesis (Bolwell *et al.*, 1995; León *et al.*, 1995; Hammond-Kosack and Jones, 1996) which in turn activates pathogenesis related (PR) gene expression (Lamb and Dixon, 1997). ROS are also signal molecules for systemic acquired resistance (SAR), ROS scavenging enzymes such as glutathione S-transferase (GST), glutathione peroxidase and polyubiquitin (Levine *et al.*, 1994; Lamb and Dixon 1997) and synthesis of some phytoalexins (Jabs *et al.*, 1997; Ebel and Mithöfer, 1998). Other ROS scavenging enzymes and molecules are ascorbate peroxidases (APXs), catalases (CATs), ascorbic acid and glutathione (Noctor and Foyer 1998, Mittler 2002).

Another early defense signal generated is reactive nitrogen species nitric oxide (NO), which induces HR, oxidative burst and defense-related genes such as *PR-1*, phenylalanine ammonium lyase (PAL) and GST (Durner *et al.*, 1998; Delledonne *et al.*, 1998; Hancock *et al.*, 2002). NO, which may be produced by nitric oxide synthase, nitrate reductase and xanthine oxidoreductase, may react with O_2- to form the very reactive and damaging molecule peroxynitrite, ONOO– (Hancock *et al.*, 2002).

Transmitting the receptor signal *via* protein kinases cascades and protein phosphatases to activate defense genes is also an early event (Ebel and Mithöfer, 1998; Cvetkovska *et al.*, 2005). MAPKs (Mitogen Activating Protein Kinase) and CDPKs (calcium-dependent

protein kinases) rapidly amplify the signal (Peck, 2003; Ludwig *et al.*, 2005; Kariola, 2006; Zhang and Klessig, 2001; Jones and Takemoto, 2004). Other important kinases are SIPK (salicylate inducible protein kinase) and WIPK (wound-inducible protein kinase). MAPK cascades are common for both host-specific and non-host resistance and activated by specific/nonspecific elicitors, oxidative burst and NO (Zhang and Klessig, 2001; Jones and Takemoto, 2004; Clarke *et al.*, 2000; Hanckok *et al.*, 2002; Hammond-Kosack and Parker, 2003). MAPK pathway, which is in the upstream of receptors and downstream of the targets, consists of several kinases (Cvetkovska *et al.*, 2005). They phosphorylate transcription factors (TFs), which finally lead to transcription of other genes, such as ERF (ethylene-responsive factor) in rice (Cheong *et al.*, 2003; Cvetkovska *et al.*, 2005). Arabidopsis MAPK4 is involved in regulation of SA and jasmonic acid (JA)/ethylene (E)-dependent responses by means of EDS1 and PAD4 (see below; Nielsen *et al.*, 2006). In defense responses, several transcription factors such as TGA-bZIP, ERF, Myb and WRKY have role in gene activation (Cvetkovska *et al.*, 2005). For example, WRKYs activate PR-1 (which controls SA synthesis and SAR), PR-2 and some receptor-like-kinases (RLKs) (Rushton *et al*., 1996; Du and Chen, 2000; Cvetkovska *et al*., 2005).

Programmed cell death (PCD) plays role in both susceptibility and resistance responses of plants. Susceptible plant cells are killed directly *via* toxins of the pathogens or indirectly *via* virulence factors or PCD-triggering specific signal molecules (Greenberg, 1997; Khurana *et al.*, 2005). The well-known example is the AAL toxin of necrotrophic fungal pathogen *Alternaria alternaria* f. sp. *lycopersici* causing Alternaria stem canker disease in tomato (*Lycopersicon esculentum*): AAL toxin blocks sphingolipid biosynthesis in susceptible plants having homozygous recessive genotype *asc/asc* plants -having unfunctional *Asc-1* gene (ceramide synthase) and kills the host cells by inducing accumulation of 3-ketosphinganine and sphinganine long chain bases which trigger apoptosis. On the other hand; functional *Asc-1* protein releases this block and results in resistance response (Brandwagt *et al.*, 2000; Spassieva *et al.*, 2002; Khurana *et al.*, 2005). The incompatible biotrophic pathogens may trigger a specific type of PCD in the host plant, called HR, and become arrested at the infection site (Heath, 2000). After recognition of an avirulent pathogen *via* R gene or non specific receptors, HR may be triggered *via* ion fluxes, ROS, NO, SA, MAPK cascades either alone or in coordination (Heath, 2000; Khurana *et al.*, 2005; Hammond-Kosack and Parker, 2003) Mitochondria voltage-dependent-gated anion channels (VDAC), (Lacomme and Roby, 1999; Khurana *et al.*, 2005), actin-mediated organelle rearrangements/signaling and activation of cysteine

proteases (caspases) are other elements of HR (Heath, 2000). HR also activates SAR (Alvarez *et al.*, 1998; Heath, 2000). HR may facilitate colonization of necrotrophic pathogens, for example, *B. cinerea* triggers oxidative burst and HR in the host (Govrin and Levine, 2000). However, non-host/necrotrophic pathogen interaction (*Pinus pinaster* and *B. cinerea*) was shown to be ROS-mediated and based on activation of Type II non-host resistance characterized by HR (Mysore and Ryu, 2004; Azevedo *et al.* 2008).

One of the early events upon pathogen recognition is lipid oxidation mediated either by ROS or by lipoxygenases (LOX). cis-jasmonic acid (JA) and 12-oxo-phytodienoic acid, which are produced from linolenic acid (Mueller *et al.*, 1993; Ebel and Mithöfer, 1998), are messengers of intracellular signaling pathway triggered upon pathogen and pest induced wounding (Farmer and Ryan 1992; Ebel and Mithöfer 1998). LOX generated lipid peroxides have role in signaling for SA accumulation and act coordinately with signal molecules JA and methyl-JA (MJ) (Léon *et al.*, 1995; Hammond-Kosack and Jones, 1996). LOX activity also mediates HR *via* membrane damage and produces fatty acid-derived secondary metabolites toxic to pathogens (Keppler and Novacky, 1986; Croft *et al.*, 1993; Hammond-Kosack and Jones, 1996).

Local and systemic signaling networks in plant defense response depend on coordinative work of SA, NO, ROS, JA and E. SA and JA or E and JA may counteract on the expression of some defense genes, whereas E and JA may act synergistically or individually (Thomma *et al.*, 2001; Hammond-Kosack and Parker, 2003; Glazebrook, 2005). An overview of these pathways is shown in Figure 1.4.

SA, which is associated with HR, usually accumulates upon incompatible pathogen infection and it is toxic to pathogens (Raskin, 1992; Hammond-Kosack and Jones, 1996). In Arabidopsis, SA is produced in phenylpropanoid pathway *via* isochorismate synthase coded by *SID2* (Wildermuth *et al.*, 2001; Glazebrook, 2005). RAR1 and SGT1 are regulators of R-gene-mediated resistance in plants (Azevedo *et al.*, 2002; Austin *et al.*, 2002). Most CC-NB-LRR-type R proteins require NDR1 (Hammond-Kosack and Parker, 2003). EDR1 (a MAP kinase kinase, Frye *et al.*, 2001), MAPK4 and SSI2 (a stearoyl-ACP desaturase, Kachroo *et al.*, 2001) negatively, but SABPs positively regulate SA signaling (Hammond-Kosack and Parker, 2003).

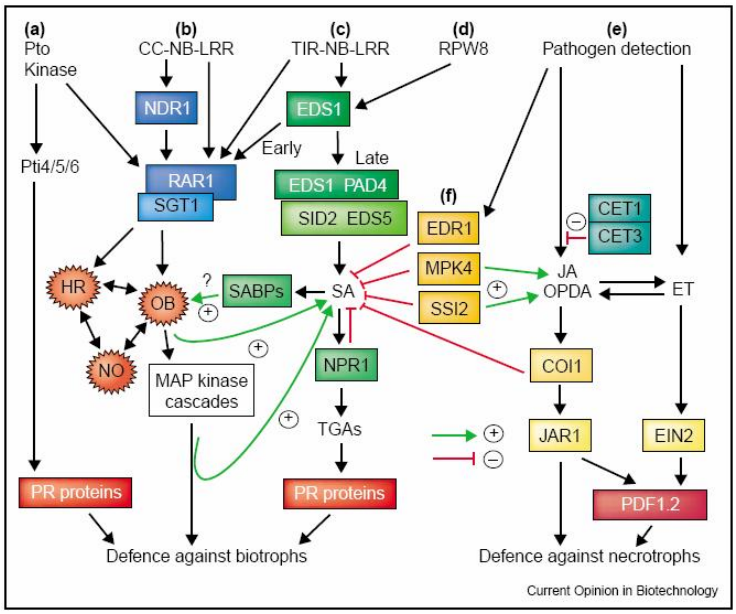

Figure 1.4 Local signaling networks controlling activation of local defense responses. R proteins: Pto-kinase; CC-NB-LRR-type, TIR-NB-LRR-type, RPW8 (non-race-specific). Abbreviations: Pti4/5/6, Pto-interacting 4, 5 and 6; NDR1, non-race specific disease resistance 1; EDS1/EDS5 enhanced disease susceptibility 1/5; RAR1, required for Mla-dependent resistance 1; SGT1, suppressor of G2 allele of SKPI; PAD4, phytoalexin-deficient 4; SID2, SA induction deficient 2; SA, salicylic acid; SA induction deficient 2, EDR1, enhanced disease resistance 1, MPK4, MAPK 4; SSI2, suppressor of salicylate insensitivity of NPR1-5; SABPs SA-binding proteins; NPR1 non-expressor of PR1; TGAs, TGACG DNA motif; CET1/CET3, constitutive expression of thionin 1/3; JA, jasmonic acid; OPDA, 12-oxophytodienoic acid; COI1, coronatine insensitive 1; JAR1, JA resistance 1; ET, ethylene; EIN2, ethylene-insensitive 2; PDF1.2, plant defensin 1.2. HR, hypersensitive response; OB, oxidative burst; NO, nitric oxide. From Hammond-Kosack and Parker (2003).

TIR-NB-LRR proteins require actions of both EDS1 (Lipase-like protein, Falk *et al.*, 1999), PAD4 (Lipase-like protein; Jirage *et al.*, 1999), SID2, EDS5 (MATE transporter; Nawrath *et al.*, 2002), SA and NPR1 (Hammond-Kosack and Parker, 2003). The ankyrin repeat protein NPR1, whose translocation into nucleus is stimulated by SA, interacts with TGA transcription factors to induce expression of PR genes (Kinkema *et al.*; 2000; Fan and Dong, 2002; Glazebrook, 2005).

JA and derivatives such as MJ (catalyzed by an S-adenosyl-L-methionine) are signal molecules involved in biotic or abiotic stimuli. No JA receptor was identified yet (Devoto and Turner, 2005). E is synthesized from methionine where two of its biosynthetic enzymes (1-aminocyclopropane-1-carboxylic acid (ACC) synthase and ACC oxidase) are affected by environmental and internal stimuli (Van Zhong and Burns, 2003). In *Arabidopsis*, there are five families of E receptors (ETR1, ETR2, ERS1, ERS2, and EIN4) and a repressor, CTR1, a Raf-like kinase (Kieber *et al.*, 1993; Stepanova and Ecker, 2000). Signal transduction and gene expression is achieved *via* EIN2, which is an integral membrane protein similar to a mammalian family of NRAMP metal-ion transporters (Alonso *et al.*, 1999), nuclear EIN3 proteins (plant-specific transcription factor) and consequently induction of transcription factors called EREBPs (ethylene-response-binding proteins) such as ERF1 (ethylene response factor 1) (Stepanova and Ecker, 2000; Van Zhong and Burns, 2003). JA and E dependent signaling stimulate expression of defense effector genes such as PDF1.2 (plant defensin) (Glazebrook 2005). JA is negatively regulated by CET1 and CET3 in the upstream; whereas by COI1 (F-box protein; Xie *et al.*, 1998) and JAR1 (JA-amino synthetase; Staswick *et al.*, 2002) are located in the downstream, both of which are required for resistance to necrotrophic pathogens (Hammond-Kosack and Parker, 2003). Nonetheless, E requires only EIN2 to stimulate expression of PDF1.2 (Hammond-Kosack and Parker, 2003). ERF1, whose over expression increases resistance to some necrotrophs, is located at the crossing point of JA and E signals, and it is dependent on both COI1 and EIN2 (Lorenzo *et al.*, 2003; Berrocal-Lobo *et al.*, 2002a; Glazebrook, 2005). There are also interactions between SA and JA/E signaling. EDR1, MPK4 and SSI2 transcription factor WRKY70 (Li *et al.*, 2004) communicate in between SA and JA/E signaling network (Hammond-Kosack and Parker, 2003; Glazebrook, 2005).

Systemic defense mechanisms arise *via* triggering of local responses by pathogens or non-pathogens. HR activates SA signaling throughout the plant to induce SAR against

subsequent responses (Hammond-Kosack and Parker, 2003; Glazebrook, 2005). Similarly, pre-treatment of plants with a chemical compound such as benzo-(1,2,3)-thio-diazole-7-carbothionic acid S-methyl ester (BTH) may also induce SAR (Buchanan *et al.*, 2000; Hammond-Kosack and Parker, 2003). The fact that *son1* mutant also has systemic resistance independent of SA, this phenomenon is called as SAR-independent resistance or SIR (Kim and Delaney, 2002; Hammond-Kosack and Parker, 2003). Non-pathogenic rhizobacteria induce JA/E dependent but SA independent systemic defense called induced systemic resistance (ISR) (Pieterse *et al.*, 2002; Hammond-Kosack and Parker, 2003). NPR1 is required for both SAR and ISR (Hammond-Kosack and Parker, 2003). SAR is mostly effective to biotrophic pathogens whereas ISR is effective against necrotrophic pathogens (Hammerschmidt, 2004).

Considering Arabidopsis and its pathogens; in general, "gene-for-gene" or R/*avr* controlled resistance including HR associated activation of defense by SA signaling are generally effective against biotrophs whereas JA/E signaling and quantitative traits regulated defense responses are generally effective against necrotrophs (Oliver and Ipcho, 2004; Hammerschmidt, 2004; Glazebrook, 2005). However, Glazebrook (2005) emphasized that this idea is an "*over-simplistic view*": For example, "gene-for-gene" resistance associated with SA-dependent signaling and SAR is important for resistance against biotrophs such as *Phytophthora parasitica, Erisyphe* spp and *P. syringae*; besides, JA-dependent resistance mechanisms seems to be not important or contradictory. However, artificially triggered JA-/E-dependent defenses are also effective for *P. parasitica* and *Erisyphe* spp. *P. syringae* inhibits SA defenses of *Arabidopsis* by inducing JA/E signaling *via* virulence factor toxin coronatine. On the other hand, generally, neither "gene-for-gene" resistance nor SA-dependent and SAR are expected for necrotrophs. Although JA signaling is shown to be required against *A. brassicicola* and *B. cinerea* as shown in *coi1* mutants, *jin1* mutants are more resistant to *B. cinerea* showing that generalization even for the same pathogen may not be true. SA- and E-signaling are not important for *A. brassicicola* as shown by defected SA (*eds5, npr1*, *pad4*, *sid2*) and E defected (*ein2*) mutants. The role of SA signaling for *B. cinerea* resistance is complex: exogenous SA treatment increases the resistance. Besides, resistance to *B. cinerea* requires E signaling as shown by susceptible *ein2* mutants and increased resistance by ERF1 over expression. As a result, JA and E signaling pathways are co-regulated in *B. cinerea* resistance (Glazebrook, 2005).

Protein folding, activation and degradation are also important in signaling during plant resistance (Hammond-Kosack and Jones, 1996). Van der Hoorn and Jones (2004) suggested various roles of proteases in defense of plants: release elicitors/toxins, be activated by elicitors, activate downstream signaling components/enzymes or activate/degrade regulators. Polyubiquitinated proteins *via* ubiquitination system-consisting of ubiquitin activating (E1), ubiquitin conjugating (E2) and ubiquitin protein ligating (E3) enzymes (Glickman and Ciechanover, 2002; Devoto *et al.*, 2003)- are recognized and degraded by 26S proteasome (Devoto and Turner, 2005). Ubiquitination is also involved in activation of kinases, localization signals, signaling and removing of oxidatively damaged proteins during H_2O_2-induced cell death (Pickart, 2001; Devoto *et al.*, 2003; Vandenabeele *et al.*, 2003). The ubiquitin-proteasome pathway modulates R-gene-triggered resistance (Hammond-Kosack and Parker, 2003). One of the E3 ligase involved in plant defense system is the well-known SCF (SKPI, CDC53p/CUL1 F-box) complexes which have a RING-finger domain (Weissman, 2001; Devoto *et al.*, 2003). Two Arabidopsis F-box proteins COI1 (Xie, *et al.*, 1998) and SON1 (suppressor of NIM1-1, Kim and Delaney, 2002) were shown to interact with SCF: SCF/COI1 complex regulates JA-mediated responses positively (Xu *et al.*, 2002) whereas SCF/SON1 complex regulates plant defense response negatively (Devoto *et al.*, 2003). RAR1 and SGT1 are involved in R-gene-mediated resistance related to ubiquitination (Azevedo *et al.*, 2002; Liu *et al.*, 2002). The chaperon HSP90 (heat shock protein 90) is required for several R-mediated defense pathways by forming a complex with RAR1 and SGT1 both of which are structurally similar to HSP90 co-chaperones (Hubert *et al.*, 2003; Dreher and Callis, 2007).

Proteins produced upon pathogen attack are regarded as defense related proteins, some of which are collected under the name of "pathogenesis-related, PR" proteins. Yet, defense related proteins are generally grouped as; i) the structural proteins and enzymes for resisting pathogen attack such as cell wall structural proteins (hydroxyproline-rich proteins, HRGP or extensins, glycine-rich proteins (GRP), peroxidases for lignin and syberin synthesis, callose synthase and enzymes for phenol synthesis); ii) enzymes having antimicrobial effect such as ones for lectin/thionin/tannin/phytoalexin synthesis and amylases, proteinases, thaumatins, β-1,3-Glucanase (PR-2), chitinases (PR-3) (discussed in Prell and Dey, 2001). Induction of PR proteins was already stated in relation to SA, ROS and transcription factor mediated signaling.

Since pathogens penetrate through the plant cuticle and cell wall, mainly through wounds or openings, fortifying the cell wall is a defense response to prevent hyphal growth, diffusion of toxins and enzymes of pathogens especially of necrotrophs (Hammond-Kosack and Jones, 1996). Lignin precursors and free radicals produced during polymerization reactions in the cell wall affect pathogen membrane, enzymes, toxins or elicitors negatively (Hammond-Kosack and Jones, 1996). Necrotrophs degrade plant cell wall by mechanical pressure and hydrolytic enzymes such as polygalacturonases (PGs) which are inhibited by PG-inhibiting proteins (PGIPs) elicited by plant cell wall fragments released during this attack (Agrios, 1988; Hammond-Kosack and Jones, 1996). Formation of papillae (Heath, 1980), rapid callose deposition, extensins (Showalter, 1993), local accumulation of lignin (Whetten and Sederoff, 1995) and rapid oxidative cross-linking by preformed HRGPs accompanied with oxidative burst (Btadley *et al.*, 1992) are other mechanisms related to cell wall fortification in the incompatible interactions (Hammond-Kosack and Jones, 1996).

Plant secondary products have also role as antimicrobial substances. Although structurally similar and their roles may be variable depending on plant species, phytoalexins are inducible antimicrobial substances by pathogen attack; whereas phytoanticipins are preformed inhibitors (Van Etten *et al.*, 1994; Dixon, 2001). Phytoalexins can be detected rapidly after pathogen recognition and HR. They are derived from different compounds such as flavonoids/isoflavonoids, isoprenoids (terpenes), stilbenes and indole from primary metabolic precursors such as phenylalanine (Dixon, 2001). Their biosynthetic enzymes such as PAL, that control key branching point, are induced *de novo* upon pathogen attack (Hammond-Kosack and Jones, 1996). For example, the inducible *Arabidopsis* phytoalexin camalexin synthesized by a cytochrome P450 monoxygenase (P450) coded by the gene *PAD3*, is required for defense responses against necrotrophic pathogens *A. brassicicola* and *B. cinerea* but not against biotrophs (Thomma *et al.*, 1999; Zhou *et al.*, 1999; Ferrari *et al.*, 2003; Glazebrook, 2005)

It is known that abscisic acid (ABA), JA and E are involved in regulation of gene expression during biotic and abiotic stresses. Anderson *et al.* (2004) showed that exogenous ABA suppressed both basal and JA-E–activated defense related genes, whereas ABA deficiency mutations resulted in upregulation of these genes which results in resistance to the necrotrophic fungal pathogen *Fusarium oxysporum*. As a result, interaction between ABA and the JA-E signaling pathways in response to biotic and

abiotic stresses is antagonistic. Similarly, ABA-deficient Sitiens (tomato mutant, *Solanum lycopersicum*), which is resistant to necrotroph *B. cinerea*, displays stronger SA-dependent defense response and earlier and stronger accumulation of H_2O_2 (Audenaert *et al.*, 2002; Asselbergh *et al.*, 2007). This shows that timely hyperinduction of H_2O_2-dependent defenses at the site of infection blocks early development of a necrotroph despite the fact that ROS formation facilitates colonization (Asselbergh *et al.*, 2007). Fujita *et al.* (2006) emphasized that several molecules such as ROS, hormones (ABA, E, JA, SA) transcription factors (such as AtMYC2, R2R3MYB, RD26 and Zat12) and kinases (MAPK) are involved in both biotic and abiotic stresses *via* synergistic and/or antagonistic actions.

Wounding and pathogen defense responses are highly overlapped: these are especially signaling-regulatory components, effector proteins (RLKs, non-receptor protein kinases, protein phosphatases, calcium-binding proteins, G-proteins, transcription factors, NPR1-like gene, NDR1, several putative R genes), enzymes required for cell wall modification, secondary metabolism and oxidative burst (Cheong *et al.*, 2002). Wounding or necrotrophic pathogen infection induce E and JA-signaling pathways: pathogen-responsive genes are regulated by cooperation of the E and JA signals *via* transcription factor ERF1 which suppresses wound responsive genes; whereas JA alone regulates wound-responsive genes *via* transcription factor AtMYC2 which repress necrotrophic pathogen-response genes (Lorenzo *et al.*, 2004).

Finally, regulation of gene expression *via* gene silencing called as RNA interference (RNAi) or post-transcriptional gene silencing (PTGS), which are triggered by double-stranded RNA (dsRNA), are also included in plant defense responses (Meister and Tuschl, 2004). Although there are many examples of RNAi in plant defense systems against viruses and transposable elements (Voinnet 2002; Yu and Kumar, 2003), there are limited reports related to bacterial and fungal pathogens (see Section 3.11). Short interfering RNAs (siRNAs), repeat-associated short interfering RNAs (rasiRNAs) and microRNAs (miRNAs) are best known small RNAs involved in RNAi (Meister and Tuschl, 2004). Long dsRNA and miRNA precursors (pre-miRNA) are converted into siRNA/rasiRNAs and miRNA respectively by the RNase-III-like enzyme Dicer (Meister and Tuschl, 2004). Then they are assembled into effector complexes such as RISC (RNA-induced silencing complex) for mRNA-target degradation (Hammond *et al.*, 2000) and miRNP (micro ribonucleoprotein particle) for translational repression (Mourelatos *et al.*, 2002) both of which have proteins called as AGO (Argonaute) (Meister and Tuschl, 2004).

1.5.2 Findings Related to the Resistance of Chickpea Against Ascochyta Blight

Studies to find the genetic basis of Ascochyta blight in chickpea involve identification of quantitative trait loci (QTL) for resistance and mapping; and investigation of transcriptional/translational and biochemical changes upon *A.rabiei* attack. Before that, information is provided related to pathogenic variability of *A.rabiei*, since it defines the virulence and the progress of the disease.

1.5.2.1 Pathogenic Variability of *Ascochyta rabiei*

There have been controversial reports on pathogenic variability and classes of pathotypes or races of *A.rabiei*. For example, in one of the pioneering study, Reddy and Kabbabeh (1985) reported 6 physiological races of *A. rabiei* in Syria and Lebanon with decreasing aggressiveness from 6 to 1 (Udupa *et al*., 1998). Dolar and Gürcan (1992) reported the existence of races 1, 4 and 6 in Turkey. Nene and Reddy (1987) reported 5 pathogenic groups and several strains from Pakistan and Turkey (Udupa *et. al.* 1998). Mmbaga (1997) proposed that, the pathogenic variability of *A.rabiei* populations can be evaluated like races on a set of chickpea differentials if characterization procedures were standardized. Navas-Cortés *et al.* (1998) identified 11 pathotypes from the isolates of India, Pakistan, Spain and the United States using differentials.

To solve the classification problem, Udupa *et al.* (1998) genotyped 53 Syrian isolates with RAPD and microsatellite markers by using three differentials. As a result, they proposed three pathotypes: pathotype I (least aggressive), pathotype II (moderately aggressive) and pathotype III (most-aggressive) with abundance of 24.5%, 9.5% and 66.0% respectively. Udupa *et al.* (1998) explained that the high abundance of pathotype III resulted from genetic drift due to selection pressure of resistance chickpea cultivars. Since different pathotypes did not form a single phylogenic group, they proposed that several genes control the pathogenicity which could be the reason for not observing a "gene-for-gene" relationship in *A.rabiei*-chickpea pathosystem. Although they noted that the genetic diversity was highest in pathotype I, followed by pathotype II and pathotype III; Santra *et al.* (2001) stated that no correlation was found in between genetic diversity and pathogenicity of Indian *A.rabiei* isolates. Chen *et al.* (2004) studied the pathogenic variability of telemorph *Didymella rabiei* in western United States and found that pathotype I (low-pathogenicity group) caused a bimodal distribution of disease severity

showing a major gene controlling the resistance; and pathotype II (high-pathogenicity group) caused a continuous distribution (ranging from 2 to 9) of disease severity showing multiple genes controlling the resistance. Pathotype I was represented by isolates AR19, AR21 and races 1, 2, 3, 4 and 5; whereas pathotype II was represented by isolates A2-11 L, A3-2S, AR628, CAB02-14, and race 6. They also proposed that few chickpea differentials can be used for pathogenicity assays. They suggested that pathotypes I and II were in line with the three-pathotype system of Udupa *et al.* (1998), except the lack of pathotype III in USA and few contrasting reactions (Chen *et al.*, 2004). On the other hand, Vail (2005) concluded that it is not possible to classify *A.rabiei* isolates into physiological races or pathotypes based on disease reaction of differentials due to quantitative nature of the disease resistance. She argued that this may be misleading due to selection of biased pathogen population consisting of highly aggressive isolates and small population size; both of which give rise to a discontinuous distribution frequency (Vail, 2005) .

Phytotoxins produced by *A.rabiei* are solanapyrones A, B, C (Höhl *et al.*, 1991; Latif *et al.*, 1993) and cytochalasin D (Latif *et al.*, 1993). Kaur (1995) stated that solanapyrones A, B and C work in a host-selective and concentration dependent manner and exhibited higher toxicity in susceptible genotypes. Therefore, solanapores are proposed to be possible virulence factors (Kaur, 1995). Other virulence factors may be enzymes such as plant cell wall hydrolyzing enzymes or suppressors of defense reactions (Strange, 2006).

1.5.2.2 Identification of Quantitative Trait Loci and Mapping for Resistance to Ascochyta Blight

The ideas related to the inheritance of *A.rabiei* resistance was changed from single genes to quantitative traits over the years. Crino (1990) summarized the work done in between years 1953 to 1986 and concluded that the resistance of chickpea against *A.rabiei* may be controlled either by a single dominant or single recessive gene. Dey and Singh (1993) suggested that chickpea-*A.rabiei* interaction system is governed by epistatic interactions of two dominant complementary genes in two genotypes and by one dominant and one recessive gene in another genotype. In another study, Van Rheenen and Haware (1994), evaluated the resistance of 19 chickpea varieties infected with different sources of *A.rabiei* in 5 different locations of India and concluded that the resistance is quantitative.

Winter *et al.* (1999) constructed a size-selected library in chickpea to screen microsatellites for designing STMS markers. In the further study, Winter *et al.* (2000) prepared a high density genetic map consisting of 11 linkage groups (LG) on the F7-F8 RIL population from the interspecific cross of Fusarium wilt resistant and susceptible lines (ICC-4958 X PI 498777; *C. reticulatum*). The map consisted of 303 markers (STMS, DAF (DNA amplification fingerprinting), AFLP, ISSR (Inter-simple sequence repeat), RAPD (Random Amplified Polymorphic DNA), isozymes, RFLP (Restriction Fragment Length Polymorphism) and SCAR (Sequence-Characterized Amplified Region). They also mapped genes for resistance to races 4 and 5 of Fusarium wilt, *Foc4* and *Foc5* respectively, in between closely linked markers (*Foc4*/TA96/*Foc5*/TA27) on LG2. The map of Winter *et al.* (2000) is regarded as "reference map" of chickpea (Millan *et al.*, 2006) and this terminology was also used in this study.

Tekeoglu *et al.* (2000) constructed three RIL populations by single-seed descent from the F2 to the F7: two intraspecific crosses, namely CRIL-3 (FLIP84-92C(2), resistant X PI 359075(1), susceptible) and CRIL-6 (Dwelley resistant X Blanco Lechoso susceptible), one interspecific cross, CRIL-7 (FLIP84-92C(3) resistant X *C. reticulatum* (PI 599072) susceptible). CRIL-3 and CRIL-7 were used as mapping population in many other studies as well as in this study. Analysis of disease development in the nursery revealed three major recessive and complementary genes with several modifier genes controlling blight resistance; with a possible epistasis (Tekeoglu *et al.*, 2000), i.e., "*non-reciprocal interactions between genes*" (Klug and Cummings, 2003). Santra *et al.* (2000) analyzed disease development in CRIL-7 in the nursery and identified two major QTLs and one minor QTL without epistatic interactions i.e., QTL-1 (LG6; markers UBC733b/UBC181a), QTL-2 (LG1; markers UBC836b/*Dia4*) and QTL-3 (LG4; markers UBC681a/UBC858b). Tekeoglu *et al.* (2002) integrated 50 STMS and one RGA on the reference map by using CRIL-7. The QTL-1 and QTL-2 reported by Santra *et al.* (2000) were shown to be located on LGVII (LG6; markers UBC733b/UBC181a/Gaa47) and LGIV (LG1; markers UBC836b/*Dia4*/Ta72s/Ta146/Ga2), respectively. Highly polymorphic co-dominant STMS were shown to be important and useful to join several genetic maps of chickpea despite of the different mapping populations (Tekeoglu *et al.*, 2002).

In contrast to above mentioned studies, Collard *et al.* (2003) used an interspecific F2 population whose resistant parent was a wild accession (*C. echinospermum*) to evaluate seedling and stem resistance to Ascochyta blight in glasshouse conditions (Collard *et al.*,

2003; Millan *et al.*, 2006). The linkage map constructed by RAPD, STMS, ISSR, and RGA markers was transferred to the reference map *via* STMS markers. Both major QTL 1S (linked to STMS11/GA2/UBC836b/TR20) and a second QTL 2S (RGA marker XLLRb280) for seedling resistance were located on LG4. They observed polygenic control, additive but no epistatic interactions for seedling resistance. They suggested two loci (but no significant QTL) without epistatic interactions controlling the stem resistance. QTL 1S occupied the same position as QTL-2 found by Santra *et al.* (2000), as well as locus *ar2b* found by Udupa and Baum (2003) and another QTL (*QTLs 4/5/6*) defined by Flandez-Galvez *et al.* (2003b). Consequently, they proposed that LG4 is an important region for resistance to Ascochyta blight (Collard *et al.*, 2003).

Due to segregation distortions, Flandez-Galvez *et al.* (2003a) criticized the use of RILs and interspecific crosses for mapping chickpea genome since such crosses might not represent the "*recombination-distance map*". Indeed, segregation distortion towards the wild parent was reported by Winter *et al.* (2000), Tekeoglu *et al.* (2002) and Collard *et al.* (2003). For that reasons, they established a blight segregating F2 population from an intraspecific cross (ICC12004 X Lasseter) and constructed a genetic map by means of STMS, ISSR, RAPD and RGA markers. The distorted segregation was in favor of maternal alleles (Flandez-Galvez *et al.*, 2003a). Afterwards, Flandez-Galvez *et al.* (2003b) identified 6 QTLs against a virulent *A.rabiei* isolate in the controlled environment and field: *QTL 1* (LG I, markers TS12b) and *QTL4* (LGIII, markers TA30/TA146/TR20) only in the field, QTLs *2* and *3* (LG II, markers TA3a/TA3b) and *QTL6* (LGIII, markers TA30/TA146/TR20) only in the controlled environment and the major *QTL5* (LG III, markers TA30/TA146/TR20) in both conditions. They proposed that QTL-2 (Santra *et al.*, 2000) or QTL for seedling resistance 1S found by Collard *et al.* (2003) resolute into three QTLs as *QTLs 4/5/6*; probably due to different mapping populations inoculated with different pathotypes (Flandez-Galvez *et al.*, 2003b). Bian *et al.* (2007) proposed *QTL 1*, *QTL3/2* and *QTL4/5/6* are located on LG3, LG8 and LG4 of the reference map. Flandez-Galvez *et al.* (2003b) also found that epistatic interactions were important. Further more, all the QTLs were mapped near a RGA marker (Flandez-Galvez *et al.*, 2003b).

Only Udupa and Baum (2003) and Cho *et al.* (2004) disclosed pathotype specific QTLs, namely, pathotype I and pathotype II (Chen *et al.*, 2004). The former scientists used a F6:7 RIL population from an intraspecific cross of chickpeas ILC 1272 (susceptible to pathotype I and II) and ILC 3279 (resistant to pathotype I and II) under controlled

environment by microsatellite markers and transferred them into the reference map. They mapped a major recessive locus *ar1* (marker GA16) on LG2 for resistance to pathotype I; and two independent recessive major loci, namely *ar2a* (marker GA16) on LG2 and *ar2b* (marker TA72) on LG4 acting complementary for resistance to pathotype II. *ar2a* was observed to be linked to *ar1*. They also indicated a possibility of two closely linked loci instead of *ar2b* (Udupa and Baum, 2003). On the other hand, Cho *et al.* (2004) used CRIL-3 population (Tekeoglu *et al.*, 2000) for evaluating resistance to pathotypes I and II and STMS markers by joining previous maps (Winter *et al.*, 2000; Cho *et al.*, 2002; Tekeoglu *et al.*, 2002). They observed that *Ar19* (called as *ar1a* by Millan *et al.* 2006) located on LG2A+6B (markers GA20/GA16) was required for major resistance to pathotype I and partially to pathotype II; whereas the second QTL (called as *ar1b* by Millan *et al.*, 2006) controlled resistance against pathotype I. These two QTLs on the LG2A+6A (*Ar19* or *ar1a*) overlapped with the region of recessive Fusarium wilt resistance genes *foc4* and *foc5* (Tullu *et al.* 1998; Winter *et al.* 1999; Cho *et al.* 2004). Finally, the last QTL on LG4A (markers GA24/GAA47) was responsible for the majority of the resistance against pathotype II (Cho *et al.*, 2004) and was called as *ar2a* by Millan *et al.* (2006). Since RILs resistant to pathotype I segregated for resistance to pathotype II (QTL *ar2a*), Cho *et al.* (2004) proposed unknown additive interactions between two or more genes for *ar2a*.

Using a RIL F6:7 population from an intraspecific cross (ILC3279 X WR315) under field conditions and flower color, RAPD, ISSR, STMS and SCAR as markers; Iruela *et al.* (2006) mapped two QTLs, QTL_{AR1} and QTL_{AR2} into LG4a and LG4b respectively, into the reference map. QTL_{AR1} (STMS marker GAA47) coincided with QTL *ar2a* for pathotype II (Cho *et al.*, 2004); whereas QTL_{AR2} might coincide with QTL-2 reported by Santra *et al.* (2000), a QTL by Millan *et al.* (2003), *QTL 4/5/6* by Flandez–Galvez *et al.* (2003b) and *ar2b* conferring resistance to pathotype II reported by Udupa and Baum (2003). The SCAR marker SCK13603 (tightly linked to QTL_{AR2}) was shown to be similar to R genes, such as rice *Xa21*, tomato Cf2 and *Arabidopsis* RPS2, whereas another one (SCM02935 on QTL_{AR2}) was similar to selenium-binding proteins conferring resistance to the blast fungus, hemibiotroph *Magnaporthe grisea*, and bacterial blight caused by *Xanthomonas oryzae pv. oryzae* (*Xoo*) (Sawada *et al.*, 2004; Iruela *et al.*, 2006).

Since LG2 of the reference map harbors QTLs for Ascochyta blight resistance and genes for Fusarium wilt, Iruela *et al.* (2007) evaluated this LG by using a RIL population of intraspecific cross of ILC3279 (resistant to blight but susceptible to wilt) and WR315

(resistant to all races of wilt and susceptible to blight). They showed that *foc5* and a QTL for resistance to Ascochyta blight (QTL_{AR3}) are closely located on LG2 which also has linked wilt resistance gene clusters: *foc1–foc4* cluster (STMS markers GA16/TA96) and *foc3–foc5* cluster (markers TA96/TA27) (Millan *et al.*, 2006; Iruela *et al.*, 2007). As mentioned before, blight resistant loci *ar1* (marker GA16) for pathotype I and *ar2a* (marker GA16) for pathotype II are also on LG2 close to *foc* gene clusters (Udupa and Baum, 2003; Millan *et al.*, 2006). Therefore, LG2 is very important for both Ascochyta blight and Fusarium wilt resistance (Millan *et al.*, 2006; Iruela *et al.*, 2007).

Although Millan *et al.* (2006) criticized the confusing situation of QTLs for Ascochyta blight resistance by questioning whether the reported resistance genes are on the same loci or not; they made the following conclusion: pathotype I resistance seems to be governed by a major QTL on LG2 linked to STMS marker GA16. This QTL or another one adjacent to this is responsible for resistance to pathotype II partially (Millan *et al.*, 2006). LG2 may have a pathotype I specific QTL linked to STMS marker TA37. Another region on LG4, which contains a QTL linked to markers STMS11 and TR20, is required for the resistance to pathotype II and resistance during seedling stage (Millan *et al.*, 2006).

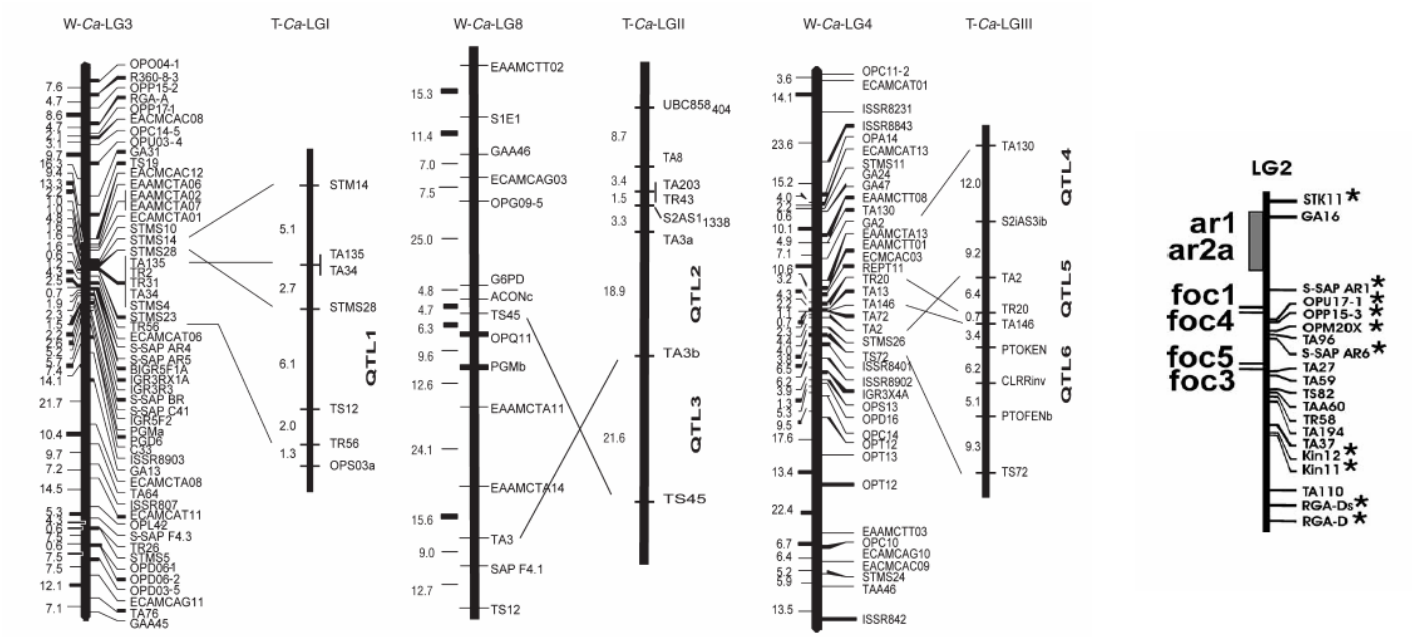

Figure 1. 5 Chickpea LGs having QTLs for *A.rabiei*resistance. Right: colinear alignment done by Bian *et al.* (2007) for chickpea LGs harbouring QTLs for *A.rabiei* resistance found by Flandez-Galvez *et al.* 2003 (T-Ca-LGI, T-Ca-LGII, T-Ca-LGIII) with the chickpea genomic map of Winter *et al.* 2000 (W-Ca-$_{LG3,\ W}$-Ca-LG4 and W-Ca-LG8). From Bian *et al.* (2007). Left: LG2 showing Fusarium resistance (foc) genes and QTLs for Ascochyta blight resistance (*ar1, ar2a*), indicated in the text. From Millan *et al.* (2006).

1.5.2.3 Resistance Gene Analog (RGA) and Gene Specific Markers for Genetic Mapping

Conserved motifs of the resistance gene analogues (RGAs) or candidate resistance genes can be used to construct degenerate oligonucleotide primers to amplify putative RGAs or resistance genes. Isolated RGA sequences may be part of the candidate R-genes such as *A. thaliana* RGA sequences in the *RPP5* gene (Aarts *et al.*, 1998; Rajesh, 2001).

There are many studies that made use of such primers: Kanazin *et al.* (1996) designed primers LM638 (S2 primer) and LM637 (AS primer) targeting to the conserved P-loop (tobacco N, flax L6 and Arabidopsis RPS2 genes) and GLPL(T/A) amino acid motif (RPS2 gene) sequences, respectively. By means of these RGA primers, they detected 9 classes of RGAs in soybean. Speulman *et al.* (1998) designed primers for P-loop motif as a part of NBS and a hydrophobic motif for isolation of R gene homologs from *Arabidopsis*. Yu *et al.* (1996a) designed degenerate primers targeting two conserved motifs on NBS (NBS-F1 for P-loop and NBS-R1 for kinase-3 domains based on N and RPS2 sequences) to isolate subfamilies of NBS-containing genes from soybean. Seah *et al.* (1998) designed primers from the kinase-2a and a conserved amino acid motif derived from NBS-LLR domain of Cre3 gene (Lagudah *et al.* 1997) to isolate RGAs from wheat and barley. Mago *et al.* (1999) designed several primers from P-loop (S1 and S2) and hydrophobic domain (AS1, 2, 3, and 4) based on the conserved motifs NBS-LRR R genes (N, RPS2, L6) and isolated RGAs from 14 categories from rice. Leister *et al.* (1996) designed a primer set (Pto-FenS and Pto-FenAS) from the conserved protein kinase domains of tomato Pto and Fen gene family to isolate RGAs from potato.

Rajesh *et al.* (2002) carried out Bulk Segregant Analysis (BSA) using RGA primers to analyze CRIL-7 (Tekeoglu *et al.*, 2000) and its parents. Out of 48 different combinations, some of which were also used in this study, only one pair of RGA marker (Ptokin1 and Ptokin2) showed polymorphism and mapped onto LG3 of the reference map. Since amplified fragment of this marker showed similarity to ankyrin repeats, Rajesh *et al.* (2002) proposed a possible link to signaling. The fact that this marker was not linked to already mapped R genes was explained by Rajesh *et al.* (2002) as follows: since Fusarium wilt and Ascochyta blight shows recessive inheritance (Tekeoglu *et al.*, 2000; Santra *et al.*, 2000), NBS-LRR primers may not amplify candidate NBS-LRR type R genes which are dominant in character. In addition, conserved domain regions (P-loop, kinase-domain etc.)

on the primers may result in amplification of genes related to signal transduction which are probably minor genes affecting resistance (Singh and Reddy, 1989; Rajesh *et al.*, 2002).

RGA markers were also mapped on chickpea genetic maps. Hüttel *et al.* (2002) studied RGAs of chickpea (ICC4958) and *C. reticulatum* (PI489777) using two degenerate primer pairs (Yu *et al.*, 1996a; Kanazin *et al.*, 1996) targeting NBS domain. Isolated RGAs were classified into 9 distinct classes which collected into two groups of R genes: TIR-NBS-LRR and CC-NBS-LRR (Hüttel *et al.*, 2002). Using RILs of interspecific population (ICC4958 X PI489777) segregating for three race-specific Fusarium wilt resistance, they mapped two NBS-LRR clusters, linked to RFLP probe CaRGA-D, on LG2 harboring *Foc4* and *Foc5* on the reference map (Tullu *et al.*, 1998; Hüttel *et al.*, 2002). Other RGA markers were mapped on LG3, LG5 and LG6. As stated before, Tekeoglu *et al.* (2002) mapped one RGA marker (LRR region of the R gene *Xa21* of rice, Chen *et al.*, 1998), shown to be the only polymorphic one out of 10 RGA markers, onto LG III (LG 3 of reference map). Flandez-Galvez *et al.* (2003a) mapped 12 RGA markers (LRR regions of *Xa21,* Chen *et al.*, 1998) that clustered on LG III (LG4, reference map) and the central region of LG I (LG 2+3, reference map). One of these markers, XLRRb, was linked to QTL 2S on LG4 for seedling resistance by Collard *et al.* (2003) and proposed to be in a resistance gene cluster by Millan *et al.* (2006). Since no R gene for Fusarium or Ascochyta resistance has been isolated up to now due to low level of polymorphism in chickpea, more variable LRR coding regions should be targeted (Millan *et al.,* 2006).

To identify and localize corresponding sequences of chickpea, Pfaff and Kahl (2003) mapped 47 gene specific markers related to plant defense responses on the reference map by using a RIL population segregating for Fusarium wilt resistance. Gene specific primers (GSP) were designed from chickpea and other plants' sequences related to defense, metabolism, oxidative stress responses, ion channel regulation, RNA/DNA regulation, flavonoid biosynthesis, HR, and signaling. Although GSP markers were distributed throughout the map, none of them were linked to *Foc*4 or *Foc*5 (Tullu *et al.*, 1998) on LG2 or the major locus for Ascochyta blight resistance on LG 4. Only PR protein 5 and a glucanase GSPs were mapped on LG2 (Pfaff and Kahl, 2003).

Cato *et al.* (2001) stated that EST markers mapped to a trait of interest may be the actual target gene and are more conservable and transportable across species than non coding ones. Using the synteny between chickpea and *M. truncatula*, Bian *et al.* (2007) mapped

ESTs upregulated upon *A.rabiei* infection (Coram and Pang, 2006) onto *M. truncatula* pseudochromosomes *in silico* to find positions of QTLs reported by Flandez-Galvez *et al.* (2003b). They showed that a small GTP-binding protein gene and a mitochondrial carrier protein gene were in the regions orthologous to chickpea *QTL4/5/6*; a glutamate dehydrogenase gene and an unknown gene were in the regions orthologous to the chickpea *QTL2/3* regions and a putative fatty acid desaturase gene was in the region orthologous to the *QTL1* region in chickpea (Bian *et al.*, 2007).

1.5.2.4 Physical Mapping of Chickpea Genome

Bacterial artificial chromosome (BAC) libraries are tools for understanding the structure, composition and function of the plant genome (Millan *et al.*2006). The first chickpea BAC library was constructed by Rajesh *et al.* (2004) from the nuclear DNA of resistant chickpea line, FLIP84-92C (*Hin*dIII digestion; 23,780 colonies and 3.8 haploid genome equivalents). Screening of this library with a STMS marker Ta96 tightly linked to the Fusarium wilt resistance gene *Foc3* resulted in two overlapping clones, having sequences similar to a ribosomal protein of *M. truncatula* and to a zinc finger-like protein motif of Arabidopsis, respectively. Both proteins have role in gene regulation (Rajesh *et al.*, 2004). Another BAC library and a plant-transformation-competent binary BAC (BIBAC) library were constructed by Lichtenzveig *et al.* (2005) from the nuclear DNA of chickpea variety Hadas (*Hin*dIII and *Bam*HI digestion respectively; 14,976 and 23,040 clones respectively; 7.0Xgenomes equivalents collectively). They also noticed the abundance of SSR motifs $(TAA)^n$ and $(GA)^n$ and non-random distribution of SSR motifs (Lichtenzveig *et al.*, 2005). Another library from the Fusarium-resistant chickpea cultivar (5X genome equivalents) in the binary vector V41 (ICC 4958) was spotted onto high-density nylon filters to be used in hybridization experiments (Millan *et al.*, 2006).

By summarizing studies of Gortner *et al.* (1998), Staginnus *et al.* (1999), Vlacilova *et al.* (2002), and others, Millan *et al.* (2006) informed that LG1 was identified as chromosome F (or G), LG2 as F (or G), LG3 as C (or D), LG4 as B, LG5 as C (or D), LG6 as E, LG7 as A, and LG8 as chromosome H. Using the synteny between chickpea and *M. truncatula,* Bian *et al.* (2007) located QTLs found by study of Flandez-Galvez *et al.* (2003b) for Ascochyta blight resistance on the orthologous pseudochromosomes of *M. truncatula* and on the chromosomes of chickpea as follows: *QTL1* is on the subcentromere region of

chromosome C, *QTL2* and *QTL3* are on the long arm of chromosome H, *QTL4/5/6* are on the subcentromere region of chromosome B.

1.5.2.5 Biochemical and Transcriptome/Proteome Related Studies of Chickpea Ascochyta Blight Resistance

Early studies to understand the basis of defense mechanism underlying the Ascochyta blight resistance were focused on using chickpea cell cultures for different enzymatic analysis. Earlier studies described the induction of oxidative burst and related events in chickpea culture cells upon *A.rabiei* attack. Yeast glucan elicitor on cell cultures resulted in rapid insolubilization of two cell wall structural proteins (HRGP and proline rich proteins, PRPs) due to H_2O_2 mediated oxidative cross-linking (Otte and Barz, 1996). Protein kinases and phosphatases were shown to counteract in the signal transduction pathway for the induction of a probably plasma membrane located NADPH-oxidase-dependent oxidative burst which leads to cross-linking of the cell wall proteins by a putative peroxidase (POD; located downstream of H_2O_2 supply) (Otte and Barz, 1996; Pachten and Barz, 1999). N-termini sequence motifs of PRP and extensin families were shown to be responsible for formation of Tyr-bridges for this oxidative cross-linking through H_2O_2 and POD activity (Otte and Barz, 2000). Mackenbrock *et al.* (1993) and Otte *et al.* (2001) proposed that besides oxidative burst, elicited chickpea cells rapidly undergo extracellular alkalinization with simultaneous deactivation of H+-ATPase; followed by acidification and transient K+ efflux regulated by K+/H+ exchange and finally activation of defense related genes like acidic and basic chitinases, thaumatin-like proteins (TLPs), isoflavone reductase and other genes involved in biosynthesis of phytoalexins medicarpin and maackiain.

Another defense enzyme related to oxidative burst in chickpea is copper amine oxidase (CuAO) which catalyzes oxidative deamination of active amines and produce H_2O_2. Rea *et al.*, (2002) reported that CuAO expression was shown to be upregulated upon both wounding and infection with *A. rabiei* both locally and systemically; but higher amounts in resistance chickpea genotypes than in susceptible ones. They showed that JA induced significantly both the basal and wound-inducible CuAO expression whereas SA blocked both wound- and JA-induced CuAO expression. The same pattern was observed with ABA. CuAO inhibition after inoculation of resistant chickpea genotype resulted in extended necrotic lesions, susceptibility and reduced mechanical resistance against fungus

due to low H_2O_2 production. High basal CuAO activity may be a constitutive defense response of chickpea upon wounding and pathogens (Rea *et al.*, 2002).

Otte *et al.* (2001) proposed the involvement of Ser/Thr kinases in the signal cascade leading to activation of PR genes. Supporting this, a Ser/Thr kinase gene close to a major QTL for Ascochyta resistance was also mapped on LG2 (STK11; Hüttel *et al.*, 2002). Moreover, rab and rac type small GTP-binding proteins were also proposed to be involved in the defense responses of chickpea (Ichinose *et al.*, 1999).

Fortification of cell wall and PR or defense related proteins are also important in the defense responses of chickpea. ESTs encoding two GRPs probably involved in fortification of cell walls are induced highly upon *A.rabiei* attack in chickpea (Cornels *et al.*, 2000). After infection with *A.rabiei*; strengthening of xylem tissues, increased levels of peroxidase, diamine oxidase, putrescine and chitinase levels were observed in both susceptible and resistance cultivars; but enhanced levels in resistant ones (Angelini *et al.*, 1993; Nehra *et al.*, 1994). Although fungal conidia germination, hypal development and appressoria formation were observed to be similar in resistant and susceptible chickpea genotypes, lignosuberizied barriers were observed to be thicker and wider in resistant ones (Angelini *et al.*, 1993). PR-5a and PR-5b (or TLP, linked to *foc4* and *foc5*) genes were shown to be elicited in resistant cultivar more rapidly than in a susceptible one (Hanselle *et al.*, 2001; Millan *et al.*, 2006). β-1,3-glucanase and several chitinases were identified for chickpea (Vogelsang and Barz, 1993a; Vogelsang and Barz, 1993b). Although β-1,3-glucanase accumulation in the intercellular fluid of susceptible and resistant cultivar were observed to be similar, the growth of *A.rabiei* in a resistant cultivar was suppressed, which means that other factors play role for resistance (Hanselle and Barz, 2001; Vail, 2005). Cervantes *et al.*(2001) isolated an ethylene-induced chickpea cysteine proteinase cDNA which may be involved in developmental PCD events and homologous to *rd21*gene of *Arabidopsis*. Jaiswai *et al.* (2003) identified a clone homologous to a known nematode resistance gene (Hs1pro1) of sugar beet. Other antifungal peptides produced by chickpea are cicerin, arietin having antifungal activity toward *Mycosphaerella arachidicola*, *Fusarium oxysporum* and *Botrytis cinerea* and cicerarin having antifungal activity against *B. cinerea*, *M. arachidicola*, and *Physalospora piricola* (Ye *et al.*, 2002; Chu *et al.*, 2003).

Phytoalexins are reported to be involved in the defense reactions of chickpea. A polysaccharide elicitor of *A. rabiei* induces higher amounts of the two pterocarpan

flavanones (medicarpin and maackiain) in the cells of the resistant cultivar than in the susceptible cultivar. Similarly, glucose 6-phosphate dehydrogenase (G6PD), enzymes of the general phenylpropanoid pathway (PAL, chalcone synthase, CHS; cinnamic acid 4-Hydroxylase, C4H; etc.) and enzymes of the flavonoids are elicitor-induced (Mackenbrock and Barz, 1991). However, medicarpin and maackiain can be detoxificated by mycelial preparations and crude protein extracts of *A. rabiei* (Lucy *et al.*, 1988 and Höhl *et al.*, 1989; Pedras *et al.*, 2005). Barz and Mackenbrock (1994) investigated constitutive and elicited metabolism of isoflavones and pterocarpans in chickpea culture: under normal conditions phenolics which are constitutively derived from phenylalanine are stored as vacuolar malonylglucosides. The main regulatory step is chalcone reductase (CHR) after which two competing pathways appear; the first one is 5-deoxyisoflavones (daidzein and formononetin) and pterocarpans (medicarpin and maackiain) and the second one is 5-hydroxyisoflavones (biochanin A) and –isoflavanones (homoferreirin and cicerin). Considering the metabolism of pterocarpans *via* isoflovane, elicitation of cell cultures leads to increases in the activities of biosynthetic enzymes such as PAL and C4H. However, elicitation of malonylglucoside metabolism is differential depending on elicitor amount: Low elicitor doses favor pterocarpan conjugate formation whereas high doses lead to pterocarpan aglycone accumulation accompanied by vacuolar efflux of formononetin and pterocarpan malonylglucosides. Elicitor-induced changes in enzyme activities and vacuolar efflux of conjugates are inhibited by treatment with cinnamic acid; the substrate of C4H (Barz and Mackenbrock, 1994). Vogelsang *et al.* (1994) proposed a putative protein in the crude culture filtrate (CCF) of *A. rabiei* inducing a rapid browning response in chickpea cell cultures of a resistant cultivar. Besides, HR-like necrotic browning response was observed only in the resistant cultivar cell cultures (Vogelsang *et al.* 1994). They observed no lignification in these browned cells; in addition to this, cinnamyl alcohol dehydrogenase (CAD) activity, the enzyme of the lignin biosynthesis pathway, was not induced in resistant cells. However, enzymes of the biosynthesis of medicarpin (PAL, C4H, CHS, and 2'-hydroxyisoflavone reductase-IFR) were rapidly and highly induced in resistant cultivar. They proposed that CCF elicitation resulted in incorporation of phenolic compounds into the cell wall and degradation of this polyphenolic barrier by fungal enzymes (tannase) liberated phytoalexins. Other studies indicated that an NADPH: isoflavone oxidoreductase and P450 proteins involved in isoflavone synthesis were induced upon elicitor treatment in cell cultures (Tiemann *et al.*, 1991; Overkamp *et al.*, 2000).

The key enzyme of the branch point diverging either to isoflavonoid or flavonoid metabolisms is flavanone 3-hydroxylase (F3H) which was determined in the cDNA-AFLP study of Cho *et al*. (2005) who tested the findings of Cho and Muehlbauer (2004) showing that blight resistance in chickpea might be conferred by constitutive resistance mechanisms. Cho *et al.* (2005) observed 12,000 constitutive and 38 differential cDNA fragments by comparison of PI 359075(1) (susceptible to pathotype I and II) and FLIP84-92C(2) (resistant to pathotype I and II), but only one clone similar to F3H was confirmed. Considering a time course (6 to 72 h), F3H expression was detected during early infection and then downregulated in resistant line, meaning that higher constitutive F3H expression before infection is necessary for blight resistance. On the other hand, induction of F3H in both lines was observed upon exogenous SA treatment but not by Me-JA; showing that the resistance is independent from SA-mediated systemic signal (Cho *et al.*, 2005). No correlation of expression of F3H with blight resistance was observed among CRIL-3 population (Tekeoglu *et al.*, 2000), meaning that F3H contributes to the resistance partially (Cho *et al.*, 2005). As a result, they proposed that FH3 may probably represent an unknown mechanism in chickpea defense response.

There are also some studies related to cDNA expression profile of chickpea upon *A.rabiei* infection. Using suppression subtractive hybridization (SSH) of cDNA libraries from Ascochyta blight- infected and non-infected plants, Ichinose *et al.* (2000) identified 35 candidate defense-related transcripts related to primary metabolism, regulation of gene expression (transcription factors and translation), defense-related (such as reinforcement of cell walls, PR-proteins, phytoalexin biosynthetic enzymes and ROS scavenging enzymes), signal transduction and catabolic pathways (Ichinose *et al.*, 2000).

Coram and Pang (2005a) constructed a cDNA library consisting of 1021 ESTs from pooled samples (24 and 48 h) of a resistant accession (ICC3996) inoculated with *A. rabiei.* Most abundant unigenes were chloroplast and ribosome related ESTs. They provided a set of defense related ESTs such as transcription factor EREBP-1, Avr9/Cf9 rapidly elicited protein 65, pathogen-induced transcriptional activator, bZIP transcription factor, putative disease resistance protein, multi-resistance transporter protein, SNAKIN2 antimicrobial peptide precursor, nematode resistance protein, CAD1 and caffeoyl-CoA-methyltransferase (CCOM). Despite the insufficient homology of chickpea transcriptome to that of model legumes, they showed that the most highly conserved categories in between chickpea and *M. truncatula* were structural, ribosomal, photosynthetic,

translational and metabolic proteins, whilst the least conserved categories were defense and stress-related and signaling proteins such as protein kinases. In the following work, Coram and Pang (2005b) constructed a small scale microarray with the set of defense-related ESTs mentioned in the above to study to test the expression profile of resistant chickpea accession (ICC3996) and a susceptible cultivar (Lasseter) upon *A.rabiei* attack. They observed that up-regulation of chickpea ESTs was peaked either 24 or 48 hpi corresponding a rapid simultaneous invasion of *A. rabiei*. Non-differentially expressed ESTs such as Avr9/Cf-9 rapidly elicited protein 65, nps45 and EREBP-1 supposed to be involved in signal transduction earlier than 12 hpi. In contrast to other reports in legumes (Dixon *et al.*, 2002) and all of the above mentioned studies except for Vogelsang *et al.* (1994), they observed downregulation of ESTs of phenylpropanoid pathway (such as CCOM) or constitutive expression (such as CAD) in both chickpea genotypes. Therefore, Coram and Pang (2005b) suggested that deposition of lignin and formation of phytoalexins may not be involved in effective resistance. Four ESTs (PR proteins and multi-resistance transporter protein) were up-regulated in both susceptible and resistant lines, implying that they are not involved in defense response. bZIP transcription factor (regulating the production SA to induce expression of PR proteins, Jakoby *et al.*, 2002) and SNAKIN2 antimicrobial peptide precursor (Berrocal-Lobo *et al.* 2002b) were up-regulated only in the resistant genotype showing that these proteins may be involved in resistance (Coram and Pang, 2005b).

Afterwards, Coram and Pang (2006) constructed a larger-scale microarray of 756 features composed of various functional categories of ESTs from chickpea (Coram and Pang, 2005a), grass pea (*L. sativus*) and RGA sequences from lentil (*L. culinaris*) to analyze *A.rabiei* response in four genotypes of chickpea over a time-course (6 to 72h): resistant ICC3996 (IC), moderately resistant FLIP94-508C (FL), susceptible Lasseter (LA) and moderately resistant wild relative *C. echinospermum* L.-ILWC245 (IL). The proportion of differentially expressed genes was relatively low at 6 and 12 hpi, followed by 72 hpi, but high at 24 and 48 hpi. They suggested that the transcriptional profile was consistent with the time course of infection progress of *A.rabiei* as formerly stated by Pandey *et al.* (1987), Kohler *et al.* (1995), Hohl *et al.* (1990), and Coram and Pang (2005b). Higher differential expression (at 6 and 12 hpi) observed in resistant IC indicated rapid pathogen recognition than other lines. Due to a large proportion of down-regulated genes overall, they proposed that chickpea "*sacrificed*" transcription of housekeeping and general metabolic genes in favor of defense-related genes. Despite the necrotrophic character of *A.rabiei*, they

proposed a picture of genes governed by *R*-gene-mediated resistance: induction of ROS, HR, down-regulation of housekeeping gene expression and expression of PR proteins. Comparison of compatible and incompatible *A. rabiei*–chickpea interactions lead to identify "*certain gene expression signatures*" for incompatible interactions: expression of PR proteins; up-regulation of b-1,3-glucanase, SNAKIN2, PRP, disease resistance response protein (DRRG49-C), leucine zipper protein (LZP), environmental stress-inducible protein (ESP), polymorphic antigen membrane protein (PAMP) and several unknown/unclear proteins (Coram and Pang, 2006) .

Systemic regulation and effect of hormones on defense responses of chickpea against *A.rabiei* was also studied. Bayraktar and Dolar (2002) investigated the effect of exogenous SA treatment to establish induced systemic resistance against an aggressive isolate (Race 6) in susceptible chickpea cultivar (Canıtez-87). They observed that, SA treatment was most effective if applied 2 days before inoculation with *A.rabiei*. This finding shows that timely application of SA may induce SAR. Cho and Muehlbauer (2004) studied expression patterns of some of the defense related genes, such as PR and antioxidative genes, during infection process of chickpea with pathotypes I and II of *A. rabiei*, race-specific pathogen *F. oxysporum* f.sp. *ciceri* and non-host pathogen *F.oxysporum* f.sp. *pisi.* Expression pattern of these genes after pathogen inoculation and exogenous treatments with SA and Me-JA were investigated by RT-PCR and Northern Blot analysis by using FLIP84-92C(2) (blight resistant and SA- and Me-JA-sensitive) and PI359075(1) (blight susceptible and SA- and Me-JA-insensitive) and their progeny CRIL-3 population (Tekeoglu *et al.*, 2000; Cho and Muehlbauer, 2004). A significant differential expression pattern of some genes after *A. rabiei* inoculation was observed in resistant parent whereas susceptible parent was insensitive. Since anti-oxidative genes such as SOD, APX, GR (glutathione reductase), did not show differential expression patterns between lines, they proposed that HR initiation by accumulation of oxidative burst does not exist contrary to previous report of Otte *et al.* (2001). This fact was previously suggested by Porta-Puglia *et al.* (1996) such that the resistance of chickpea to *A.rabiei* is not related to an HR, but resistant cultivars show necroses as disease symptoms of reduced extent as compared to susceptible ones. Cho and Muehlbauer (2004) observed an upregulation in β-glucosidase but downregulation in putative glucosytransferase after infection; as confirmed by the rapid metabolism of glucoside-conjugated isoflavonoids to produce antifungal aglycons shown by Mackenbrock and Barz (1991). Cho and Muehlbauer (2004) observed that only resistant FLIP84-92C(2) was sensitive to SA and MJ showing that signal transduction pathways

mediated by SA and JA may be simultaneously regulated in chickpea. Due to identical LOX2 expression in both lines, they concluded that resistance mechanisms mediated by JA might not be involved in blight resistance. Besides, the expression patterns of the defense related genes in CRIL-3 population against infection to both pathotypes of *A.rabiei* or with respect to SA and MJ treatment showed no correlation with resistance. Similar results were obtained for the case of fusarium wilt (*F. oxysporum* f.sp. *ciceri*) and non-host resistance (*F. oxysporum* f.sp. *pisi*). As a result, they concluded that systemic regulation of the defense-related genes at transcription level might not be important for resistance of chickpea against these two important necrotrophic pathogens. Induction after fungal infection should be regarded as immediate responses to pathogens, not as defense-related mechanisms. As a conclusion, constitutive or unknown defense systems independent of SA- and JA-mediated systemic resistance mechanisms might be involved in defense reactions of chickpea (Cho and Muehlbauer, 2004).

Microarray chips (Coram and Pang, 2006) was used in the study of Coram and Pang (2007) to profile potential changes in the above mentioned lines (IC, FL and LA) after treatment with SA, MJ and E precursor ACC (3 and 27 h post treatment, hpt), for identifying putative signaling pathways after *A. rabiei* inoculation. They observed down-regulation of genes in general for all treatments, especially housekeeping genes. Proportions of differentially expressed genes were 69.7 %, 57.6 % and 15.8 % for ACC, SA, and MJ respectively. Only the resistant IC genotype showed a higher induction of defense-related transcripts upon MJ treatment. Co-regulation of transcripts was not common; but co-down-regulation of SOD in resistant IC (MJ-SA) and moderately resistant FL (ACC-SA) showed accumulation of ROS for an oxidative burst. ACC treatment induced transcripts related to some PR proteins (indicating widespread presence of E mediated GCC-box promoters), controlling oxidative burst, phenylpropanoid pathway and EREBP transcription factors. They observed no synergistic induction of similar genes by JA and E; however, some important induced transcripts were regulated by all treatments in resistant line IC. So, they concluded that signaling pathways mediated by ACC/MJ/SA are all involved in resistance response. Since several of the important IC transcripts were not regulated by any treatment, they proposed that other *A. rabiei*-specific signaling events may be required for their induction (Coram and Pang, 2007). Similarly, Cho and Muehlbauer (2004) and Bayraktar and Dolar (2002) reported PR-like proteins were upregulated in susceptible line only by SA, showing that SA regulation of defense-related genes are independent of *A. rabiei* resistance (Coram and Pang, 2007). The observations of

this study indicate that, although E, JA and SA are partially involved in the signaling of defense responses to *A. rabiei*, they are not responsible for mediating the entire response leading to resistance (Coram and Pang, 2007). This fact was also reported by Cho and Muehlbauer (2004) for the case of SA- and JA-mediated regulation. Consequently, Coram and Pang (2007) suggested a simplified model of the defense-related pathways controlled by SA, JA and E in *A. rabiei* resistant chickpea genotypes shown in Figure 1.6. Recently, Coram *et al.* (2007) proposed a hypothetical model for chickpea defense responses to Ascochyta blight by summarizing all functional genomics data available for chickpea; shown in Figure 1.6. They suggested using NILs (near-isogenic lines) or RILs to eliminate the misleading gene expression responses owing to background genetic differences of chickpea genotypes. They emphasized the limited ESTs available for chickpea to date, leading to under representation of expression profile (Coram *et al.*, 2007).

Buhariwalla *et al.* (2005) used them as markers for diversity analysis of *Cicer*. They suggested that this database provides a preliminary profile of some differentially expressed genes and constitutive mechanisms related to stress tolerance as well as means for identification of candidate accessions for new sources of resistance to biotic and abiotic stresses. Very recently, SuperSAGE method was used in chickpea to investigate salinity, drought and cold stresses and more then >3000 genes responding to stress were identified (Kahl *et al.* 2007).

Other studies related to chickpea but not to Ascochyta blight are represented briefly. Mantri *et al*. (2007) realized transcriptional profile of chickpea genes upon drought, cold and high-salinity treatments with different tissue types by using a microarray consisted of chickpea and grasspea ESTs and lentil RGAs. Significant transcriptional changes in ESTs were observed for all treatments. 38 ESTs were differentially expressed in all treatments. Overall, more genes were found to be repressed than induced. Furthermore, Jayashree *et al.* (2005) and Buhariwalla *et al.* (2005) constructed an SSH library over 2800 ESTs from drought tolerant chickpea genotypes and made it available in ICRISAT database.

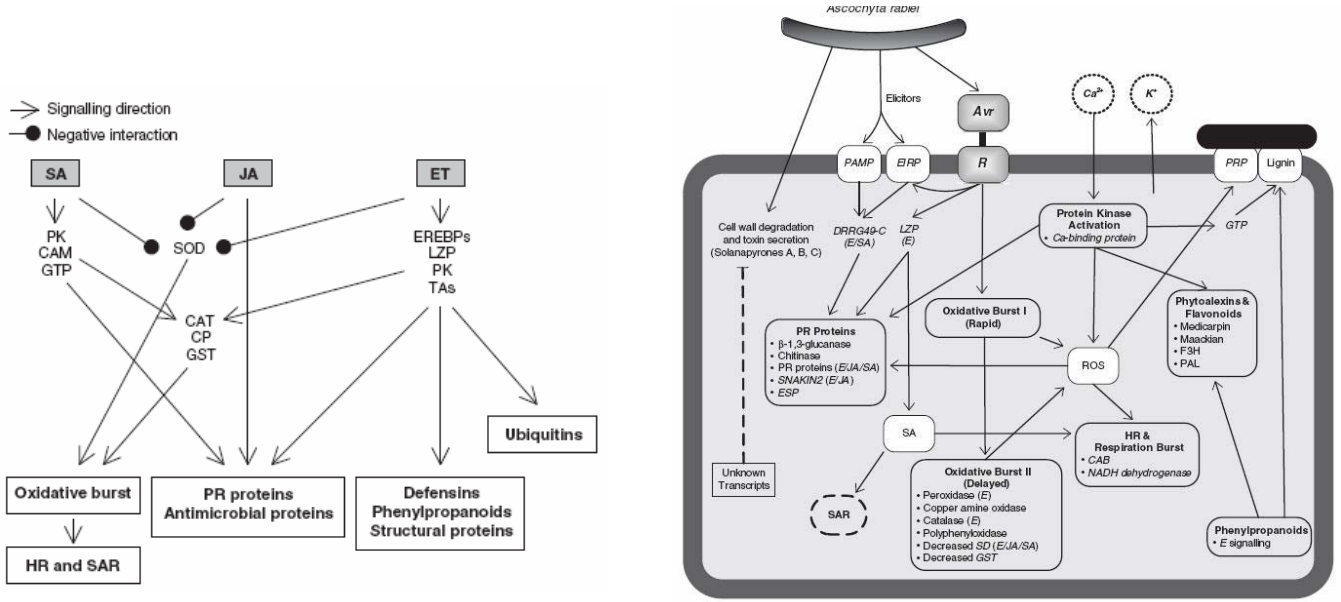

Figure 1.6 Models for defense-related signalling and responses of chickpea. Left: model of the defence-related pathways controlled by SA, JA and ET/E$_{in}$ *A. rabiei* resistant chickpea drawn by Coram and Pang (2007). Right: hypothetical model of chickpea defence response to Ascochyta blight drawn by Coram *et al.* (2007). CAM, calmodulin-like protein; CAT, catalase; CP, cationic peroxidase; GS, glutathione *S*-transferase; GTP, GTP binding protein; LZP, leucine zipper protein; PK, protein kinase; TA, translational activator. CAB, chlorophyll a/b binding protein; EIRP, elicitor-induced receptor protein; GTP, guanosine triphosphate binding protein; PAMP, polymorphic antigen membrane protein; SD or SOD, superoxide dismutase. Other abbreviations were mentioned in the text.

Proteomics studies on chickpea are very limited. By studying extracellular matrix (ECM; i.e. cell wall), Bhushan *et al.* (2006) proposed that ECM serves as the first mediator in cell signaling to perceive and transmit extra- and inter-cellular signals in many pathways including defense. The functional classification of proteins observed were as follows: 9% development, 13% cell signaling, 10% transport, 19% metabolism, 6% stress responsive, 36% unidentified and 7% no significant match. Among stress responsive proteins; peroxidases, NBS-like putative resistance protein and NBS-LRR type resistance protein and among signaling proteins calcium dependent protein kinases, 14-3-3-like protein, calmoludin protein and E responsive protein are important. Moreover, most of the proteins common among plant species have been implicated either in stress response (including disease resistance proteins) or cell signaling. This shows the role of ECM as the first defense step against various stress factors (Bhushan *et al.* 2006).

1.6 The Aim of This Study

In this thesis, it was aimed to identify a wide variety of chickpea ESTs expressed in a tolerant chickpea line ILC195 upon infection with *A.rabiei* isolates of varying pathogenicity level by means of PCR amplification of resistance gene analogs (RGAs), differantial display reverse transcriptase PCR (DDRT-PCR) analysis and PCR amplification with gene specific primers (GSPs); so that chickpea genes probably having some roles in the resistance could be obtained. It was also aimed to compare differential expression of some of the putative ESTs in between infected ILC195 and any other well-defined chickpea-pathotype partners (FLIP84-92C(3) and pathotypes I and II) to see whether or not the expression profile is universal. Genetic mapping of some of the putative ESTs found in this study on the known chickpea genetic maps was set as another objective to find out any EST markers linked to QTLs of Ascochyta blight resistance.

CHAPTER 2

MATERIALS AND METHODS

2.1. General Flow Chart of the Procedure

In this study, different materials and methods were used in orderly fashion. Figure 2.1 shows the flow chart of the overall procedure which consists of methods explained in this chapter.

2.2 Preparation of Infected Plant Materials

In the study several plant materials and fungal isolates were used for preparation of several infected materials for different experimental purposes.

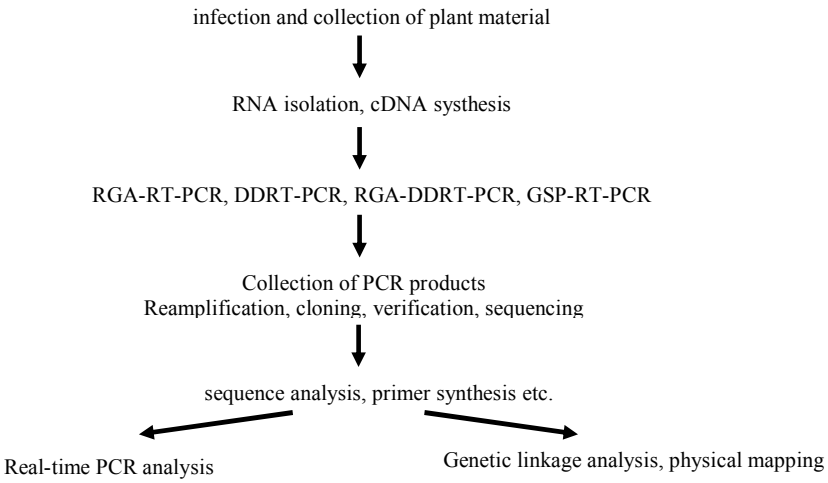

Figure 2.1 Illustration of general flow chart of procedures.

2.2.1 Preparation of Infected Plant Materials for RGA-RT-PCR, DDRT-PCR, and RGA-DDRT-PCR Experiments

The chickpea material for RGA-DDRT-PCR-trials, RGA-RT-PCR, DDRT-PCR, and RGA-DDRT-PCR experiments is *C. arietinum* L. cultivar ILC195 (Singh *et al.* 1993). This variety was developed by ICARDA by mass selection from the variety Vysokoroshyji-30 from Krasnodar in the former USSR (Singh *et al.* 1993). ILC195 is known as having a moderate resistance (tolerant) to Ascochyta blight. It was released by national program in Turkey in 1986 for winter sowing (Singh *et al.* 1993). Fungi material used for these experiments is composed of various isolates of *A.rabiei*. Pathogenicity tests were conducted on ILC195 at Ankara University, Department of Plant Protection, by the supervision of Prof. Dr. Sara Dolar. Based on their study, two aggressive (*ank6, çor1*), and two mild (*ayaç5, elmalı*) were used. In the first infection experiment (Table 2.1), isolates *ank6*, *çor1* and *ayaç5* were used. In the second infection experiment (Table 2.2), *ank6, çor1*, *ayaç*5 and *elmalı* were used. Fungal materials were grown on chickpea seed meal dextrose agar (CSMDA, 4 % chickpea flour, 2 % dextrose, 2 % agar in 1 L distilled water) at $22^{\pm}1^{o}C$ in 14 h day light for 2 weeks.

Conditions of the infection experiments were as follows: 8-10 seeds were surface sterilized with 1% sodium hypochlorite solution and was planted in 16.5 cm diameter pots. Seedlings were grown in incubation room for 2 weeks at $23^{\pm}1$ ^{o}C under 260 $\mu molsec^{-1}m^{-2}$ watts. Inoculums were prepared at concentration 500.000 spores/mL and sprayed on plants. Control plants were not inoculated. Pots were kept in plastic bags to maintain humidity till the end of harvesting of plants at the relevant time point. Harvested plant material placed in 50 mL falcon tube was soaked into liquid N_2 immediately and kept in -80 ^{o}C freezer.

Table 2.1 ILC195 plant groups collected in the first infection experiment.

	***A.rabiei* isolate**				
Time point	***Ank6***	***Çor1***	***Ayaç5***	***Control***	
3rd day (72 h) group no	4	5	6	8	**Sample abbreviation**
23rd day group no	12	13	14	16	

Table 2.2 ILC195 Plants collected in the second infection experiment.

	A.rabiei isolate					
hour	***Ank6***	***Çor1***	***Ayaç5***	***Elmalı***	***Control***	**Sample abbreviation**
10	22	23	24	25	26	
24	32	33	34	35	36	
36	47	48	49	50		
48	56	57	58	59		
72	60	61	62	63		

2.2.2 Preparation of Infected Plant Materials for Real Time qRT-PCR Experiments

The first set of plant material and the fungal material for Real Time qRT-PCR were cultivar ILC195 and isolate *ank6*, respectively. Conditions of these infection experiments were as follows: 8 - 10 seeds were planted in 16.5 cm diameter pots and seedlings were grown in incubation room for 2 weeks at 12 h day light (260 μmol.sec^{-1}m^{-2} light strength) at 23$^{\pm}$1 ^{o}C. *ank6* was grown as before. Inoculums were prepared in distilled water at concentration of 500.000 spores/mL and sprayed on plants. Control plants were sprayed with distilled water. Temperature was decreased to 19$^{\pm}$1^{o}C and the day light was changed to 14 h. Pots were kept in plastic bags to maintain humidity till the end of harvesting of plants at varying time points (Table 2.3). The harvested plant material placed in 50 mL falcon tube was soaked into liquid N_2 immediately and kept in -80 ^{o}C freezer. After 4 days all the plastic bags were removed, and evaluation of infection was carried out after 3 weeks of inoculation according to 1 - 9 rating scale (Singh *et al*., 1981): 0 – 3.0 as resistant, 3.1 – 5.0 as tolerant and 5.1 – 9.0 as susceptible.

Table 2.3 ILC195 plants infected with isolate *ank6* collected for Real-Time qRT-PCR.

Hour	***Control***	***Ank6***	
6	C6	in6	
12	C12	in12	
24	C24	in24	
36	C36	in36	
48	C48	in48	
72	C72	in72	**sample abbreviation**

The second experimental plant material for Real Time qRT-PCR was line FLIP84-92C(3). This line was developed from the cross of two different chickpea variety of ICARDA: ILC72 (resistant) and ILC215 (susceptible). FLIP84-92C(3) is known to be resistant to Ascochyta blight (Tekeoglu *et al.*, 2000); i.e., to pathotype I and pathotype II. Chickpea varieties named "Spanish white" (susceptible to both pathotype I and II) and Dwelley (resistance to pathotype I but susceptible to pathotype II) were used to observe early disease symptoms. One seed was planted in one Deepot (6.3 cm diameter, 25.5 cm height, Figure 2.2) and grown in USDA-ARS greenhouse for two weeks. Conditions of the greenhouse were: day length 17 h, day temperature of 22 ^{o}C, night temperature 16 ^{o}C, 20-50 % humidity, 1000 watt light (when no sunlight), by watering each day and watering with nutrient solution (100 ppm nitrogen) once a week (Sheri McGrew; personnel communication).

Pathotype I (least aggressive) and pathotype II (moderately aggressive) were described by Chen *et al.* (2004). The two pathotypes were first grown from the stock culture on Potato Dextrose Agar (PDA) for about 2 weeks, then conidial spores were harvested and spread onto V8 Agar plates (200 mL V8 Juice, 3g calcium carbonate, 20 g agar, 800 mL H_2O; total 1 L) and grown for 2 weeks at $22^{\pm}1^{o}$C, 12 h photo period, under fluorescent light (Tony Chen; personnel communication). Two isolates for pathotype I (AR19 and AR20) and two isolates for pathotype II (AR628 and MSR9A) were combined for preparing inoculums to produce 4.10^5 spores/mL in distilled water. Infection was based on "Minidome assay" (Chen and Muehlbauer, 2003): Each plant was sprayed with appropriate spore suspension, control plants were sprayed with distilled water; and then pods were covered with plastic cups. After last sample time (72 h), plastic cups were removed. The harvested plant material (Table 2.4) collected in 50 mL falcon tubes was frozen with liquid N_2 immediately and kept in -80^{o}C freezer. After 2 weeks, disease development was assayed according to 1 - 9 rating scale (Singh, *et al.*, 1981).

Table 2.4 FLIP84-92C(3) plants infected with pathotype I and pathotype II.

Hour	Control	Pathotype I	Pathotype II	sample abbreviation
6	F6	PI-6	PII-6	
12	F12	PI-12	PII-12	
24	F24	PI-24	PII-24	
36	F36	PI-36	PII-36	
48	F48	PI-48	PII-48	
72	F72	PI-72	PII-72	

Figure 2.2 Infection experiments on FLIP 84-92C(3). Pods with chickpea plants (left), "Minidomes" covered and uncovered (Chen and Muehlbauer, 2003) (right).

2.2.3 Plant Materials for Genetic Linkage Experiments

For genetic linkage analysis, DNA of CRIL-7 population (Tekeoglu *et al*., 2000) available in the UDS-ARS Legume Genetics and Physiology Unit was used. The parents of this population are FLIP84-92C(3) (resistant) and *C.reticulatum* PI 599072 (susceptible). Also DNA available for the parents of CRIL-3 (FLIP84-92C(2), resistant and *C. arietinum* L. PI 359075(1), susceptible) of Tekeoglu *et al*. (2000) in the same Unit was used. Further information of these populations was provided in the Introduction in Section 1.5.2.2.

2.3 Total RNA Isolation, Qualification and Quantification

8-10 seedlings inoculated with the same isolate were bulked for each time point. For all experiments (RGA-RT-PCR, DDRT-PCR, RGA-DDRT-PCR, GSP-RT-PCR and Real-Time qRT-PCR) Total RNA from all plant samples was isolated by using TRIzol reagent as described in manufacturers manual. Only exception is that; because of high number of plant groups in the second infection, 750 µL TRIzol was used for isolation of total RNA instead of 1 mL. The procedure of TRIzol method was presented in Table 2.5. The integrity of total RNA samples was checked in 1% agarose gels containing ethidium bromide (EtBr) prepared in 1X phosphate buffer (10X phosphate buffer: $Na_2HPO_4.H_2O$ (0.05 M), $NaH_2PO_4.H_2O$ (0.05 M); dilute to 1 L with DEPC (Diethylpyrocarbonate) treated autoclaved distilled water (1/1000 v/v); 10 mM, pH 6.8) RNA concentrations were determined by UV spectrophotometer. After determination of integrity and concentration, total RNA was used either for mRNA isolation or directly for cDNA construction.

Table 2.5 Method of total RNA isolation by TRIzol reagent with minute modifications.

Step	Procedure
1	Collect plant samples and freeze in liquid N_2 and keep at -80 ^oC until use.
2	Take plant samples from -80 ^oC, crush them in liquid N_2 to powder in a pre-cooled sterile mortar and pestle.
3	Take 50-100 mg of plant powder in a pre-cooled sterile 2 mL eppendorf tube.
4	Add 1 mL TRIzol into eppendorfs and shake vigorously (or vortex), incubate 5min at room temperature.
5	Add 200 µL chloroform, shake vigorously (or vortex) and incubate 2-3 min at room temperature.
6	Centrifuge for 20 min at 12.000Xg at 4 ^oC.
7	Take aqueous phase (very clear upper solution) without disturbing lower phases into another sterile eppendorf.
8	Add 500 µL isopropanol into this upper phase, incubate 10 min at room temperature while shaking by inverting very slowly 1-2 times.
9	Centrifuge for 20 min at 12.000Xg at 4 ^oC.
10	Discard upper phase and add 1 mL ice cold 75 % ethanol. This solution can be kept at -80^oC for a very long time.
11	Dissolving RNA: Mix the sample in step 10 gently and centrifuge for 10-20 min at 12.000Xg at 4 ^oC.
12	Discard supernatant, air dry RNA pellet for a short time, add suitable amount of autoclaved DEPC treated (1/1000 v/v) or molecular grade distilled water (30-100µL).
13	Incubate samples in water bath at 65 ^oC for 10 min.
14	Keep samples at -80 ^oC for further use.

2.4 mRNA Isolation, Qualification and Quantification

mRNA was used as a template of cDNA in RGA-RT-PCR, DDRT-PCR, and RGA-DDRT-PCR experiments. To isolate mRNA, Dynalbeads Oligo$(dT)_{25}$ magnetic particles produced by DYNAL Biotech was used. The procedure given by the manufacturer was followed (Table 2.6). Dynalbeads Oligo$(dT)_{25}$ magnetic particles can be used 4 times for the same RNA sample without regeneration. mRNA concentration was determined by a semi-quantitative method described by Sambrook *et al.* (1989): A very thin layer of 1 % agarose gel containing EtBr was prepared in 1X phosphate buffer (10 mM, pH 6.8; Section 2.3). A dilution series for both mRNA samples and control mRNA (standard mRNA from human skeletal muscle, 1 µg/µL (Clontech) were prepared. 1 µL from each dilution series was dropped onto gel. After absorption, the relative abundance of mRNAs was visualized on UV transilluminator.

Table 2.6 mRNA isolation according to Dynal Biotech-Dynalbeads, for 75 µg total RNA.

Step	Procedure*
1	Transfer 75µg of total RNA, adjust to 100 µL with autoclaved DEPC treated distilled water.
2	Heat total RNA 65 ^{o}C for 2 min.
3	Meantime resuspend dynalbeats, take 200 µL for each sample in another tube; magnet.
4	Wash beats once with 100 µL of 2X binding buffer (20 mM Tris-HCL, 1.0 M LiCl, 2 mM EDTA; pH 7.5). Magnet them, remove supernatant.
5	Resuspend beats in 100 µL of 2X binding buffer.
6	Add total RNA on magnets (total volume: 200 µL or higher).
7	Mix by inverting gently for 3-5 min at room temperature.
8	Magnet, remove supernatant.
9	Wash twice with 200 µL Washing Buffer-B (10 mM Tris-HCl, 0.15 M LiCl, 1mM EDTA; pH 7.5). Resuspend beats thoroughly and magnet remove supernatant.
10	Elute mRNA by dissolving in elution solution (10 mM Tris-HCl, 80 ^{o}C) by incubating at 80 ^{o}C for 2 min and take the supernatant (mRNA). Store at -80 ^{o}C.

2.5 Primers for Trials, RGA-RT-PCR and RGA-DDRT-PCR Experiments

There are two types of primers used in this study: primers disclosed in literature and primers designed in this study. Primers were designed using Primer Detective (Version 1.01, Todd M.J.Love, 1990) and ClustalX programmes (to find conserved regions). As a reference gene, actin was used as other authors (Cho and Muehlbauer, 2004; Coram and Pang, 2006) to check the integrity and relative intensities of cDNAs indirectly. PCR conditions for actin is stated in Table 2.7. Table 2.8 shows the total list of primers for RGA-RT-PCR, DDRT-PCR, RGA-DDRT-PCR and trial experiments. Different combinations of primer pairs were used in these experiments.

Table 2.7 PCR reaction conditions for actin.

Reaction mix (Total Volume: 30 µL)		**Final Concent.**	**Reaction conditions**		
10X PCR buffer	3 µL	1X	94 ^{o}C	3 min	1 cycle
$MgCl_2$ (25mM)	1.8 µL	1.5 mM	94 ^{o}C	1 min	
dNTP (25mM)	0.1-0.3 µL	0.083-0.25 mM	53 ^{o}C	1 min	35 cycle
Taq polimerase	0.5-1.0 U	0.017-0.033 U µL	72 ^{o}C	1 min	
Act-F (10 pmol/µL)	1 µL	0.33 pmol/µL	72 ^{o}C	15 min	1 cycle
Act-R (10 pmol/µL)	1 µL	0.33 pmol/µL			
cDNA	required µL	-			
Molecular grade distilled water	Up to final volume				

Table 2.8 Primers used for RGA-RT-PCR and RGA-DDRT-PCR experiments.

Forward and Reverse	Sequence 5'-3'	Target region	Template gene and primer reference
Act-F Act-R	GTAACATTGTCTTGAGTGGTGG CTCTACAACAAAATGAGGGG	Actin	(Housekeeping) *Cicer arietinum* mRNA for actin, partial., Accession no:AJ012685 Expected amplicon length: 465bp. Designed in this study
S2	GGIGGIGTIGGIAAIACIAC	P-loop	*N* ve *RPS2* genes of *Arabidopsis* and flax NBS domain of *L6* gene (*Linum usitatissimum)* (Leister *et al.*, 1996)
NLRRfwd NLRRrev	TAGGGCCTCTTGCATCGT TATAAAAAGTGCCGGACT	LRR domain	*Tobacco N* gene, gene (Chen *et al.*, 1998).
RLRRfwd RLRRrev	CGCAACACTAGAGTAAC ACACTGGTCCATGAGGTT	LRR domain	*Arabidopsis RPS2* gene (Chen *et al.*, 1998).
Ptokin-1 Ptokin-2 (rev)	GCATTGGAACAAGGTGAA AGGGGGACCACCACGTAG	Ser/thr protein kinase gene	*Lycopersicon esculentum* (tomato) *Pto* gene (Chen *et al.*, 1998, Leung *et al.*, 2002)
PtoFen-S PtoFen-AS	ATGGGAAGCAAGTATTCAAGG TTGGCACAAATTCTCATCAAGC	protein kinase domain of Pto and Fen genes	*Lycopersicon esculentum* (tomato) *Pto* gene and fenthion resistance gene (Leister *et al.*, 1996)
Cicerkin2-fwd Cicerkin2-rev	TNHTTGTICTKGATGATGTGR YCACATCATCMAGHACAADIA	Conserved regions	Designed from the conserved region of the sequences disclosed in Hüttel *et al.* (2002) .
WipK-1 WipK-2 (rev)	GGTCGTGGTGCTTATGGAAT CCATGAAGATGCAACCGAC * is not classified as RGA primer	MAP kinase	MAP kinase of *Tobacco, Arabidopsis, Petroselinum crispum* (Parsley), *Medicago sativa* (Ligterink *et al.*, 1997)
NBSrev	YCTAGTTGTRAYDATDAYYYTRC	NBS domain	*Arabidopsis RPS2* gene and *Tobacco N* gene (Yu *et.al.* 1996a)
AS1 AS2 AS3 AS4 AS5 AS6 AS7 AS8	IAGIGYIAGIGGIAGIAGICC IAGIGYIAGIGGIAAICC IAGIGYIAAIGGIAGICC IAGIGYIAAIGGIAAICC IAAIGYIAGIGGIAGICC IAAIGYIAGIGGIAAICC IAAIGYIAAIGGIAGICC IAAIGYIAAIGGIAAICC	GLPL(A/T)L motif	Arabidopsis RPS2 gene and Tobacco N gene, Linum usitatissimum (flax) L6 gene *Mago et al.,* (1999)
T1	CATTATGCTGAGTGATATCTTTTTTTTTAA		polyA tail T primers of Delta Differential Display Kit (Clonetech, Cat. K1810-1)
T2	CATTATGCTGAGTGATATCTTTTTTTTTAC		
T3	CATTATGCTGAGTGATATCTTTTTTTTTAG		
T4	CATTATGCTGAGTGATATCTTTTTTTTTCA		
T5	CATTATGCTGAGTGATATCTTTTTTTTTCC		
T6	CATTATGCTGAGTGATATCTTTTTTTTTCG		
T7	CATTATGCTGAGTGATATCTTTTTTTTTGA		
T8	CATTATGCTGAGTGATATCTTTTTTTTTGC		
T9	CATTATGCTGAGTGATATCTTTTTTTTTGG		

2.6 Procedures for Preliminary Trials: RGA-DDRT-PCR Trial Experiments

Preliminary trials for setting up experimental procedures were collected under the name of RGA-DDRT-PCR trials. Several trials were carried out to set up the experimental procedure and to obtain cDNA efficiently. Total RNA source was the RNA samples obtained from first infection experiment (Table 2.1):

a) In the first trial, first strand cDNA was constructed from total RNA samples (Table 2.1, 3rd day infected samples) separately and then cDNA samples were bulked. cDNA synthesis was performed like this: 0.2 μL of 100 pmol/μL of cDNA synthesis primer (CDS primer) (final con. 1 pmol/μL), 0.5 μL of 25 mM dNTP (final con. 0.625 mM) and 6 μL of gel equalized total RNA were mixed and incubated for 5 min at 65 ^{o}C. Mixture was incubated on ice for 2-3 min. Then, 4 μL 5X first strand buffer (final con. 1X) (Invitrogen), 2 μL of 0.1 M DDT (final con. 0.01 M) (Invitrogen), 1 μL of 200 U/μL of Superscript III (final con. 10 U/μL) (Invitrogen), 0.5 μL of 40 U/μL RNaseOUT (Invitrogen) (final con. 1 U/μL) and DEPC treated sterile distilled water up to total reaction volume of 20 μL were added. The reaction mixture was incubated at 42 ^{o}C for 2 h. Reaction was terminated by incubating at 70 ^{o}C for 10 min. cDNA integrity and equality of infected and uninfected cDNA samples were done by PCR amplifying of actin gene. Sequence of the CDS primer is: 5' AAG CAG TGG TAA CAA CGC AGA GTA CTT TTT TTT TTT TTT TTT TTT TTT TTT YVN 3'

b) In the second trial, cDNA was constructed after bulking of total RNA samples (Table 2.1, 3rd day infected samples) to eliminate experimental deviations during cDNA synthesis and so that each RNA sample may be represented better in the bulk: 2 μL of 10 pmol/μL of cDNA synthesis primer (CDS primer) (final con. 1 pmol/μL), 1 μL of 25 mM dNTP (final con. 1.25 mM) and 12 μL of gel equalized total RNA were mixed and incubated for 5 min at 65^{o}C. Mixture was incubated on ice for 2-3 min. Then 4 μL 5X first strand buffer (final con. 1X) (Invitrogen), 2 μL of 0.1 M DDT (final con. 0.01 M) (Invitrogen) and 1 μL of 200 U/μL of Superscript III (final con. 10 U/μL) (Invitrogen) and DEPC treated sterile distilled water up to total reaction volume of 20 μL were added. The reaction mixture was incubated at 25 ^{o}C for 5 min, and then at 50 ^{o}C for 1 h. Reaction was terminated by incubating at 70 ^{o}C for 15 min.

c) In the third trial, first strand cDNA is constructed after bulking of RNAs as in paragraph (b) and reaction conditions were changed slightly aiming to produce cDNA more efficiently: 0.375 µL of 50 pmol/µL of oligodT primer (Invitrogen) (final con. 1.25 pmol/µL), 0.3 µL of 25 mM dNTP (final con. 0.5 mM), gel equalized total RNA and DEPC treated sterile distilled water up to 9.75 µL of total reaction volume were added. This mixture was incubated for 5 min at 65°C and taken on ice for 2-3 min. Then, 3 µL 5X first strand buffer (Invitrogen) (final con. 1X), 0.75 µL of 0.1 M DDT (Invitrogen) (final con. 0.005 M), 0.75 µL of 200 U/µL of Superscript III (Invitrogen) (final con. 10 U/µL) and 0.75 µL of 40 U/µL RNaseOUT (Invitrogen) (final con. 1 U/µL) was added (total volume 15 µL). The reaction mixture was incubated at 50°C for 1 h. Reaction was terminated by incubating at 70 °C for 15 min.

The basic reaction of RGA-DDRT-PCR trials was a PCR reaction carried out using a RGA primer as forward primer and polyA tail T primer as reverse primer (Figure 2.3). The PCR reactions were carried out according to the conditions given in the following Table 2.9. PCR products were separated and visualized as indicated in Section 2.10.

Table 2.9 Summary of PCR reactions of several trials of RGA-DDRT-PCR trials.

	Template and primers	PCR mix	PCR conditions
First trial (a)	Uninfected cDNA from plant group 8; infected cDNA of bulked cDNAs of plant groups 4, 5, 6 (Table 2.1). For PCR reaction, 5 µL cDNA from 1/5 diluted stock cDNA was used. Primer pairs: S2 (forward), T primers (reverse) from T1 to T7	10 µL reaction was prepared by using any commercial components: 1 µL from 10X buffer (final 1X), 1µL from 25mM $MgCl_2$ (not added if 10X buffer had $MgCl_2$) (final 2.5mM), 0.1 µL from 25mM dNTP, *Taq* polymerase (1 Unit), 1 µL from forward primer (10 pmol/µL), 1 µL from reverse primer, cDNA, radioactive dNTP. ([α^{32}P]CTP(10Mbq=0.025cm^3) or [α^{33}P]-dATP(40Mbq=0.100cm^3)), molecular grade sterile distilled water to complete 10 µL.	Cycle 1* 1 cycle of: step 1: 94 °C (5min) step 2 : 40 °C (5min) step 3 : 72 °C (5min) Cycle 2* 2 cycles of: step 3: 94 °C (2min) step 4: 40 °C (2min) step 5: 72 °C (2min) Cycle 3 30-35 cycles of step 1: 94 °C (1min) step 2: 42 °C (1min) step 3: 72 °C (1min) * These cycles facilitate primer binding
Second trial (b)	Uninfected cDNA from plant group 8; cDNA from bulked total RNA of infected plant groups 4, 5, 6 (Table 2.1). 3 µL cDNA from 1/5 diluted stock cDNA was used. Primer pairs: S2 or NLLRfwd (forward), T primers (reverse)		
Third trial (c)	Uninfected cDNA from plant group 8; cDNA from bulked total RNA of infected plant groups 4, 5, 6 (Table 2.1). 3 µL cDNA from 1/5 diluted stock cDNA was used. Primer pairs: Ptokin-1 or RLLRfwd (forward), T primers (reverse)		

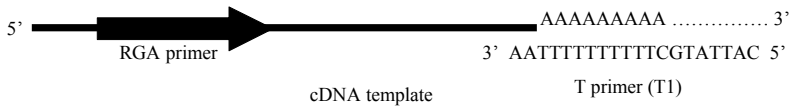

Figure 2.3 Illustration of PCR reaction in RGA-DDRT-PCR-trials amplification.

2.7 Procedures for RGA-RT-PCR Experiment

This experiment was performed to identify a wide variety of chickpea expressed RGAs in ILC195 upon infection with *A.rabiei* isolates of varying pathogenicity level. mRNA was used as a template for cDNA synthesis. Total RNA from plant groups shown in Table 2.2 was bulked as shown in Table 2.10. For construction of cDNA, RevertAid^{TM} M-MuLV reverse transcriptase (Fermentas) was used with the procedure disclosed in Table 2.10. According to manufacturer manual, 0.1 – 5.0 μg total RNA or 10 ng-0.5 μg mRNA or 0.01 pg - 0.5 μg specific RNA can be used as a template. In this experiment, 40 μg total RNA was used as starting material for mRNA isolation by Dynalbeads Oligo$(dT)_{25}$ (Table 2.6). Approximately 0.5 μg mRNA was used for cDNA construction. Table 2.11 shows the reaction conditions of cDNA synthesis. The integrity and equality of cDNA samples at different dilutions were tested by carrying out PCR amplifying of actin gene. cDNA amounts giving approximately the same actin band intensity were used for RGA-RT-PCR reaction. The basic reaction of RGA-RT-PCR is a PCR reaction on cDNA using a forward RGA primer in combination with a polyA-tail T or a reverse RGA primer as shown in Figure 2.4. PCR reaction conditions of RGA-RT-PCR which were modified from Rajesh *et al.* (2002) are summarized in the Table 2.12. The PCR products were separated and visualized as indicated in Section 2.10.

Table 2.10 Bulking of total RNA samples for RGA-RT-PCR.

	A.rabiei isolate					Sample numbers
H	***Ank6***	***Çor1***	***Ayaç5***	***Elmalı***	***Control***	
10	22	23	24	25	26	
24	32	33	34	35	36	
36	47	48	49	50		
48	56	57	58	59		
72	60	61	62	63		
BULK	all above BULK 1 = B1 mixed isolate bulk		all above BULK2 =B2 mild isolate bulk		all above BULK3 = B3 uninfected bulk	

Table 2.11 cDNA construction for RGA-RT-PCR, DDRT-PCR and GSP-RT-PCR.

STEP	TREATMENT
1	Add following components into 200 µL reaction tube: mRNA (10 ng-0.5 µg), 1 µL Oligo $dT_{(18)}$ (50 pmol/µL; final 2.5 pmol/µL); molecular grade water to complete total volume 11 µL.
2	Incubate at 70 ^{o}C for 5 min
3	Incubate on ice for 2-3 min
4	Add following components: 4 µL 5X reaction buffer (final con. 1X) 0.8 µL dNTP (25 mM; final con. 1 mM); 2.7 µL molecular grade water; 0.5 µL Ribonuclease inhibitor (RNaseOUT; 40 U/µL; final con. 1 U/µL). FINAL VOLUME: 19 µL
5	Incubate at 37 ^{o}C for 5 min
6	Add 1 µL RevertAid^{TM} M-MuLV reverse transcriptase (Fermentas, 200 U/µL; final conc. 10 U/µL). FINAL VOLUME: 20 µL
7	Incubate at 42 ^{o}C for 1.5 h.
8	Incubate at 70 ^{o}C for 10 min
9	Add 0.5 µL RNase H (5000 U/µL; final con. 122 U/µL)
10	Incubate at 37 ^{o}C for 20 min
11	store cDNA at -80 ^{o}C or -20 ^{o}C until use

Table 2.12 RGA-RT-PCR reaction conditions

Reaction mix (Total Volume: 25µL)		**Final Concent.**
10X rxn buffer	2.5 µL	1X
MgCl2 (25 mM)	2.5 µL	2.5 mM
dNTP (25 mM)	0.1 µL	0.1 mM
Taq polimerase	0.3 µL (1.5U)	0.06 U /µL
Forward primer (10 pmol/µL)	2.5 µL	1 pmol/µL
Reverse primer (10 pmol/µL)	2.5 µL	1 pmol/µL
Molecular grade distilled water	required µL	-
cDNA (1/20 diluted)	required µL	-
[α^{33}P]-dATP(40Mbq=0.100cm^{3})	(0.02-0.04) µL	

Reaction conditions		
94 ^{o}C	5 min	1 cycle
94 ^{o}C	1 min	45 cycle
45 ^{o}C	1 min	
72 ^{o}C	2 min	
72 ^{o}C	7 min	1 cycle

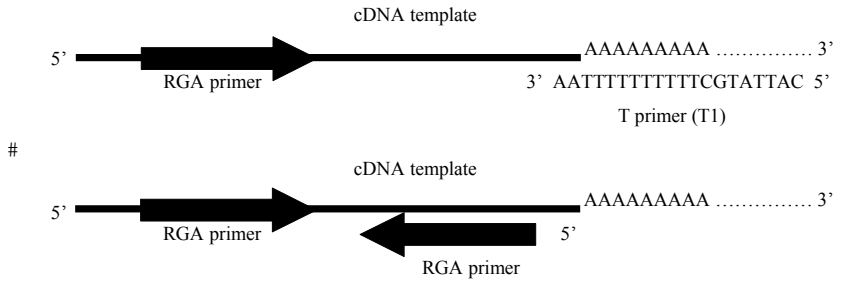

Figure 2.4 Illustration of PCR reaction in RGA-RT-PCR amplification.

2.8 Procedures for DDRT-PCR Experiment

mRNA Differential Display (DD) allows detection of altered gene expression which was first described by Liang and Pardee (1992). The procedure is based on amplification of the 3' terminal part of mRNAs and resolution of those fragments on a DNA sequencing gel. PCR amplification using combination of forward arbitrary primers with primers designed to bind to the 5' boundary of the poly-A tails of cDNAs derived from mRNAs by reverse transcription is the basis of the method (Figure 2.5) (Delta Differential Display Kit Manual, Clonetech, Cat.K1810-1). In this study, DDRT-PCR analysis was done to identify differentially expressed gene sequences of tolerant chickpea ILC195 upon various *A. rabiei* isolates (*ank6, cor1, ayac5*) infection in a time-based manner (10 h, 24 h, 3rd day). DDRT-PCR was performed by arbitrary upstream primers (P) and T primers (Table 2.13) designed for polyA-tail disclosed in Delta Differential Display Kit (Clonetech, Cat. K1810-1) and conditions were derived from kits manual. mRNA was used as a template for cDNA synthesis. 20 μg of bulked total RNA (description of bulks in Section 3.3, Figure 3.3) was used as starting material for mRNA isolation (Table 2.6; Section 2.4). Approximately 200 ng mRNA was used to construct cDNA with Fermentas RevertAid^{TM} M-MuLV reverse transcriptase (Table 2.11; Section 2.7). The integrity and equality of cDNA samples was tested by carrying out PCR amplifying of actin gene at different dilutions. cDNA amounts giving approximately the same actin band intensity were used for DDRT-PCR experiment. DDRT-PCR was performed by modification of conditions disclosed in Delta Differential Display Kit Manual (Clonetech, Cat. K1810-1) as shown in Table 2.14. The PCR products were separated and visualized as indicated in Section 2.10.

Table 2.13 Primers used in DDRT-PCR experiment.

Name	Sequence 5'-3'	Target region	Reference
P1	ATTAACCCTCACTAAATGCTGGGGA	Forward Arbitrary Primers	Delta Differential Display Kit (Clonetech, Cat. K1810-1)
P2	ATTAACCCTCACTAAATCGGTCATAG		
P3	ATTAACCCTCACTAAATGCTGGTGG		
P4	ATTAACCCTCACTAAATGCTGGTAG		
P5	ATTAACCCTCACTAAAGATCTGACTG		
P6	ATTAACCCTCACTAAATGCTGGGTG		
P7	ATTAACCCTCACTAAATGCTGTATG		
P8	ATTAACCCTCACTAAATGGAGCTGG		
P9	ATTAACCCTCACTAAATGTGGCAGG		
PI0	ATTAACCCTCACTAAAGCACCGTCC		
T1	CATTATGCTGAGTGATATCTTTTTTTTTAA	Reverse polyA tail T primers	Delta Differential Display Kit (Clonetech, Cat. K1810-1)
T2	CATTATGCTGAGTGATATCTTTTTTTTTAC		
T3	CATTATGCTGAGTGATATCTTTTTTTTTAG		
T4	CATTATGCTGAGTGATATCTTTTTTTTTCA		
T5	CATTATGCTGAGTGATATCTTTTTTTTTCC		
T6	CATTATGCTGAGTGATATCTTTTTTTTTCG		
T7	CATTATGCTGAGTGATATCTTTTTTTTTGA		
T8	CATTATGCTGAGTGATATCTTTTTTTTTGC		
T9	CATTATGCTGAGTGATATCTTTTTTTTTGG		

Table 2.14 DDRT-PCR reaction conditions

Reaction mix		Final Concent.	Reaction conditions		
10X rxn buffer	1 µL	1X	94 ºC	5 min	
MgCl2 (25 mM)	0.6 µL	1.5 mM	40 ºC	5 min	1 cycle*
dNTP (25 mM)	0.1 µL	0.25 mM	72 ºC	5 min	
Taq polimerase	0.3 µL (1.5 U)	0.15 U/µL	94 ºC	2 min	
P primer (10 pmol/µL)	1 µL	1 pmol/µL	40 ºC	5 min	2 cycle*
T (10 pmol/µL)	1 µL	1 pmol/µL	72 ºC	5 min	
Molecular grade distilled water	required µL	-	94 ºC	1 min	
cDNA (1/20-1/25 diluted)	required µL	-	58 ºC	1 min	35 cycle
[α^{32}P]-dCTP(10Mbq=0.025cm^3)	(0.01-0.025) µL	-	72 ºC	2 min	
Total volume	10 µL		72 ºC	7 min	1 cycle

* These cycles facilitate primer binding

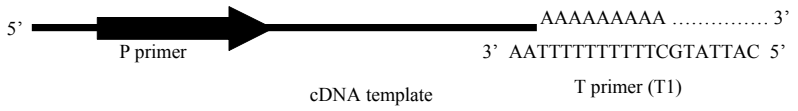

Figure 2. 5 Illustration of PCR reaction in DDRT-PCR amplification.

2.9 Procedures for RGA-DDRT-PCR Experiment

To test whether or not there are differentially expressed RGAs or some more genes (which could be amplified by RGA primers) in chickpea upon infection, the RGA-DDRT-PCR experiment was designed like a DD experiment to detect altered gene expression. The primer pairs which showed efficient amplification in RGA-RT-PCR were selected as primer sets of this experiment. 40 μg total RNA was used as starting material for mRNA isolation (Table 2.6 in Section 2.4). Approximately 150 ng mRNA was used as a template for cDNA by using Fermentas RevertAid^{TM} M-MuLV reverse transcriptase (Table 2.11 in Section 2.7). The integrity and equality of cDNA samples was tested by carrying out PCR amplifying of actin gene at different dilutions. cDNA amounts giving approximately the same actin band intensity were used for RGA-DDRT-PCR experiment. Reaction conditions of this experiment were similar to reaction conditions of RGA-RT-PCR (Table 2.12 and Figure 2.4 in Section 2.7). The PCR products were separated and visualized as indicated in Section 2.10.

2.10 Running and Developing of Radiolabeled PCR products

After adding loading buffer, PCR products were heated at 94^{o}C for a few min before loading into 6% denaturating polyacrlyamide electrophoresis gel (PAGE) casted as a sandwich between glass plates. 6X PAGE gel was prepared by adding 480 g urea, 57 g acrylamide, 3 g bisacrylamide, 200 mL 5X TBE buffer and diluted to 1 L distilled water (final con. 1X) (5X Tris-Borate-EDTA buffer: 54 g Tris base, 27.5 g boric acid, 20 mL 0.5 M EDTA, distilled water up to 1 L). Before casting; 650 mL 10% w/v APS (ammoniumpersulfate) and 25 mL TEMED (N, N, N’,N’-Tetramethyl ethylene diamine) was added into 60 mL of gel solution. After heating gels by running about half an hour,

PCR products were loaded and run for at least 3.5 h. After that, the gel sandwich was set to cool to room temperature and the glass plates were separated. The gel was taped onto a filter paper (Whatmann, 3 mm) and edges were labeled with radioactive PCR mix. The gel was covered with stretch film and dried by vacuuming. After completely dried, the gel was set in a developing cassette and an X-ray film (Agfa, 100NIF) was fixed on it in the dark. After keeping for a few days depending on the freshness of the radioactive material, the film was developed in the Radiology Unit of METU Health Center.

2.11 Obtaining and Reamplification of PCR Product Fragments From PAGE Gels

By placing the developed X-ray film onto the gel, selected bands were pointed with a needle and cut out of the gel by a sterile blade. Gel fragments were put into a sterile 500 μL eppendorf tube. After adding 30-40 μL molecular grade distilled water, they were incubated at 99-100 ^{o}C for 5 min. This suspension was used as template for reamplification of fragments by PCR. The same primer pair from which the bands were produced was used for reamplification. The conditions of this PCR reaction were given in Table 2.15. The last step of PCR was a 30 min elongation reaction at 72 ^{o}C, to let *Taq* polimerase to add polyA residues at the 3' end of DNA fragments to mediate T/A cloning. Reamplified PCR products were run in EtBr containing 1 % agarose gel in 0.5 X TBE buffer (TBE buffer in Section 2.10). Bands showing the original size profile as in the denaturating PAGE gel was cut by a sterile blade from the agarose gel under UV transilluminator. These agarose fragments were directly used for cloning.

Table 2.15 Reamplification conditions. Annealing temperature (Ta) is 42 ^{o}C for RGA-RT-PCR and RGA-DDRT-PCR, 58 ^{o}C for DDRT-PCR fragments. Cycle number is 45 for RGA-RT-PCR and RGA-DDRT-PCR fragments, 35 for DDRT-PCR fragments.

Reaction mix (Total Volume: 30μL)		**Final Concent.**	**Reaction conditions**		
10X rxn buffer	3 μL	1X	94 ºC	3 min	1 cycle
MgCl2 (25 mM)	1.8 μL	1.5 mM	94 ºC	1 min	
dNTP (25 mM)	0.3 μL	0.25 mM	Ta	1 min	35-45 cycle
Taq polimerase	0.2-0.3 μL (1-1.5 Units)	0.033-0.05 U/μL	72 ºC	2 min	
Forward primer (10 pmol/μL)	3 μL	1 pmol/μL	72 ºC	30 min	1 cycle
Reverse primer (10 pmol/μL)	3 μL	1 pmol/μL			
Molecular grade distilled water	required μL	-			
Band suspension	required μL (5-20μL)	-			

2.12 PCR Amplification of Disease Related Genes with Gene Specific Primers (GSPs)

Many different PCR conditions and cDNA bulks were used for obtaining chickpea copies of known plant disease related genes. These PCRs were performed with gene specific primers (GSPs) and called as GSP-RT-PCR. The list of anonymous primers available in Prof. Dr. M.S. Akkaya laboratory and GSPs which were designed in this thesis are also shown in Table 2.16. Primers were designed by means of Primer Detective (Version 1.01, Todd M.J.Love, 1990) and ClustalX programmes by considering conserved regions of the target gene copies of relatives of chickpea.

PCR Amplification: Different PCR conditions were tested to obtain any products from cDNA bulks. In a 25-30 μL reaction volume the final concentrations of components varied as: from 1.5 mM to 2.5 mM for $MgCl_2$, from 0.25 mM to 0.4 mM for dNTP, from 0.3 pmol/μL to 1 pmol/μL for primers, from 1 to 1.5 units for *Taq* polymerase, 1X for PCR buffer, various amounts for cDNA and molecular grade distilled water. PCR thermal cycles were designed as follows: 1 cycle of 94 ^{o}C (3 or 4 min); 35 to 40 cycles of 94 ^{o}C (1 min) – Tm (1 min) – 72 ^{o}C (1 or 2 min); 1 cycle of 72 ^{o}C (10 to 15 min). Several Tm values were tested according to melting temperatures of primers. PCR products were run either on 1 or on 2 % agarose gel containing EtBr. Product bands were cut by a sterile blade from the agarose gel under UV. This agarose fragments were directly used for cloning.

Touch-Down PCR Amplification: Touch-Down PCR is a modified PCR method which aims to increase the specificity of PCR by applying an increment to the annealing temperature. It starts with a number of successive cycles from high annealing temperature to a lower final one by decreasing the temperature in each cycle. The rest of cycles are completed with the lowest annealing temperature. An example for reaction conditions for Touch-Down PCR is provided in Table 2.17. PCR products were run on 2% agarose gel containing EtBr. Product bands were cut by a sterile blade from the agarose gel under UV. This agarose fragments were directly used for cloning.

Table 2.16 Gene Specific Primers (GSPs).

Anonymous GSPs available in Prof. Dr. M.S. Akkaya laboratory			
Gene	**Sequence 5'-3'**	**This gene is disclosed in**	**Target Size (bp)**
RAR1	RAR1-317 fwd GRAAGCAYACAACWGARAAACC RAR1Hv-769 rev TCACACAGCATCAGCATTGTG	required for Mla12 resistance-1 barley, Shirasu *et al.*, 1999	452
SGT1	SGT1-175 fwd ACACTGAGGCTGTAGCTGATGC SGT1-698 rev CTTWGAAAACAGACGAGGCTGG	Suppressor of the *G2* allele of *SKPI*, barley, Azevedo *et al.*2002	523
EDS1	EDS1-612 fwd CCTCGGATKATGCTTGCTC EDS1-1313 rev GACRTTWGCWTTGAAGTCAT	Enhanced Disease Susceptibility, Arabidopsis, Parker *et al.*1996	701
NPR1	NPR1-333 fwd CGGTGCRTTTTGTCRGCGAG NPR1-1075 fwd GGGGATAYACGGTGCTKCATG NPR1-1712 rev TTCCATGTACCTTTGCTTCTT	non-expressor of PR gene Arabidopsis, Cao *et al.*, 1997	742 and 1379
NDR1	NDR1-31at fwd TGGTCGAAAYTGYTGYACTTGC NDR1-31at rev GCCACTRCCTCAATTYCGAGTC	Non-race specific resistance Arabidopsis, Century *et al.*1995	526

GSPs designed in this study			
Gene	**Primers 5' to 3'**	**Designed for conserved regions by alignment of**	**Target Size (bp)**
Fungal pathogen-induced protein **(FPIP)** (Defense)	FPIP-fwd AGGGCATGTACTAGAACCAC FPIP-rev CAAGTCGAAAATAAGCCTCGCC	**gi: 508843** = *Pisum sativum* disease resistance response protein 206-d (DRR206-d) gene, complete cds.	604
Hypersensitive response protein **(HRP)** (Defense)	HRP-fwd AGATGTTCTTGAGCCTGGTTGC HRP-rev AGCGAAACCCAAAACACTGTCG	**gi: 17065547** = *A. thaliana* hypersensitive-induced response protein (MQB2.6) mRNA, complete cds (template sequence for primers); **gi: 57834177** = Lotus corniculatus var. japonicus Lj-HIR1 mRNA for hypersensitive-induced response protein, complete cds.	592
Kinase-associated protein phosphatase **(KAPP)** (Signaling)	KAPP-fwd TGAAGGACTCAGAAGTCTCTGG KAPP-rev ATGCTGAATCCCCAAGATTGGC	**gi: 1709235** = *A. thaliana* kinase associated protein phosphatase mRNA, complete cds. (template sequence for primers); **gi: 3328363**=*Oryza sativa* kinase associated protein phosphatase (kapp) mRNA, complete cds.	642
Formate dehydrogenase **(FDH)** (Defense)	FDH-fwd ACAGAGCTTATGATCTTGAAGG FDH-rev GTCCTTAGGAGCTGGGTGTGGG	**gi: 6681407**= *A. thaliana* FDH mRNA for formate dehydrogenase, complete cds. (template sequence for primers);**gi: 3863 6525** = Quercus robur mRNA for FDH (ORF1); **gi: 56562180** = *Lycopersicon esculentum* mRNA for FDH gene; **gi: 11991526** = Solanum tuberosum mRNA for FDH. **gi: 16995760** = Glycine max cDNA clone SOYBEAN CLONE ID:Gm-c1066-4491 5' similar to TR:Q9ZRI8 Q9ZRI8 FDH. mRNA sequence.	404
Pectin esterase **(PE)** (Cell wall and defense)	PE-fwd GTGGCAAAAGATGGCAGTGG PE-rev CAGTCACGGTAGAATTGGCG	**gi: 21061** = *Phaseolus vulgaris* PvVPE3 mRNA for PE (template sequence for primers); **gi: 30695594** = *A. thaliana* ATPME1; PE AT1G53840 (ATPME1) mRNA, complete cds; **gi: 886129** = *Medicago sativa* putative PE mRNA, complete cds.	419
non-host resistance gene **(NHO1)** (Resistance)	GxNho1-Fwrd-106 GAGTTCACCCAGTTCTACC GxNho1-Rv748 GYTGATCMCCWARACATCCAGC GxNho1-Rv1300 ATCWGCCTGAATCTGCATCAG	**gi: 40457262** = *Glycine max* glycerol kinase (GK) mRNA, complete cds.	642 and 1194

Table 2.17 Reaction conditions for Touch-Down PCR.

Reaction mix (Total Volume: 25μL)		**Final Conc.**	**Reaction conditions**		
10X rxn buffer	2.5 μL	1X	94°C	4 min	1 cycle
MgCl2 (25 mM)	2.5 μL	2.5 mM	94°C	1 min	10 cycles
dNTP (25 mM)	0.3 μL	0.3 mM	Ta = from 52°C to 42°C	1 min	1 °C decrease in each cycle
Taq polimerase	1.0-1.5U	0.04-0.06 U/μL	72°C	2 min	
GSP fwd (10 pmol/μL)	2.5 μL	1 pmol/μL	94°C	1 min	
GSP rev (10 pmol/μL)	2.5 μL	1 pmol/μL	42°C	1 min	30 cycle
cDNA	required μL	-	72°C	2 min	
Mol. grade distil. water	required μL	-	72°C	15 min	1 cycle

2.13 Cloning

Agarose gel fragments obtained from reamplification of RGA-RT-PCR, DDRT-PCR, RGA-DDRT-PCR and GSP-RT-PCR were put into a sterile 1.5 mL eppendorf tube and frozen in liquid N_2. With a blunted sterile tip, these fragments were crushed until became liquified. This suspension was used as inserts for ligation into vectors. pGEM-T-Easy vector (Promega) ligation system is based on T/A cloning (Appendix B). 10 μL ligation reaction mix for pGEM-T-Easy vector was composed of the following: 0.05 μL of pGEM-T-Easy (50 ng/μL; final conc.: 0.25 ng/μL), 5 μL of 2X ligation buffer (final conc. 1X), 0.5-1 Units of T4 DNA ligase (final conc. 0.05 – 0.1 U μL), required μL of liquified agarose fragment to complete total volume of the reaction. T4 DNA ligase and its buffer were supplied from various companies. The ligation mix was incubated at 16 -18 °C overnight. Transformation was carried out as described in Chung *et al.*(1989) with minute modifications. Table 2.18 discloses the steps of transformation of vectors into *E.coli* DH5-α cells. White colonies were picked up by a sterile wooden stick and grown overnight in LB broth at 37 °C (LB broth: 10 g Tryptone, 5 g NaCl, 5 g yeast extract up to 1 L distilled water; after autoclaving filter sterilized ampicilin was added with final concentration 100 μg/mL). 50 % (v/v) sterile glycerol was added into an equivalent volume of these stock cultures and stored at -80 °C. In case of any crowded plates, colonies were picked and streak plated to obtain single white colonies. The existence of the expected insert was confirmed by colony PCR using M13 phage primers, by either shaking the contaminated wooden stick into PCR mix or by adding 2 μL of grown stock culture (Table 2.19). Only for clones obtained from first trial (a) of RGA-DDRT-PCR trials, pTZ57R/T plasmid (Fermentas, Appendix B) was used for cloning by means of Manufacturer's kit.

Table 2.18 Transformation procedure of Chung *et al.* (1989) with some modifications.

Step	Procedure
1	Grow *E.coli* DH5-α cells in 2-5 mL LB at 37°C overnight in a shaker.
2	Take 10-20 µL culture from step 1 and inoculate 1 mL LB in eppendorfs. Grow at 37 °C for 2-2.5 h by shaking.
3	Cool down the culture on ice for 20 min.
4	Centrifuge and collect cells at 4°C 1500 rpm 5 min.
5	Discard supernatant, suspend cells in ice cold 150 µL TSS (Transformation and Storage Solution) (100 mL TSS: 85 % (v/v) LB, 10 % PEG (w/v, MW 8000), 5 % DMSO (v/v), $MgCl_2$ (50 mM final conc., pH 6.5). Filter sterilize).
6	Add ligation product <=10 µL.
7	Incubate cells in ice while occasional inverting for 30 min.
8	Make a heat shock at 42 °C for 2 min.
9	Cool down cells on ice for a few min.
10	Add 0.8 mL LB into cell suspension.
11	Incubate at 37 °C for 1 h .
12	Spread plate on selective LB agar media and grow at 37 °C overnight (Selective LB agar plate: 10 g Tryptone, 5 g NaCl, 5 g yeast extract, 15 g agar up to 1 L distilled water. After autoclaving, filter sterilized IPTG, X-gal and ampicilin was added with final concentrations of 40 µg/mL, 40 µg/mL and 100 µg/mL, respectively).
13	Select white colonies and confirm them by PCR of M13 phage primers.

Table 2.19 Conditions for confirmation of recombinants by PCR with M13 phage primers.

Reaction mix (Total Volume: 30µL)		**Final Concent.**	**Reaction conditions**		
10X rxn buffer	3 µL	1X	94 °C	3 min	1 cycle
MgCl2 (25 mM)	1.8 µL	1.5 mM	94 °C	1 min	
dNTP (25 mM)	0.3 µL	0.25 mM	53 °C	1 min	35-40 cycle
Taq polimerase	1 Ų	0.033 Ų/µL	72 °C	1 min	
Forward primer (10 pmol/µL)	1 µL	0.33 pmol/µL	72 °C	15 min	1 cycle
Reverse primer (10 pmol/µL)	1 µL	0.33 pmol/µL			
Molecular grade distilled water	required µL	-			
Cell suspension in water or in LB	required µL	-			

2.14 Sequencing

Only for clones obtained from first trial (a) of RGA-DDRT-PCR trial experiment (Section 2.6), plasmid isolation by using QIAGEN QIApred Spin Miniprep Kit was performed: cells were grown overnight in 2-2.5 mL LB (200 µg/µL ampicilin) and centrifuged for 5

min to collect cells. After that the kit procedure was followed. Plasmids were eluted with 50 μL elution buffer and stored at -20 ^{o}C or -80 ^{o}C. Inserts were checked by double digestion using *PST*I (Fermentas) and *Eco*RI (Fermentas). Sequencing service was taken commercially. For all other clones, commercial sequencing service was taken from the facility of Genome Sequencing Center of Washington University of Medicine. This facility uses high-throughput sequencing method for large number of clones, which should be prepared as glycerol-LB (at least 100 μg/μL ampicilin) cell stocks 96-well plates. For that reason, 200 μg /μL of glycerol-LB clone stocks were loaded into 96-well plates, sealed and sent by post for sequencing. The facility also accepts clones in LB (at least 100μg/μL ampicilin). Results were downloaded from the website of this facility as SCF (Standard Chromatogram Format) and FASTA formats.

2.15 Analysis of Sequence Results

The quality of SCF chromatogram and sequence data of cDNA sequences (ESTs here after) were evaluated and worked on BioEdit program (BioEdit version 7.0.5.3). Firstly, vector sequences were removed by searching the following insert sites of pGEM-T-Easy vector: 5'…GGAATTCGAT/insert/ATCACTAGTG…3' and when these sites were not found "align two sequences" (aligning vector and sequence) option of National Center for Biotechnology Information (NCBI) BLAST web site was used. Insert sequences were subjected to BLASTN (Viridiplantae kingdom), BLASTX and "Conserved Domain Search" analysis of NCBI BLAST program. Sequences were translated by a web-based program called fr33net. In case of no BLASTX result, BLASTP option was also used. Clones having the same BLAST results were aligned by the "Pairwaise Alignment/Align two sequences (optimal GLOBAL alignment)" function of BioEdit program to find the percent of similarity. Functional analysis was arranged according to three main classes of AmiGO (Gene Ontology): "Molecular Function", "Biological Process" and "Cellular Component". The data for these classes were obtained from information of closely related hits of BLASTX and conserved domains by using their Interpro, SMART, Pfam, AmiGO and Uniprot data. In case of poor information, BLASTN hits were also used to find out necessary data. Table 2.20 shows the list and websites of programs and databases used in the analysis of EST data.

Table 2.20 Programs and databases used in the analysis of EST sequences.

Program or Database	Source and Citation
Bioedit	http://www.mbio.ncsu.edu/BioEdit/BioEdit.html Bioedit Version 7.0.5.3.(Hall, 1998). Last updated 6/27/07
NCBI	National Library of Medicine. National Center for Biotechnology Information. http://www.ncbi.nlm.nih.gov/ Revised: July 23, 2008
fr33net	http://www.fr33.net/translator.php
CLUSTALW	Life Sciences Tools. Tools for DNA, RNA and protein sequences. Pawlowski, N. http://align.genome.jp/ Kyoto University Bioinformatics Center, Institute for Chemical Research, Kyoto University. Accession date: 26 June 2008
The Gene Ontology (AmiGo)	http://amigo.geneontology.org/cgi-bin/amigo/go.cgi The Gene Ontology Consortium. Gene Ontology: tool for the unification of biology. Nature Genet. (2000) 25: 25-29.
Interpro	InterPro - Integrated Resource of Protein Domains And Functional Sites. http://www.ebi.ac.uk/interpro/ The European Bioinformatics Institute. Copyright (C) 2001 The InterPro Consortium
Pfam	http://pfam.sanger.ac.uk/ The Wellcome Trust. Sanger Institute The Pfam protein families database*:* R.D. Finn, J. Tate, J. Mistry, P.C. Coggill, J.S. Sammut, H.R. Hotz, G. Ceric, K. Forslund, S.R. Eddy, E.L. Sonnhammer and A. Bateman Nucleic Acids Research (2008) Database Issue 36:D281-D288. Release 1.1 (18th September 2007) Release 1.2 (15th October 2007)
SMART	http://smart.embl-heidelberg.de/ Schultz et al. (1998) *Proc. Natl. Acad. Sci. USA* 95, 5857-5864 Letunic et al. (2006) *Nucleic Acids Res* 34, D257-D260 SMART (a Simple Modular Architecture Research Tool) European Molecular Biology Laboratory. Bork Group.
Uniprot	http://www.expasy.uniprot.org/ The UniProt Consortium. The Universal Protein Resource (UniProt) Nucleic Acids Res. 36:D190-D195(2008).

2.16 Genetic Linkage Analysis

Genetic linkage analysis was carried out by primers designed for some of the EST sequences of clones (Table 2.23). Information for mapping population CRIL-7 (Tekeoglu *et al.*, 2000) and parents were presented in Section 2.2.3 and in the Introduction Section 1.5.2.2. DNA material available in USDA-ARS Legume Genetics and Physiology Unit isolated by Norm's Mini Mix method (Table 2.22) was used. For obtaining at least a single PCR product, various annealing temperatures and cycle numbers were tested for each of primer set using bulked cDNA material of ILC195 and DNA material of CRIL-7 parents and/or CRIL-3 parents. An example of conditions is disclosed in the Table 2.21.

Table 2.21 PCR conditions for linkage analysis.

Reaction mix (Total Volume: 20 µL)		**Reaction conditions**		
PCR Master Mix (2 units of Promega Taq, 1X Promega PCR buffer (A), 1.5 mM $MgCl_2$, 0.2 mM dNTP)	14µL	94°C	3 min	1 cycle
Forward primer (10 pmol/µL)	1 µL (0.5 pmol/µL final)	94°C	1 min	
Reverse primer (10 pmol/µL)	1 µL (0.5 pmol/µL final)	Ta°C	1 min	35-40 cycle
cDNA or DNA	required µL	72°C	1 min	
Molecular grade distilled water	required µL	72°C	15 min	1 cycle

Table 2.22 Norm's Mini Mix: DNA Isolation Miniprep (15 mL tubes). Procedure From WSU USDA-ARS Grain Legume Genetics and Physiology Laboratory, 24.01.2007.

Step	**Procedure**
1	Prepare DNA Extraction Buffer (Norm's Mini-Mix): For 100 mL, add 2 g CTAB into 58 mL of HPLC grade water and stir overnight. Then, add 10 mL of 1 M Tris pH 8.0, 28 mL of 5 M NaCl and 4 mL of 0.5 M EDTA.
2	Collect plant samples and freeze in liquid N_2 and keep at -70 °C until use.
3	Take plant samples from -70 °C crush them in liquid N_2 to powder in a pre-cooled sterile mortar and pestle; take 2-3mL of ground tissue into the 15 mL tube.
4	Extraction buffer: For 1 sample add 5 mL Norm's Mini Mix and 20 µL 2-mercaptoethanol. Norm's Mini Mix: Pipette desired amount of Norm's Mini Mix into a Corning bottle. In the fume hood, add 2-mercaptoethanol to the extraction buffer in the Corning bottle to make a 0.4 % 2-mercaptoethanol solution (equals 40 µL/10 mL of buffer).
5	Add 5 mL of buffer (Norm's Mini Mix plus BME) into each sample tube, mix by inverting, and heat for 30-45 min in a 65 °C in water bath by inverting tubes every 5-10 min.
6	Cool samples for a few min.
7	Add 5 mL of chloroform:iso-amyl alcohol (24:1) in the fume hood. Mix by inverting the tubes gently.
8	Centrifuge at 2200 rpm for 15 min. Take upper aqueous phase into a fresh tube.
9	Add 30 µl of RNase (10 mg/ mL), mix by inverting. Incubate at 37°C for 30 min.
8	Add another 5 mL of chloroform:iso-amyl alcohol (in hood). Mix by inverting and spin for another 10 min. Centrifuge at 2200 rpm for 15 min. Take upper aqueous phase into a fresh tube.
9	Add cold 95 % ethanol (2X the amount of sample in the tube). Mix gently and see precipitated DNA. Incubate samples at 4 °C for 20 min to aid precipitation of DNA.
10	Wash the DNA by hooking out the DNA with glass hooks and placing it in 5 mL of 70 % ethanol for 10 min.
11	Dry the DNA by hooking out the DNA again, inverting the hook, and allowing the ethanol to evaporate from the DNA for 5-10 min.
12	Place 300-500 mL of sterile TE buffer into 1.5 mL tubes, place the DNA into the TE hydrate DNA for at least an h or overnight. Store in the refrigerator or freezer.

Table 2.23 EST specific primers designed for genetic linkage and Real-Time qRT-PCR.

EST info Band no/Source/ 96-well plate no	**Forward (SN) and Reverse (ASN) Primers**	**BLASTX** (1st hit of BLASTX and 2nd hit and 1st hit of blastN and BlastP if necessary') **Done in September-December 2006**
Band no: 104 RGA-RT-PCR U_HW-Plate1a05.b1	104-SN160 5'CTACCATACATACTgAggTTgTCACCT3' 104-ASN202 5'gCACTTgTAgATTATTCAggTCACTgg3' expected amplicon size on cDNA: 69bp	gi|18378095|emb|CAC86495.1| RGA-F protein [Cicer arietinum] expect =1e-48 Identities = 110/122 (90%),
Band no: 116 RGA-RT-PCR U_HW-Plate2a02.b1	116-SN21 5'ggggCAATTTCTgTTCCTATgTgACT3' 116-ASN140 5'CACAAgACAATCCTCgTAgACCTT3' expected amplicon size on cDNA: 141bp	gi|18034123|gb|AAL57365.1|AF404866_1 neuronal nitric oxide synthase protein inhibitor [Arabidopsis thaliana] Expect = 4e-08 Identities = 28/34 (82%)
Band no: 179 RGA-RT-PCR U_HW-Plate2a03.b1	179-SN20 5'ggTCgCAATTTCggTTCgTATg 3' 179-ASN165 5'ACAAgAgTTAATATAgCAgCAT3' expected amplicon size on cDNA: 167bp	gi|66391201|ref|YP_239367.1| hypothetical protein 3 [Microplitis demolitor bracovirus] Expect = 3e-14 Identities = 42/56 (75%) gi|18034123|gb|AAL57365.1|AF404866_1 neuronal nitric oxide synthase protein inhibitor [Arabidopsis thaliana] Expect = 5e-12 Identities = 33/34 (97%)
Band no: 304 RGA-RT-PCR U_HW-Plate2a04.b1	304-SN75 5'ggATAATTCgTTggATATgCCCgCTACA3' 304-SN136 5'CCTTTTgCCgTTTgggAgATTT3' expected amplicon size on cDNA: 83bp	gi|26335581|dbj|BAC31491.1| unnamed protein product [Mus musculus] Expect = 2.1 Identities = 24/100 (24%),
Band no: 1558 DDRT-PCR U_HW-Plate2b06.b1	1558-SN10 5'CACTAAATgCgggTAgAggTAgCA3' 1558-ASN124 5'CCCTACTgTgTTCgACgTTATCTC3' expected amplicon size on cDNA: 138bp	gi|108873360|gb|EAT37585.1| conserved hypothetical protein [Aedes aegypti] Expect = 5.7 Identities = 17/45 (37%),
Band no: 1619 DDRT-PCR U_HW-Plate2b07.b1	1619-SN68 5'TTCCAAAAgACCCTgTTTCCAC3' 1619-SN228 5'gCTCCTCCCATAgAAggAAgAA3' expected amplicon size on cDNA: 184bp	gi|4689475|gb|AAD27911.1| putative ribonuclease E [A. thaliana] Expect = 4e-22 Identities = 67/104 (64%),
Band no: 1769 RGA-DDRT-PCR U_HW-Plate2b10.b1	1769-SN75 5'gCTTTCAAAgATAAgCgAgAACTAA3' 1769-ASN167 5'CCACAAAgTCCTTTTTTTCATAgg3' expected amplicon size on cDNA: 116bp	BlastP gi|115475155|ref|NP_001061174.1| Os08g0191900 [Oryza sativa (japonica cultivar-group)] gi|40253652|dbj|BAD05595.1| putative pentatricopeptide (PPR) repeat-containing protein [Oryza sativa (japonica cultivar-group)]
Band no: 1868 RGA-DDRT-PCR U_HW-Plate2b11.b1	1868-SN54 5'CACTgTgTTCCAAgCAAACTTg3' 1868-ASN178 5'TCCTCTCAACAAACCCCTTgTCAT3' expected amplicon size on cDNA: 148bp	gi|21593731|gb|AAM65698.1| putative serine carboxypeptidase II [Arabidopsis thaliana] Length=471 Expect = 9e-46 Identities = 93/140 (66%),
Band no: R46 RGA-DDRT-PCR	R46-SN32 5'TCgCAACCACTAgAgTAACATg 3' R46-ASN210 5'TggAgTgATCCCCTAAAgATAACC3' expected amplicon size on cDNA: 202bp	gi|10177448|dbj|BAB10839.1| receptor-like protein kinase [A. thaliana] Length=580 Expect = 3e-26 Identities = 57/69 (82%) gi|30697726|ref|NP_201077.2| ATP binding / protein binding / protein kinase/ protein serine/threonine kinase/ protein-tyrosine kinase [Arabidopsis thaliana]
Band no:R50 RGA-DDRT-PCR U_HW-Plate2c11.b1	R50-SN77 5'gTTTTgCggTgTAATggTAggA3' R50-ASN148 5'gAATACTCAgCTTACTCTTATgCg3' expected amplicon size on cDNA: 95bp	gi|14716946|emb|CAC44142.1| putative polyprotein [Cicer arietinum] Expect = 7e-17 Identities = 43/49 (87%)
Band no: 2 RGA-RT-PCR U_HW-Plate3a02.b1	2-SN29 5'CgATATTgCACACggAgAAgAACg3' 2-ASN103 5'TTACTCTCgCCCAgCATACgCTTAgC3' expected amplicon size on cDNA: 100bp	gi|92885696|gb|ABE87884.1| Serine/threonine protein kinase, active site [Medicago truncatula] Length=371 Expect = 1e-77 Identities = 148/177 (83%)

Table 2.23 Continued.

EST info Band no/Source/ 96-well plate no	**Forward (SN) and Reverse (ASN) Primers**	**BLASTX** (1st hit of BLASTX and 2nd hit and 1st hit of blastN and BlastP if necessary') **Done in September-December 2006**
Band no: 447 RGA-RT-PCR U_HW-Plate3a09.b1	447-SN188 5'AgCTgTggACCATCTTgCggCT3' 447-ASN356 5'CATgCgACTAgCAggATTTg 3' expected amplicon size on cDNA: 187bp	gi|22327992|ref|NP_200901.2| ATP binding / microtubule motor [A. thaliana] Expect = 2e-04 Identities = 42/86 (48%)
Band no: 505 RGA-RT-PCR U_HW-Plate3a10.b1	505-SN48 5'gATgATgATgACgATgATgATggT3' 505-ASN185 5'CCTCCACACCgTTTTCCACAggTTCA3' expected amplicon size on cDNA: 80bp	gi|18401985|ref|NP_564517.1| unknown protein [A. thaliana] gi|12323095|gb|AAG51532.1|AC051631_12 hypothetical protein; 76492-75272 [A. thaliana]
Band no: 1089 RGA-RT-PCR U_HW-Plate3b05.b1	1089-SN87 5'gTTATCggCATACCCTTCCTCTgg3' 1089-ASN187 5'CCCACTggTAgATTTCCgTATAgg5' expected amplicon size on cDNA: 124bp	gi|118009650|gb|EAV23718.1| binding-protein-dependent transport systems inner membrane component [Pseudomonas mendocina ymp] Length=302 Expect = 3e-29 Identities = 76/83 (91%)
Band no: 1465a DDRT-PCR U_HW-Plate3b06.b1	1465a-SN160 5'AgTTCggTACAgCCAgAgTgggAgTCg3' 1465a-ASN282 5'CTgCCTTTTCACCATgCgggAgTgCT3' expected amplicon size on cDNA: 147bp	gi|18411065|ref|NP_565129.1| unknown protein [Arabidopsis thaliana] gi|15292905|gb|AAK92823.1| putative auxin-independent growth promoter protein [A. thaliana]
Band no: 1468 DDRT-PCR U_HW-Plate3b08.b1	1468-SN250 5' TgCTACTgTCCAgCATAATCAA 3' 1468-ASN430 5' CACTCggggTATATTAgATATTCg 3' expected amplicon size on cDNA: 206bp	gi|115444821|ref|NP_001046190.1| Os02g0196000 [Oryza sativa (japonica cultivar-group)] gi|49388124|dbj|BAD25255.1| putative Zinc transporter zupT [Oryza sativa (japonica cultivar-group)]
Band no: 1508 DDRT-PCR U_HW-Plate3c04.b1	1508-SN214 5' ggATAgAAACAAgTCgCCAgCA 3' 1508-ASN265 5' CgAgATgTCCAATCgggCACATTTC 3' expected amplicon size on cDNA: 76bp	gi|108706378|gb|ABF94173.1| ReMembR-H2 protein JR702, putative, expressed [Oryza sativa (japonica cultivar-group)] Expect = 1e-16 Identities = 59/160 (36%)
Band no: 1528 DDRT-PCR U_HW-Plate3c06.b1	1528-SN214 5' CTTgCATgggCTTAgTAAggTTgC 3' 1528-ASN253 5' gCTCgTggTgTTACAAATgAAg 3' expected amplicon size on cDNA: 61bp	gi|56718873|gb|AAW28145.1| hAT-like transposase [A. thaliana] Length=696 Expect(2) = 4e-49 Identities = 87/137 (63%),
Band no: 1571 DDRT-PCR U_HW-Plate3c10.b1	1571-SN204 5' CTgCACTCAAAACTgACTTTCC 3' 1571-ASN369 5' ggATAACTggATTCCCTCCCCCATgT 3' expected amplicon size on cDNA: 191bp	gi|2465008|emb|CAA04767.1| ripening-induced protein [Fragaria vesca] Length=337 Expect = 4e-06 Identities = 35/55 (63%),
Band no: HRP GSP-PCR U_HW-Plate3d04.b1	HRP-SN131 5' gACTTCTTCCCTggTTTAATCACC 3' HRP-ASN250 5'CCCAAAACACTgTCgAAATTCCCgAAA 3' expected amplicon size on cDNA: 146bp	gi|15292873|gb|AAK92807.1| putative receptor protein kinase [A. thaliana] Expect = 0.001 Identities = 31/67 (46%)
Band no: 1612 DDRT-PCR U_HW-Plate3d06.b1	1612-SN28 5' ggATgTTgAgAAATgACATgACgg 3' 1612-SN91 5' CCCATgACAATgTTCgTACCAg 3' expected amplicon size on cDNA: 85bp	gi|30680567|ref|NM_116866.2| A. thaliana ATCSLC12; transferase, transferring glycosyl groups (ATCSLC12) mRNA, complete cds Expect = 1e-05 Identities = 101/125 (80%)
Band no: 1934 DDRT-PCR U_HW-Plate3e04.b1	1934-SN6 5' CCCTCACTAAATgCTgTATgTg 3' 1934-ASN68 5'CATAAgTCAAACCACACTTgCCACAg3' expected amplicon size on cDNA: 88bp	gi|92870550|gb|ABE79841.1| Ubiquitin; Ribosomal protein S27a [Medicago truncatula] gi|92895634|gb|ABE92781.1| Ubiquitin; Ribosomal protein S27a [Medicago truncatula] Expect = 5e-12 Identities = 30/33 (90%),
Band no: 1940 DDRT-PCR U_HW-Plate3e06.b1	1940-SN117 5' ggACAACCAgTTgggCAAAAAggT 3' 1940-SN234 5' gACAgATTATgCgACATTggTC 3' expected amplicon size on cDNA: 139bp	gi|303732|dbj|BAA02117.1| GTP-binding protein [Pisum sativum] gi|738941|prf||2001457J GTP-binding protein Expect = 8e-13 Identities = 34/35 (97%)

Table 2.23 Continued.

EST info Band no/Source/ 96-well plate no	**Forward (SN) and Reverse (ASN) Primers**	**BLASTX** (1st hit of BLASTX and 2nd hit and 1st hit of blastN and BlastP if necessary') **Done September-December 2006**
Band no: 1990 RGA-DDRT-PCR U_HW-Plate3e08.b1	1990-SN137 5' CCCTTCTTCTTTCTTCTTACCAgC 3' 1990-ASN265 5' TCATCAgAgTCTCAgCAggAgTTgg 3' expected amplicon size on cDNA: 153bp	gi|110736036|dbj|BAE99990.1| peptidylprolyl isomerase [A. thaliana] Expect = 5e-49 Identities = 109/127 (85%)
Band no: 1998 RGA-DDRT-PCR U_HW-Plate3e09.b1	1998-SN162 5' gAggTTTCggCAAAgAAAggAACTCg 3' 1998-ASN341 5'gTgATCCTATTggAATgCAAgTCC 3' expected amplicon size on cDNA: 203bp	gi|15227838|ref|NP_179336.1| protein binding [A. thaliana] gi|2494144|gb|AAB86525.1| unknown protein [Arabidopsis thaliana] gi|57868152|gb|AAW57414.1| plant intracellular Ras-group-related LRR protein 5 [A. thaliana]
Band no: 2166 RGA-DDRT-PCR U_HW-Plate3g04.b1	2161-SN56 5' TCTTgTTgCATAACCAggATCTCC 3' 2161-ASN 153 5 TCgAgCTgTCCCgATTAgTTCC 3' expected amplicon size on cDNA: 120bp	gi|18397470|ref|NP_564354.1| unknown protein [A. thaliana] gi|12322128|gb|AAG51102.1|AC025295_10 unknown protein [A. thaliana] gi|14334838|gb|AAK59597.1| unknown protein [A. thaliana]
Band no: FPIP3 GSP-PCR U_HW-Plate3h02.b1	FPIP-SN57 5' CATATCgAAATAggggATCTCTgC 3' FPIP-ASN187 5' CTgTTTCAAggCTggTTgCTTCTgg 3' expected amplicon size on cDNA: 156bp	gi|92897434|gb|ABE93662.1| SAM (and some other nucleotide) binding motif [Medicago truncatula] Expect = 2e-62 Identities = 119/132 (90%),
Band no: 1531 DDRT-PCR U_HW-Plate4f07.b1	1531-SN40 5' ggACAgAAAACTTgATAACCCA 3' 1531-ASN113 5' gTgCATAgCTTACTAAgAAggAAAgC 3' expected amplicon size on cDNA: 99bp	gi|30690915|ref|NP_174274.2| dihydroxy-acid dehydratase [A. thaliana] gi|42794937|gb|AAS45834.1| PCD/DCoH-like protein 2 [A. thaliana] Expect = 0.004 Identities = 21/26 (80%)
Band no: 1881 RGA-DDRT-PCR U_HW-Plate4g01.b1	1881-SN26 5' gAgggTCAAgCgTgCTgAggTATg 3' 1881-ASN161 5' TgAAATgAgggTCTggACAC 3' expected amplicon size on cDNA: 155bp	gi|18418385|ref|NP_568355.1| S-adenosyl-methionine-dependent methyltransferase/ methyltransferase [A. thaliana]
Band no:R13 RGA-DDRT-PCR	R13-SN28 5' TACTATgACAgATATACgTTTCATgg 3' R13-ASN162 5' gACTAAAgTATggCTgATAAATgTAC 3' expected amplicon size on cDNA: 157bp	gi|47027063|gb|AAT08746.1| glucose-6-phosphate/phosphate-translocator [Hyacinthus orientalis] Expect = 1e-14 Identities = 54/93 (58%),
Band no: 1901 (or 1772) RGA-DDRT-PCR U_HW-070116_Plate5c 07 U_HW-Plate3e02.b1	1901-SN105 5'CACgAAATgTCTTgTgCTCAAAgTgCAA 3' 1901-ASN262 5' AgTCACTgATTgTCTCACCAgTAC 3' expected amplicon size on cDNA: 183bp	gi|92868048|gb|ABE78148.1| Heat shock protein DnaJ [Medicago truncatula] gi|92876072|gb|ABE83817.1| Heat shock protein DnaJ [Medicago truncatula] Expect = 8e-75 Identities = 169/180 (93%),
Band no: 1611 DDRT-PCR U_HW-Plate3d05.b1,	1611-SN5 5' ACCCTCACTAAATggAgCTggC 3' 1611-SN79 5' TgAAAACTCCAgCTTTgTCAgC 3' 1611-ASN271 5' TCTCTAACACCCTTTTgTCAAgg 3' expected amplicon size on cDNA: 216bp expected amplicon size on cDNA: 290bp	gi|92884723|gb|ABE87465.1| PDZ/DHR/GLGF; Tetratricopeptide-like helical [Medicago truncatula] Expect = 3e-51 Identities = 108/145 (74%)
Band no: FDH GSP-RT-PCR U_HW-Plate3g12.b1	FDH-SN28 5' CgATAggAACTgTTggTgCTggAC 3' FDH-ASN194 5' ggggTTgATAACAATTACATCg 3' expected amplicon size on cDNA: 188bp	gi|38636526|emb|CAE12168.2| formate dehydrogenase [Quercus robur] Length=372 Expect = 2e-65 Identities = 119/134 (88%),

2.16.1 Cleaved Amplified Polymorphic Sequences (CAPS) Analysis

To generate polymorphism from the non-polimorphic primer sets, Cleaved Amplified Polymorphic Sequences (CAPS) method was applied. The aim of this method is to find out single base mutations, i.e, Single Nucleotide Polymorphism (SNP) in a given allele. For this purpose, the original sequence data from which primers were designed were analyzed to find out any restriction enzyme (RE) sites. The RE sites of the fragment in between forward and reverse primers of the target sequence were identified by free web based program WatCut (http://watcut.uwaterloo.ca/watcut/watcut/template.php).

Restriction Enzyme (RE) analysis: REs that have a single site in the target region were used for digestion of the PCR products giving single band. 15 µL mixture of RE digestion reaction consisted of: 1.5 µL 10X RE buffer (final conc. 1X), 0.15 µL 100X BSA (required for some REs; final conc. 1X), maximum 1 unit RE, PCR product and distilled water. Depending on incubation temperature, reaction lasted for 2 h to overnight and terminated at 80 ^{o}C for 10 min. Products were run in EtBr containing 1.5-2 % agarose gel.

Finding SNPs by sequencing: SNPs can be determined by sequencing of PCR amplicons obtained by a primer pair from different parents. For that reason, bands of same size produced from CRIL-7 parents were cut from agarose gel with a sterile blade. This agarose gel fragments were cleaned up by using Qiaquick Spin Gel Extraction kit. After checking on the agarose gel, 5 µL of cleaned product was treated with 2 µL ExoSAP-IT at 37^{o}C for 20 min and reaction was terminated at 80 ^{o}C for 15 min. After that, 4 µL sequencing master mix and 1 µL forward primer (10 pmol/µL; final conc.: 0.83 pmol/µL) was added. The sequencing reaction (sensitive to light) was performed as 96 ^{o}C for 5 min, 25 cycles of 96 ^{o}C (10 sec)-Ta (15 sec)-60 ^{o}C (4 min). The PCR product was cleaned by sephadex column (Edge Biosystems) by following manufacturer's manual. Cleaned products in collection tubes were vacuum dried at least 30 min at 60 ^{o}C and then stored in dark. The product was analyzed in Fulmer Sequencing Laboratory of the Washington State University. SNPs in sequence were determined by a web based program dCaps Finder 2.0.

Screening of CRIL-7 population for polymorphism: Polymorphic banding pattern of CRIL-7 population was determined by PCR with using primers either showed a polimorphic pattern or became polimophic after digestion at SNP point with the corresponding RE. After screening, products (PCR or digested PCR products) were run in EtBr containing 2%

agarose gel. Scoring was done as giving “A” for FLIP84-92C(3) phenotype (resistant), “B” for PI 599072 (susceptible) and “AB” for heterozygous phenotype. JoinMap mapping program was used to analyze genetic linkage within marker data collected in WSU-ARS Grain Legume Genetics and Physiology Laboratory.

2.17 Real-Time qRT-PCR analysis

Quantifying gene expression through reverse transcription followed by quantitative PCR is the basis of Real-Time PCR technology. In this study Real Time qRT-PCR analysis was performed. One of the methods used in this area is real-time quantitation using SYBR® Green I dye, which become fluorescent when bound to any double-stranded DNA. The fluorescence response is measured in each cycle of PCR so that the linear increase in the amount of PCR product can be monitored (Stratagene Instruction Manual of Brilliant® SYBR® Green QPCR Master Mix cat. #600548, #929548). In this study, SYBR® Green I dye method was used to analyze differentially expressed ESTs obtained in RGA-RT-PCR, RGA-DDRT-PCR, DDRT-PCR and GSP-RT-PCR experiments with some of the primers shown in Table 2.23. Total RNA isolated according to TRIzol method shown in Table 2.5.

DNAse Treatment and LiCl precipitation: To get rid of genomic DNA contamination which hinders Real-Time qRT-PCR analysis, total RNA samples were treated with Turbo DNAse of Ambion Company. LiCl precipitation was done with the commercial LiCl solution of Ambion Company (7.5 M LiCl, 50 mM EDTA, pH 8.0). The combined procedure of DNAse treatment and LiCl precipitation of 10 μg total RNA is shown in Table 2.24. After DNAse treatment and LiCl precipitation, concentrations of total RNA samples were checked by UV spectrophotometer and equalized before running on agarose gels. The integrity of total RNA was checked in EtBr containing 1% agarose gels prepared in 1X phosphate buffer.

cDNA Synthesis: DNAse treated and LiCl precipitated total RNA was used for cDNA synthesis using Superscript III (Invitrogen). 2 μg total RNA was used as template (Table 2.25). cDNA was diluted to ½ and this dilution was used as the stock cDNA for Real-Time qRT-PCR experiments. RNAseH treatment (Invitrogen, 5000 U/μL), which was done only for bulked total RNA samples (1 μL of RNAseH, final con. 230 U/μL), was added into cDNA after synthesis and incubated at 37 ^{o}C for 20 min).

Primers of Reference Genes for Normalization and Real-Time qRT-PCR Reaction Conditions: Primers (Table 2.26) were designed for actin gene and 18S ribosomal RNA for normalization of Real-Time qRT-PCR data. Two types of equipment were used for Real-Time qRT-PCR experiments. The reaction conditions followed in Roche Light®Cycler and in Stratagene MX4000 equipment are shown in Table 2.27. Optimization of annealing temperature tests were carried out for each primer pair. At the end of the PCR reaction, a dissociation curve cycle was added to evaluate product quality. PCRs were carried out as duplicates; except some cases for Stratagene MX4000 equipment.

Analysis of Real-Time qRT-PCR Data: Analysis of Real-Time qRT-PCR data was performed according to $2^{-\Delta\Delta C_T}$ methods as explained in Livak and Schmittgen (2001). In this literature, the threshold cycle (C_T) is defined as "*the fractional cycle number at which the amount of amplified target reaches a fixed threshold"*. $2^{-\Delta\Delta C_T}$ method is a relative quantification method, to find out the relative expression level of a transcript in a treated group (sample) with respect to an untreated control (calibrator) group. This relative quantity is expressed as a fold decrease or increase as compared to control group. To get rid of experimental variations in the amount of message of sample and calibrator, a housekeeping gene expression is used to normalize data as an endogenous reference. The formulas for relative expression by $2^{-\Delta\Delta C_T}$:

$$\Delta\Delta C_T = (C_{T\text{sample}} - C_{T\text{sample-ref.}}) - (C_{T\text{calibrator}} - C_{T\text{calibrator-ref.}}) \quad [1]$$

$$\text{fold change} = 2^{-\Delta\Delta C_T} \quad [2]$$

where;

$C_{T\text{sample}}$ = C_T value of treated group for a given transcript;
$C_{T\text{sample-ref}}$ = C_T value of treated group for a reference gene;
$C_{T\text{calibrator}}$ = C_T value of untreated group for a given transcript;
$C_{T\text{calibrator-ref}}$ = C_T value of untreated group for a reference gene.

Table 2.24 DNAse and LiCl treatments of total RNA samples for Real-Time qRT-PCR.

STEP	TREATMENT
1	Add following components into 200 µL reaction tube: Total RNA (10 µg); 5 µL 10X reaction buffer; 2 µL Turbo DNAse (Ambion, 2 U/µL); Nuclease free water to complete 50 µL. FINAL VOLUME: 50 µL.
2	Incubate at 37 ^{o}C for 30 min.
3	Add 10 µL DNAse inactivation reagent (Ambion).
4	Incubate at room temperature for 2 min.
5	Centrifuge at 10Xg for 1.5 min.
6	Take supernatant into a sterile 1.5 mL eppendorf tube .
7	Add ice cold 7.5 M LiCl (1 volume LiCl into 2 volume total RNA solution; final LiCl concentration is 2.5 M).
8	Incubate at least 30 min at -20 ^{o}C (no more then 45 min).
9	Centrifuge at top speed at 4 ^{o}C for 40 min.
10	Discard supernatant (pellet is invisible).
11	Add 1 mL ice cold 70 % (v/v) ethanol.
12	Centrifuge at top speed at 4 ^{o}C for 40 min.
13	Discard supernatant (pellet is invisible).
14	Dry pellet until all ethanol evaporates.
15	Dissolve pellet in DEPC treated molecular biology grade distilled water (total volume no more then 20 µL).

Table 2.25 cDNA synthesis from DNAse/LiCl treated total RNA for Real-Time qRT-PCR.

STEP	TREATMENT
1	Add following components into 200 µL reaction tube: 2 µg DNAse treated, LiCl precipitated total RNA; 1 µL Oligo dT_{20} (Invitrogen); 1µL dNTP (10mM; final conc. 0.5 mM); Molecular grade water to complete 13 µL.
2	Incubate at 65 ^{o}C for 5 min.
3	Brief spin to collect any drops.
4	Incubate on ice for 2-3 min.
5	Add following components: 4 µL 5X reaction buffer (final conc. 1X); 1 µL 0.1M DDT (final con. 0.005 M), 1µL Ribonuclease inhibitor (Invitrogen, RNaseOUT; 40 U/µL, final con. 2 U/µL); 1 µL Superscript III (Invitrogen, 200 U/µL, final con. 10 U/µL). FINAL VOLUME: 20µL.
6	Incubate at 50 ^{o}C for 90 min.
7	Incubate at 70 ^{o}C for 15 min.
8	Brief spin to collect any drops.
9	store cDNA at -80 ^{o}C or -20 ^{o}C until use.

Table 2.26 Primers for reference genes for Real-Time qRT-PCR experiments.

Gene	Sequence 5'-3'	Template gene and primer reference	Target Region (bp)
Actin	RT-actSN383 CCATCTAGTGGTTGAGGAACTTCCA RT-actASN467 GCCTTCATGCTCTTATCCCAAC	*C.arietinum* mRNA for actin, partial., Accession no: AJ012685	106
18S	cicer18sFrd CCTAGTAAGCGCGAGTCATCAGC cicer18sRev GAACACTTCACCGGACCATTCA	*C. arietinum* 18S ribosomal RNA, clone CapR18S. Accession no: AJ011011	99

Table 2.27 Reaction conditions of Real-Time qRT-PCR experiments

Roche Light®Cycler					
Reaction mix (Total Volume: 10µL)		**Final Concent.**	**Reaction conditions**		
Master SYBR Green I (1a+1b)	1 µL	-	95 ºC	600sec	1 cycle
MgCl2 (25 mM)	1.2 µL	3 mM	95 ºC	10 sec	
Forward primer (10 pmol/µL)	0.5 µL	0.5 pmol/µL	Ta	5 sec	40
reverse primer (10 pmol/µL)	0.5 µL	0.5 pmol/µL	72 ºC	10 sec	cycle
PCR-grade H_2O	6.3 µL	-	95 ºC	0 sec	
cDNA (1/2 diluted)	0.5 µL	-	65 ºC	15 sec	
Total volume	10 µL	-	98 ºC	Stepwise increase	
			40 ºC	30 sec	

Stratagene MX4000					
Reaction mix (Total Volume: 10µL)		**Final Concent.**	**Reaction conditions**		
SYBR Green QPCR Master Mix	10 µL	-	95ºC	10 min	1 cycle
Forward primer (10 pmol/µL)	1 µL	0.5 pmol/µL	95ºC	30 sec	
Reverse primer (10 pmol/µL)	1 µL	0.5 pmol/µL	Ta	30 sec	40
PCR-grade H_2O	6 µL	-	72ºC	30 sec	cycle
cDNA (1/8 diluted)	2 µL	-	95ºC	1 min	
Total volume	20 µL	-	55ºC	30 sec	
		-	95ºC	Stepwise increase	

2.18 BAC Library Hybridization

BAC library hybridization is a method to find out physical map position of a gene. For one of the ESTs of a candidate gene (similar to formate dehydrogenase; FDH) hybridization was performed on chickpea BAC library provided by WSU-USDA-ARS Grain Legume Genetics and Physiology Unit. Fdh-3 probe prepared from FLIP84-92C(3) by PCR as explained in Section 2.16.1 and sequence was confirmed by sequencing as in Section 2.16.2. Approximately 60 ng of Qiaquick Spin Gel Extraction kit cleaned probe was labeled with [α32P]-dNTP (by Radprime Labeling kit from Invitrogen using Klenow Fragment) and used for hybridization. The procedure of hybridization is provided in Table 2.28.

Table 2.28 BAC Library Hybridization Protocol. From WSU USDA-ARS Grain Legume Genetics And Physiology Laboratory, 24.01.2007.

Step	Procedure		
1	Prepare hybridization buffer:		
	Stock solution	Volume (mL)	Final concentration
	1 M sodium phosphate, pH 7.2*	1000	0.5 Msodium phosphate
	20 % (w/v) SDS	700	7 % (w/v) SDS
	10 % (w/v) BSA	200	1 % (w/v) BSA
	0.5 M EDTA, pH 8.0	4	1 mM EDTA
	H_2O	96	
	*1 M sodium phosphate: 720 mL 1 M Na_2HPO_4 + 280 mL 1 M NaH_2PO_4, pH to 7.2 with HCl or NaOH. Store at 4 °C. Heat to 65 °C before use.		
2	Denature 0.5 mL of 10 mg/mL solution of DNA per 25 mL hybridization buffer by heating at 100°C for 5-10 minute. Chill on ice and add to the hybridization buffer.		
3	Prehybridize the filters in an incubator at 65°C with gentle shaking for at least 2 h.		
4	Prepare the probe to be used and denature by heating at 100°C for 5-10 min. Add the denatured probe to the hybridization buffer		
5	Incubate at 65°C for at least 12 h.		
6	Wash the filters in 65°C 0.5 x SSC and 0.1% (w/v) SDS at 65°C with gentle shaking for 15-20 min. Wash the filters 3 times.		
7	Remove the filters, absorb excess wash buffer on paper towel, wrap wet in Saran wrap, and expose to film.		

CHAPTER 3

RESULTS AND DISCUSSION

As stated in Section 1.6, this study aimed to identify a wide variety of chickpea ESTs expressed in a tolerant chickpea line ILC195 upon infection with *A.rabiei* isolates of varying pathogenicity level by means of PCR amplification of RGAs, differential display (DD) analysis and PCR amplification with GSPs to construct a draft picture of chickpea genes probably involved in defense response. In this chapter results will be presented starting from experimental trials by providing discussion at the same time.

3.1 Preliminary Trials: RGA-DDRT-PCR Trials

Preliminary trials for setting up experimental procedures and tests related to finding any differentially expressed RGAs were collected under the name of RGA-DDRT-PCR trial experiments. Experimental material were uninfected and infected ILC195 plant samples infected with *A.rabiei* isolates (*ank6, çor1* and *ayaç5*) separately after 3 dpi (Table 2.1). Procedures were presented in Section 2.6. Figure 3.1 part (a) shows total RNA samples for this experiment. Firstly, all three trials were presented briefly:

First trial: Experimental procedure of this trial was explained in Section 2.6. paragraph (a). cDNA of infected samples was constructed separately and than bulked by evaluating actin band intensity (Figure 3.1.b). A hot PCR reaction with [α^{32}P]CTP was carried out by using S2 (forward) primer in combination with T primers (reverse). 35 bands differentially expressed in between infected and uninfected cDNA were selected and reamplified. Figure 3.1.c shows some of the successfully reamplified bands. Reamplified products were ligated in pTZ57R/T vector and transformed into *E.coli* DH5-α. After confirming the presence of inserts by M13 PCR (Figure 3.1.d), plasmid isolation was carried out and inserts were released by restriction enzyme (RE) digestion (*Pst*I and *Eco*RI) (Figure 3.1.e). 8 clones containing inserts were sequenced. Only two of them deviated from the original size

profile (R22 and R23). Although they were probably plant origin as understood from the BLAST, this may be resulted from factors stated in Section 3.6.

Second trial: Experimental procedure of this trial was explained in Section 2.6 paragraph (b). Infected cDNA sample was prepared after bulking infected total RNA samples. Hot PCR reaction with [α^{33}P]-dATP was carried out by using S2 or NLLRfwd (forward) X T primers (reverse). By comparison of infected and uninfected cDNA only 2 differential bands were selected, one of which (R50) could be reamplified, cloned in pGEM-T-Easy vector and transformed into *E.coli* DH5-α. After confirming the presence of insert by M13 PCR, R50 was sequenced by high-throughput sequencing.

Third trial: Experimental procedure of this trial was explained in Section 2.6 paragraph (c). Infected cDNA sample was prepared after bulking infected total RNA samples. After performing hot PCR reaction with α[^{33}P]-dATP by using Ptokin-1 or RLLRfwd primers in combination with T primers (reverse), 14 differentially expressed bands were selected. Four of them (R44, R46, R48, R49) could be reamplified, cloned in pGEM-T-Easy vector and transformed into *E.coli* DH5-α. After confirming the presence of insert by M13 PCR these four clones were sequenced by high-throughput sequencing.

As a result, trial experiments were not efficient to obtain high quality cDNA which resulted in insufficient number of clones. Additionally, conditions for cDNA synthesis were not adequately optimized very well. Only few ESTs were shown similarity to relatively important genes, i.e., R46 and R50. Biological role of these ESTs were discussed in Section 3.8 and 3.11 together with other ESTs. Sequence and related data were provided in Appendix D, “Molecular Function”, “Biological Role” and “Cell Component” data were provided in Appendix E and autoradiograph pictures were provided in Appendix F.

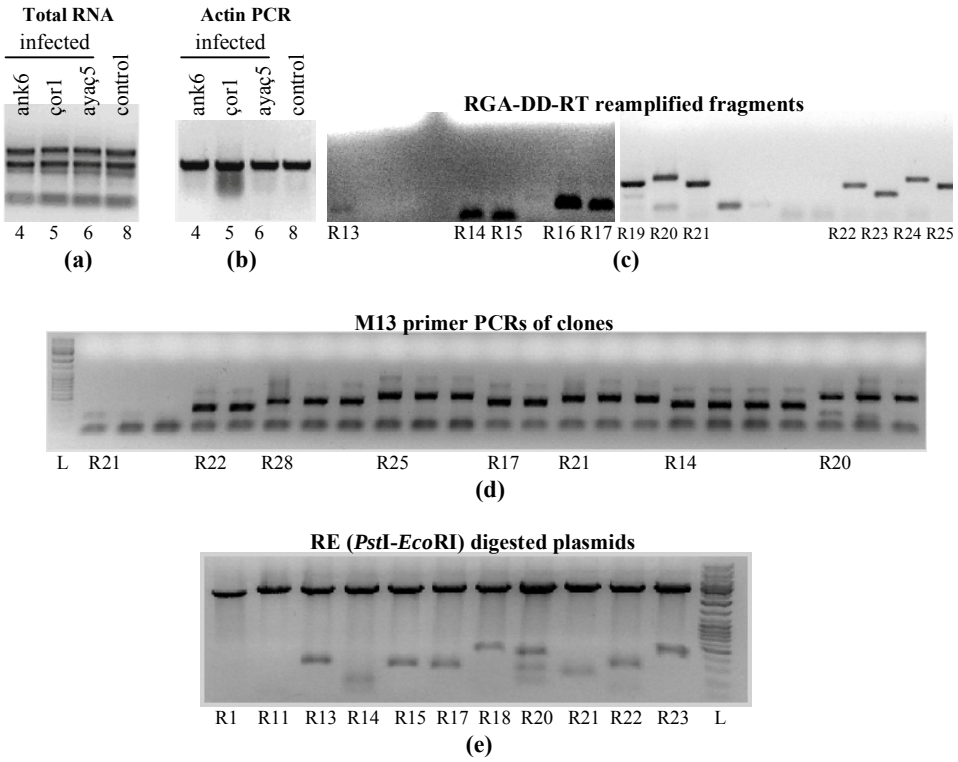

Figure 3.1 Samples and some gels of first trial. (a) Total RNA samples of 3rd day infected samples: 4 (*ank6*), 5 (*çor1*), 6 (*ayaç5*), 8 (uninfected), in 1% agarose gel in 1X phosphate buffer. (b) actin bands of cDNA samples of these total RNA samples. (c) several reamplified fragments. (d) colony M13-PCR of several reamplified fragments. (e) restriction enzyme cut plasmids pTZ57R/T having inserts. (b), (c), (d) and (e) are in 1% agarose gel in TBE buffer. L is 100 bp DNA ladder.

3.2 RGA-RT-PCR Analysis

This experiment was performed to identify a wide variety of chickpea expressed RGAs and ESTs which could be amplified with RGA primers in the tolerant chickpea line ILC195 upon infection with *A.rabiei* isolates of varying pathogenicity level. It is known that RGA primers may also amplify other important ESTs (Rajesh *et al.*, 2002). The experimental procedure was explained in various Sections (2.3, 2.4, 2.5 and 2.7). After isolation of total RNA from time-point plant samples, mRNA isolation was carried out after bulking of corresponding total RNA shown in Figure 3.2 (or Table 2.10). B1 was the bulk of total RNA from plants separately infected by isolates *ank6, çor1* and *ayaç5*; B2 was the bulk of total RNA from plants infected with isolate *elmalı*, and B3 was bulk of total RNA of uninfected plants. mRNA spots of these bulks were shown in Figure 3.2.c.

The reason why mRNA was preferred for cDNA construction is as follows: in the trial experiments it was observed that cDNA could not be constructed efficiently. Various conditions for cDNA construction were tried but none of them resulted in reproducible PCR products (trials with Superscript III and M-MuLV enzymes; several amounts of total RNA, enzyme and other constituents; changing of reaction conditions etc). This brought about the idea that there should be some impurities such as phenolic, carbohydrate and undetermined compounds which hinder the enzymatic reactions. According to literature, these compounds are known to inhibit reverse transcriptase reactions (Salzman *et al.*, 1999). Two ways for getting rid of these compounds are either using of higher amount of TRIzol during total RNA isolation or isolation of mRNA with magnetic beads. Since total RNAs were already isolated, the second solution; i.e., mRNA isolation by Dynal Biotech-Dynalbeads was applied. In addition to this, mRNA isolation also helps to get rid of contaminating DNA. After this step, cDNA construction was carried out and actin amplification and further PCR reactions were successful. Actin bands from cDNA samples at different dilutions to test the integrity and equality of cDNA were shown in Figure 3.2.d.

Hot PCR reaction with [α^{33}P]-dATP was carried out using 110 combinations of forward and reverse primers (Table 3.1). Since only a few R genes have been disclosed as increased expression after infection, in this part of the study, the aim was stated as finding a large set of chickpea expressed RGAs and related ESTs. An additional purpose was to find out any differential expression between infected plant samples, i.e. B1 bulk of RNA of plant samples separately infected with isolates *ank6, çor1* and *ayaç5* and B2 bulk of plants

infected with the isolate *elmalı*. Since there was no control plants at all sampling hours, comparison of expression between infected and uninfected plants was not considered. For these reasons, the product bands were cut as in the following:

i) Product bands that exist in all sample groups, i.e. in B1, B2 and B3: finding a large set of chickpea expressed RGAs and related ESTs.

ii) Product bands that differentially expressed between B1 and B2: finding out any differential expression between two bulks, one of which represents the response to aggressive isolates and the other to mild isolate infection.

About 750 original size band sets were cut from the developed denaturating PAGE gels. Since large DNA sequences are more informative, reamplification of comparatively large bands was carried out primarily. Among about 300 product band, approximately 200 could be successfully reamplified. Each reamplified product band was cloned in pGEM-T-Easy vector separately and transformed into *E.coli* DH5-α. The ligation products, whose transformation was unsuccessful, were bulked as two-three or four together and transformed again. After checking the presence of the insert by M13 PCR, these clones were sequenced by high-throughput sequencing. Biological role of these ESTs were discussed in Section 3.11 together with other ESTs. Sequence and related data were provided in Appendix D, "Molecular Function", "Biological Role" and "Cell Component" data were provided in Appendix E and autoradiograph pictures of differentially expressed bands in between B1 and B2 were provided in Appendix F.

Table 3.1 Primer combinations used in RGA-RT-PCR experiment.

Forward	Reverse
S2	AS1, AS2, AS3, AS4, AS5, AS6, AS7, AS8, T1, T2, T3, T4, T5, T6, T7, T8, T9, NBSrev, Cicerkin2-rev
NLRR fwd	RLRR rev, AS1, AS2, AS3, AS4, AS5, AS6, AS7, AS8, T1, T2, T3, T4, T5, T6, T7, T8, T9, NBSrev, Cicerkin2-rev
RLRR fwd	NLRR rev, AS1, AS2, AS3, AS4, AS5, AS6, AS7, AS8, T1, T2, T3, T4, T5, T6, T7, T8, T9, NBSrev, Cicerkin2-rev
Ptokin-1	Ptokin-2, T1, T2, T3, T4, T5, T6, T7, T8, T9
PtoFen-S	PtoFen-AS, T1, T2, T3, T4, T5, T6, T7, T8, T9
Cicerkin2-fwd	Cicerkin2-rev, AS1, AS2, AS3, AS4, AS5, AS6, AS7, AS8, T1, T2, T3, T4, T5, T6, T7, T8, T9, NBSrev, NLLR rev, RLLR rev
WipK-1	WipK-2 , T1, T2, T3, T4, T5, T6, T7, T8, T9

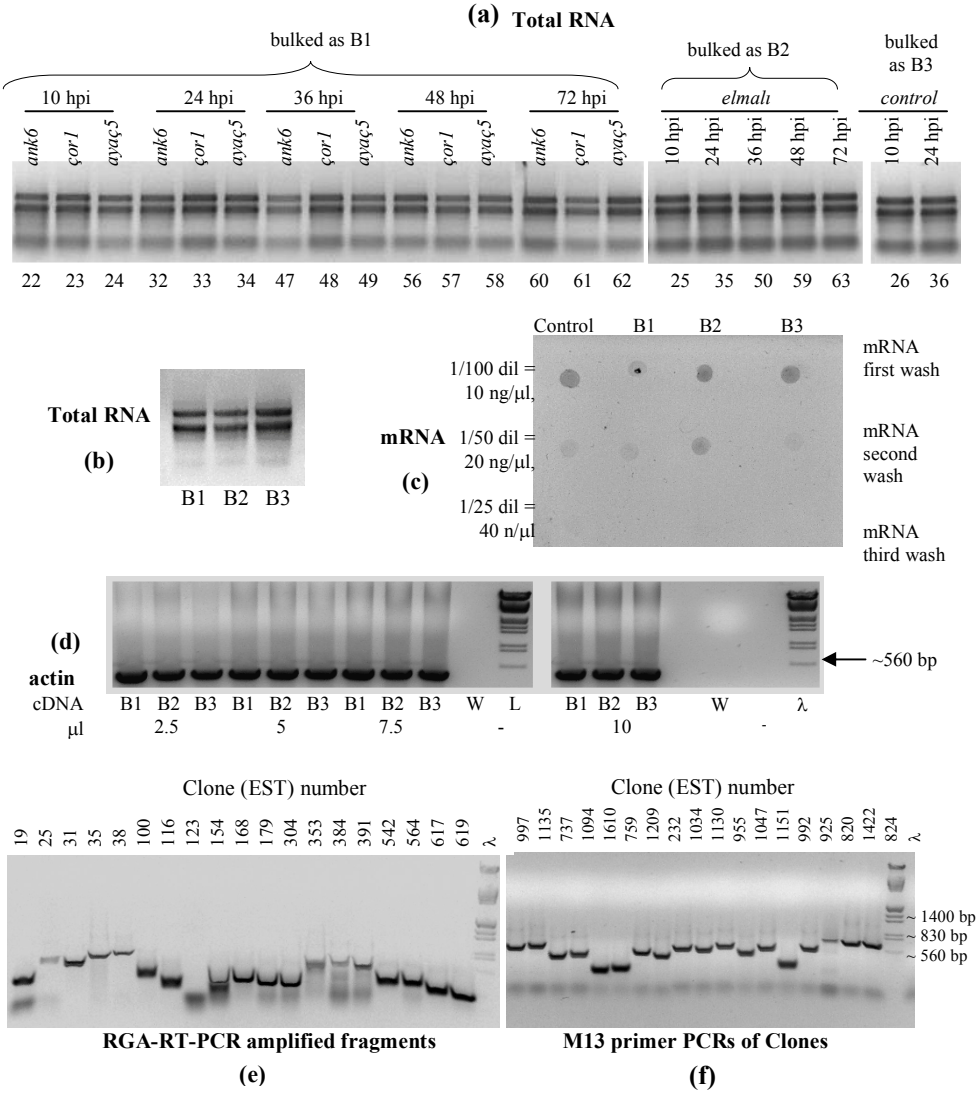

Figure 3.2 Samples used in RGA-RT-PCR experiment and some reamplification and M13 primer PCRs. (a) Total RNA of all plant samples given in Table 2.10. (b) Total RNA bulks B1, B2, and B3. (c) mRNA spots of B1, B2, B3 and standart RNA (human skeletal muscle, Clonetech, 2002). (d) actin bands from cDNA (1/5 diluted) at different µL as template. W= PCR grade water. (e) An example of gel showing reamplified products (f), and an example of gel showing M13 primer PCR confirmation. λ, λ - EcoRI-HindIII DNA Ladder. 1% agarose gel in 1X phosphate buffer for (a), (b), (c) and in TBE for (e) and (f). Numbers indicate arbitrary names of RGA-RT-PCR products.

3.3 DDRT-PCR Analysis

mRNA Differential Display (DD) allows detection of altered gene expression (Liang and Pardee, 1992). DDRT-PCR was applied in the literature for a different chickpea (resistant and susceptible)-*A.rabiei* couple and with a different approach for bulking of samples (Rajesh, 2001). Under this topic, the analysis was done to identify differentially expressed gene sequences of ILC195 upon various *A. rabiei* isolates (*ank6, cor1, ayac5*) infection in a time-based manner. Total RNA from plant groups shown in Figure 3.3 were used to construct experimental bulks. These bulks were used to isolate mRNA for cDNA construction. Experimental samples which were compared in DDRT-PCR were as follows:

i) Infected sample, H1, was cDNA constructed from mRNA from bulked total RNA of the infected plants (with *ank6*, *çor1* and *ayaç5*) collected at 10 hpi of the second infection experiment. Its control sample, C1, was cDNA constructed from mRNA of uninfected plants taken at 10 hpi of the same experiment.
ii) Infected sample, H2, was cDNA constructed from mRNA from bulked total RNA of infected plants (with *ank6*, *çor1* and *ayaç5*) collected at 24 hpi of the second infection experiment. Its control sample, C2, was cDNA constructed from mRNA of uninfected plants taken at 24 hpi of the same experiment.
iii) Infected sample, H3, was cDNA constructed from mRNA from bulked total RNA of the infected plants (with *ank6*, *çor1* and *ayaç5*) collected at 3 dpi of the first infection experiment. Its control sample, C3, was cDNA constructed from mRNA of uninfected plants taken at 3 dpi of the same experiment.

mRNA isolation was carried out after bulking of total RNA samples of corresponding plant samples (Figure 3.3) and then cDNA was constructed. For any differential expression analysis, equal cDNA amount is very important. After testing the integrity and equality of cDNA samples by amplifying of actin gene from cDNA at different dilutions (1/10, 1/20, 1/25, 1/40), hot PCR reaction with [α^{32}P]-dCTP was carried out by performing all P (from P1-P10) primers in combination with T reverse primers (from T1 to T9). All the 90 primer combinations were performed and differentially expressed bands were cut as:

i) increased gene expression upon infection: bands existing only or expressed higher than that of uninfected sample at least in two of the infected samples (H1, H2, H3).
ii) decreased/silenced gene expression upon infection: band existing only or expressed higher than infected group at least in two of the uninfected samples (C1, C2, C3).

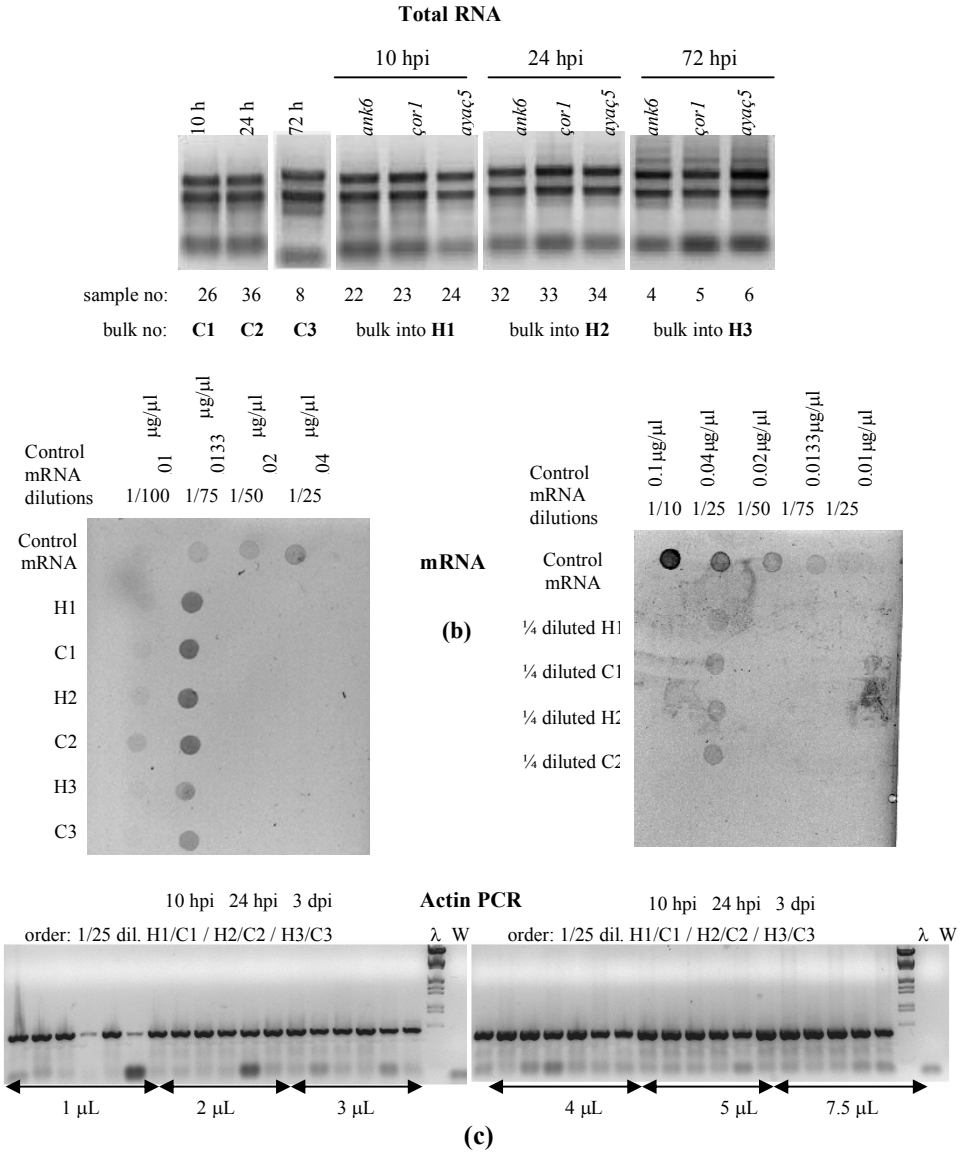

Figure 3.3 Samples of DDRT-PCR analysis. (a) total RNA samples; (b) mRNA spots of infected samples (H1, H2, H3), uninfected samples (C1, C2, C3) and control RNA (human skeletal muscle, Clonetech, 2002); (c) Examples of actin bands from 1/20 or 1/25 diluted cDNA used at different µL amounts as template. W= PCR grade water, λ = DNA ladder, λ-*Eco*RI-*Hind*III. (a) and (b) are 1% agarose gel in 1X phosphate buffer. (c) is 1% agarose EtBr gel in TBE buffer.

Totally about 100 original sets of differentially bands were cut out of the gel. About 60 product bands were successfully reamplified. Afterwards, same procedure was followed as explained for RGA-RT-PCR experiment as indicated in Section 3.2. Biological role of these ESTs were discussed in Section 3.11 together with other ESTs. Sequence and related data were provided in Appendix D, "Molecular Function", "Biological Role" and "Cell Component" data were provided in Appendix E and autoradiograph pictures were provided in Appendix F.

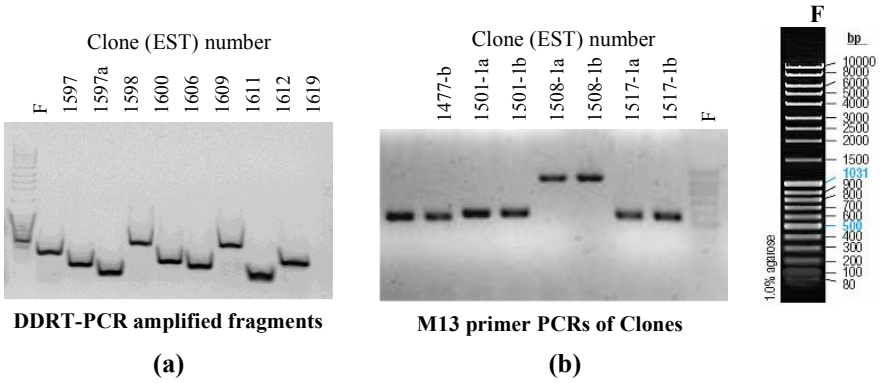

Figure 3.4 Reamplification and M13 primer PCR of DDRT-PCR analysis. (a) An example of gel showing reamplified DDRT-PCR fragments (b), and an example of gel showing M13-PCR confirmation of some product bands. F, 'DNA Massruler Fermentas SM 0403'. 1% agarose EtBr gel in TBE. Numbers indicate arbitrary numbered DDRT-PCR products. The data of succesfully sequenced ESTs and autoradiograph pictures of them were provided in Appendix D and F, respectively.

3.4 RGA-DDRT-PCR Analysis

R genes usually do not show differential expression upon infection. However, a few have been disclosed to be induced after infection. One known example is rice *Xa1* gene (Yoshimura *et al.* 1998). Some other authors (Rajesh, 2001) also used DD approach with RGA primers on chickpea infected with Ascochyta blight. In addition, RGA primers were shown to amplify other gene fragments which may be important for defense such as the ankryin repeat isolated by Rajesh *et al.* (2002). To test whether or not there are differentially expressed RGAs or some more genes upon infection in chickpea, RGA-RT-PCR reaction was repeated by using primers successfully amplified fragments and this experiment was called as RGA-DDRT-PCR. For that reason, total RNA of infected and uninfected ILC195 samples of 3 time points were used as bulk. Experimental groups were prepared as follows:

i) Infected bulk of various samples, I, was cDNA constructed from mRNA from bulked total RNA of infected (*ank6*, *çor1* and *ayaç5*) plants at 10 and 24 hpi (second infection experiment) and 3 dpi (first infection' experiment). Its control sample, u, was constructed from mRNA from bulked total RNA of uninfected plant samples taken at 10 and 24 hpi (second infection experiment) and 3 dpi (first infection experiment).

ii) Infected bulk, H3, and uninfected sample, C3, were explained before in DDRT-PCR experiment Section 3.3 paragraph (iii). Thus, a second replicate was provided to minimize false positives.

After construction of cDNA from these mRNAs, the integrity and equality of cDNA samples were tested by carrying out PCR amplifying of actin gene at different dilutions (1/5 to 1/40) (Figure 3.5.b). Hot PCR reaction with [α^{32}P]-dCTP was carried out by using 78 primer pairs which was observed to be productive in RGA-RT-PCR reaction. Bands were cut from the denaturating PAGE gel as follows:

i) increased expression of RGAs upon infection: band that exists only or expressed higher in infected samples than uninfected samples; (i.e.; in H3 and I);

ii) decreased/silenced expression of RGAs upon infection: band that exists only or expressed higher in uninfected samples than infected samples (i.e.; in C3 and u).

iii) constitutively expressed RGAs: bands that exist in all samples (H3, C3, I, u).

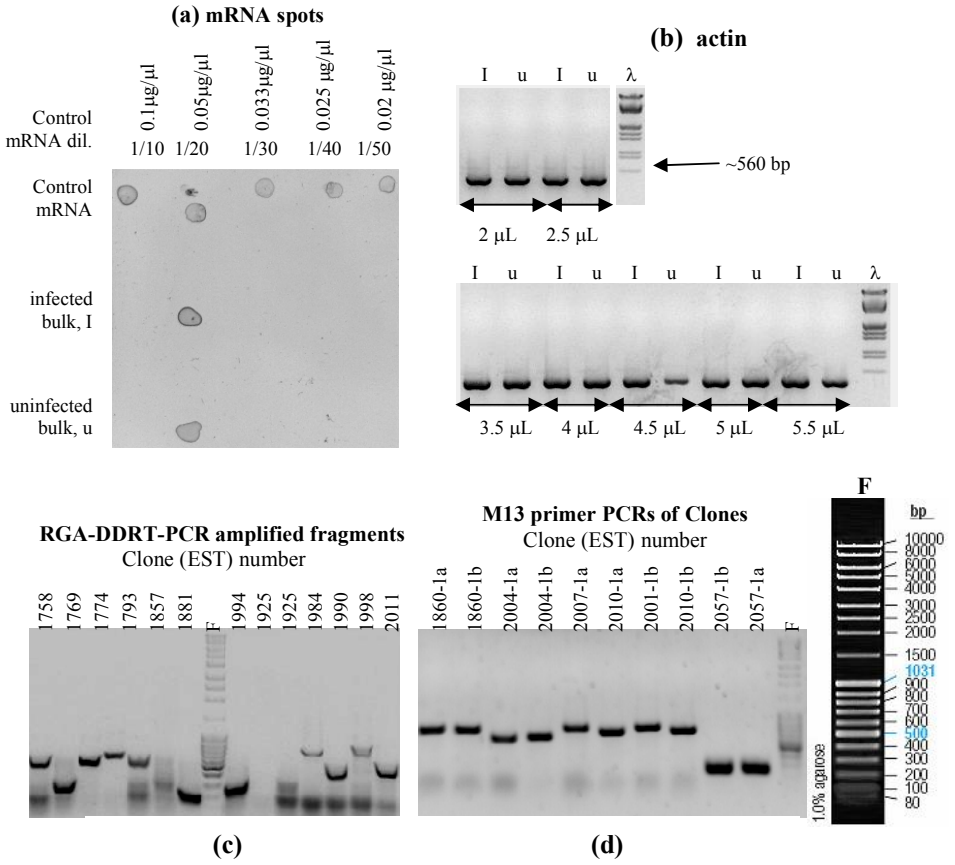

Figure 3.5 RGA-DDRT-PCR samples and some products. (a) mRNA spots of infected samples (I), uninfected samples (u) and control RNA (human skeletal muscle, Clonetech, 2002). 1% agarose EtBr gel in 1X phosphate buffer. (b) actin PCR bands of 1/40 diluted cDNA and different µL of cDNA was taken as template. 1% agarose EtBr gel in TBE buffer. W= PCR grade water. λ, λ-EcoRI-HindIII DNA ladder. (c) An example of gel showing reamplified products (d) An example of gel showing M13 primer PCR of some products bands. F, 'DNA Massruler Fermentas SM 0403'. (b), (c) and (d) are 1% agarose gel in TBE. Numbers indicate arbitrary numbered RGA-DDRT-PCR products. The data of successfully sequenced ESTs and autoradiograph pictures of differentially expressed ones were provided in Appendix D and F, respectively.

Totally about 170 original sets of bands were cut out of the gel, about 70 of them differentially expressed. Approximately, 80 were reamplified. Afterwards same procedure was followed as explained for RGA-RT-PCR experiment, in Section 3.2. Biological role of these ESTs were discussed in Section 3.11 together with other ESTs. Sequence and related data, were provided in Appendix D, "Molecular Function", "Biological Role" and "Cell Component" data were provided in Appendix E and autoradiograph pictures of differentially expressed bands were provided in Appendix F.

3.5 PCR Amplification of Disease Related Gene Copies with Gene Specific Primers

To amplify disease related gene copies in chickpea, gene specific primers (GSPs) were used with cDNA bulks. These experiments were called as GSP-RT-PCR shortly. Firstly, traditional PCR approach was applied. Secondly for the GSPs which could not amplify appropriate product by traditional PCR, Touch-Down PCR method was performed.

Clones obtained from PCR: For amplifying disease related gene copies in chickpea, several annealing temperatures were applied. PCRs were first applied to GSPs anonymous in Prof.Dr. Akkaya laboratory: SGT1 (Azevedo *et al.*, 2002), EDS1 (Parker *et al.*, 1996), NPR1 (Cao *et al.*, 1997) and finally NPR1 (Century *et al.*, 1995), which were described briefly in the Introduction, were used to amplify by using infected cDNA from 23rd day plants, or B1 or B2 bulks as template. Most of these primers were designed on the gene copies from *Arabidopsis*. However, amplification of expected fragments was not achieved. This may be probably due to low specificity of these primers on chickpea cDNA. The fragments obtained were shown in Figure 3.6. In addition to these anonymous GSPs, other primers were designed by considering some of the genes used by Pfaff and Kahl (2003) for mapping which was presented in the Introduction. For this case, some of the proposed genes in this publication were selected. These genes are fungal pathogen-induced protein (FPIP), hypersensitive response protein (HRP), kinase-associated protein phosphatase (KAPP), formate dehydrogenase (FDH) and pectin esterase (PE). Due to the lack of corresponding homolog sequences in chickpea, primers were designed for the selected genes from available sequences of closely related genera of chickpea by considering conserved regions (Table 2.16). Various annealing temperatures and cycle numbers were applied to obtain these fragments by PCR. However, some GSPs did not amplify any products. Fragments obtained were shown in Figure 3.6.

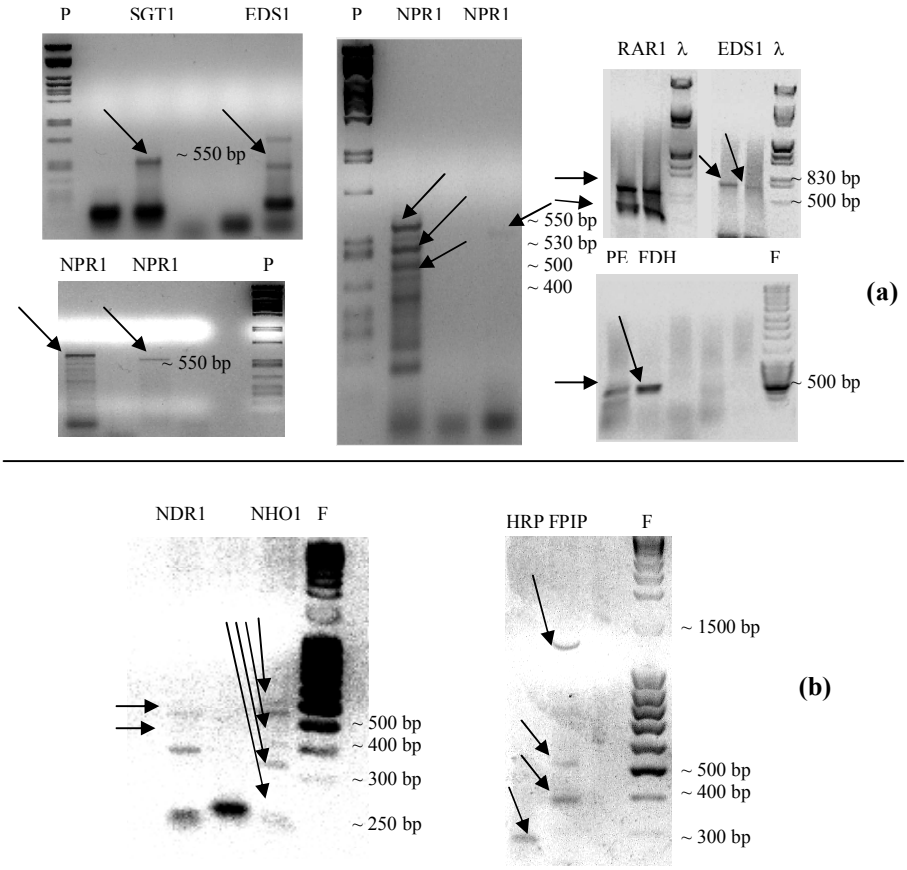

Figure 3.6 GSP-RT-PCR amplifications. (a) Traditional PCR amplification with following Tm : SGT1 (50 ^{o}C), EDS1 (50 ^{o}C), NPR1 (50 ^{o}C), RAR1 (42 ^{o}C), EDS1 (43^{o}C), PE (45 ^{o}C), FDH (42 ^{o}C). 1-2% agarose gel in TBE. (b) Some GSP amplifications by 'Touch-down' PCR with following Tm: NDR1 (from 50 ^{o}C to 42 ^{o}C), NHO1 (from 50^{o}C to 42 ^{o}C), HRP (from 52 ^{o}C to 42 ^{o}C), FPIP (52 ^{o}C to 42 ^{o}C). 2 % agarose gel in TBE. Products run on 2 % agarose gel in TBE. P, Pst1 DNA ladder ; λ, λ DNA EcoRI-HindIII ladder; F, Fermentas DNA ladder SM0403. Amplified fragment sizes are: SGT1 ~ 550 bp; EDS1 ~ 550, 800 bp; NPR1 ~ 550, 530, 500, 400 bp; RAR1 ~ 600 bp, ~ 900; NDR1 ~ 500, 300 bp; NHO1 ~ 500, 450, 300, 250 bp; HRP ~ 300 bp; FPIP ~ 1200, 550, 400 bp; PE ~ 400, 500 bp; FDH ~500 bp.

Clones obtained from Touch-Down PCR: For some of the GSPs, either no product or a smear of products were obtained. This could have resulted in cloning of unrelated DNA fragments due to low specificity. For that reason, Touch-Down PCR was applied, so that more specific products in high concentration could be obtained. Figure 3.6.b shows the fragments obtained from Touch-Down PCR.

Cloning and sequencing of GSP products: Product fragments obtained from all GSPs were cloned in pGEM-T-Easy vector and transformed into *E.coli* DH5-α. These clones were sequenced by high-throughput sequencing with other clones. Biological role of these ESTs were discussed in Section 3.11 together with other ESTs. Sequence and related data, were provided in Appendix D, "Molecular Function", "Biological Role" and "Cell Component" data were provided in Appendix E.

As a result, using of GSPs for amplifying disease related gene copies in chickpea was successful for some of the genes; however for KAPP, no product was obtained. For most of them fragment sizes were different from the expected sizes in the original organism. Both cases are not unusual; for example Pfaff and Kahl (2003) obtained no amplification product for some of the GSPs in chickpea; in addition, some of the GSPs amplified varying number of fragments in different sizes (Pfaff and Kahl, 2003).

The ESTs obtained in this study are as follows: SGT1 fragments could not be cloned. For FDH and PE, the sequenced clones yielded the expected gene fragments (discussed in Section 3.8 and 3.11 respectively). However, GSPs for EDS1, FPIP, RAR1 and HRP yielded unrelated but important gene fragments. Sequences of clones for FDH, PE, EDS1, RAR1 and HRP were provided in Appendix D. In this study, an EST showing a low similarity to a putative receptor protein kinase of *A. thaliana* was obtained from GSP experiment by using HRP primers. These primers were designed from sequences of HR-induced proteins of Arabidopsis (17065547) and Lotus (57834177) which are irrelevant to receptor protein kinases (Section 3.11). On the other hand, GSPs for EDS1 (Lipase-like protein, Falk *et al.*, 1999) yielded two different ESTs, namely, one (Eds1-1) related to actin related 3 (ARP3)/actin-like protein; the other one (Eds1-4) yielded a FKBP type peptidyl prolyl isomerase (Section 3.11). From GSPs for FPIP, an EST similar to SLL2-S9-protein which has methyltransferase activity (discussed in Section 3.7 and 3.8) was obtained. For RAR1, one of the ESTs obtained (Rar1-1) has homology to mitochondrial VDAC was obtained. As a result; except two primer sets, most of the GSPs yielded different products

than expected ones. This may be resulted from i) low similarity of the sequences in Arabidopsis and barley to that of chickpea and ii) not specific definition of genes, such as fungal-induced or HR-response that addressed variable genes. For NPR1, NHO1 and NDR1, no sequence information could be obtained as explained in Section 3.6.

3.6 Problems Related to Reamplification, Cloning, Sequencing and DDRT-PCR

In general, since the steps of RGA-RT-PCR, DDRT-PCR and RGA-DDRT-PCR are similar, same problems were noticed during reamplification, cloning and sequencing. Despite its usefulness for systematic isolation of new transcripts differentially regulated upon any treatment, there are some drawbacks of DDRT-PCR. Under this topic, problems observed in reamplification, cloning, sequencing and differential display were discussed.

Reamplification problems: Reamplification of fragments from PAGE gels is not easy to clone. Reamplification was not successful for some of the product bands cut out of the PAGE gel. Because of the low yield of the reamplification of DDRT-PCR bands, several conditions were changed to reamplify bands cut from the PAGE gel: increasing/decreasing template concentration, increasing PCR cycle numbers, increasing primer concentrations etc. Also direct ligation from PAGE gel bands was tried, but no clones were obtained. The best results were obtained by a combination of increased incubation temperature during the elution of the product band cut from the PAGE gel, avoiding of using large amounts of template in the PCR for reamplification and using fresh primers. Several authors also indicated reamplification problems in DDRT-PCR and low yield of reamplification products (Domachowske and Malech, 1997; Sanabria and Dubery, 2004; Bonnet *et al.*, 1998). These literature indicated that besides unknown reasons, inhibitors may exist (such as urea) in PAGE that may hinder *Taq* DNA polymerase during reamplification by PCR.

Cloning and Sequencing: Despite the successful reamplification, cloning may not be successful. Since T/A cloning was used in this study the blunt-ended (Sanabria and Dubery, 2004) RGA-RT-PCR, DDRT-PCR, RGA-DDRT-PCR and GSP-RT-PCR fragments should be processed to gain A-tail. For that reason, the elongation time of the reamplification PCR increased up to 30 min to let *Taq* DNA polymerase add dATPs to the PCR product. It is also reported that UV radiation during cutting the reamplified fragment from the agarose gel may degrade DNA (Sanabria and Dubery, 2004). For that reason, prolonged exposure was avoided during cutting of reamplified bands from the agarose gel.

In the ligation reaction, the amount of template (the liquified agarose gel fragment) was kept to be as much as possible. In some cases, clones not exactly white but grayish in color were obtained. Most of the time, such colonies were not recombinant. This problem was explained by Sanabria and Dubery (2004): cells that grow close to each other exhaust X-gal and ampicilin. Thus, non-recombinants may grow attached to recombinant ones. They also pointed out that these colonies could be re-plated together. For that reason, such colonies were streak plated to overcome this problem.

In this study, M13-PCR confirmed clones obtained from all experiments (RGA-RT-PCR, DDRT-PCR, RGA-DDRT-PCR and GSP-RT-PCR) were stored in -80 ^{o}C as sterile glycerol stocks until enough number of them were collected for high-throughput sequencing. Then these clones were transferred into fresh LB-ampicilin media, grown overnight (37 ^{o}C), equal amount of 50 % (v/v) glycerol was added and loaded into 96 well-plates (Plate 1, Plate 2, Plate 3 and Plate 4). These plates were sent in dry ice to Genome Sequencing Center of Washington University of Medicine for forward (only from 5' site) sequencing. This sequence data will be called as "first run of sequencing" here after. First run of sequencing data were evaluated and it was observed that most of the sequencing reaction were failed: among 336 clones, 41 of them were read fully from 5' to 3', 113 of them had only 5' site and remaining were either not sequenced or non-recombinant (pGEMT-easy self ligated) (Table 3.2). Some of the above mentioned reasons might lead to this result: i) some non-recombinant cells might outgrew due to regrowing of clones for loading into 96 well-plates prepared for sequencing; ii) cross-contamination between wells might have happened during loading, shipping and handling of 96 well-plates; iii) pGEMT-easy self ligated vectors or primer dimers might be misleading during M13-PCR confirmation; iv) other problems related with sequencing facility. Despite this situation, data of fully and partially sequenced clones were analyzed. ESTs which might have role in defense primers were used to design primers for use in Real-Time qRT-PCR and genetic linkage analysis. All these primers were designed according to rules for Real-Time qRT-PCR primer design so that primers could be used for both Real-Time qRT-PCR and genetic linkage analysis: target size was about 100 bp to 200 bp, no more than three Gs or Cs successively, high melting temperatures, no big difference in melting temperatures between forward and reverse primer, high G/C content and less or no self or 3' end complementarily (RRC Core Genomics Facility, 2003). Table 2.23 in Section 2.16 shows these primers, related clones and short BLASTX results. These are named as "EST specific primers".

The clones whose sequence could not be obtained in the first run of sequencing were streak-plated and two single white colonies were selected for each. M13-PCR re-confirmation and inoculation of glycerol-stock culture were carried out for these two colonies. To avoid transferring of glycerol stocks into fresh LB media which may result in outgrowing of non-recombinant cells, the newly prepared glycerol-stock cultures of these single colonies were directly loaded into 96 well-plates. Another replicate of these were prepared by growing clones overnight in LB-amplicin containing 96 well-plate inoculated from re-confirmed glycerol-stock cultures. So, one set of 96 well-plate containing re-confirmed glycerol-stock cultures (Plate 5 and Plate 6), another replicate of this set containing LB-ampicilin grown overnight cultures were shipped for sequencing. 96 well-plate containing glycerol-stock cultures were sequenced from forward side; their replicates (LB-amplicin grown overnight cultures) were sequenced from both sides (forward and reverse). Sequence data obtained from the same clones were compared to each other (between two replicates of Plate 5 and 6 and with previously sequenced plates Plate 1 to 4) in terms of quality of SCF chromatogram. For a given clone which had several sequence readings, data having best SCF chromatogram was selected and analyzed. Sequence data, translated sequence data, detailed BLAST data and conserved domains were provided in Appendix D. Detailed "Molecular Function', 'Biological Role' and 'Cell Component" data were provided in Appendix E. Biological role of all ESTs was discussed in Section 3.11. Another problem was that, independent from the quality of SCF chromatogram, about 1/3 of the forward reactions of Plate 5 and Plate 6 gave a hit related to "chlorite dismutase" (gi71847815). This may be a contamination after preparation of 96-well plates or an unknown problem related to sequencing. These sequence data were not evaluated.

Table 3.2 Efficiency of sequencing reactions of the first four 96-well trays.

Plate no	Number of clones in plates	Number of fully sequenced clones	Number of partially sequenced clones	Number of clones failed to be sequenced or sequenced very poorly or not recombinant
1	96	1	35	60
2	48	10	24	14
3	96	25	45	26
4	96	5	9	82
Total	**336**	**41**	**113**	**182**

Inherent problems of DDRT-PCR: Most of the authors agree that DDRT-PCR produces high number of false positives especially due to low stringency conditions; i.e., at low annealing temperatures (40-42 ^{o}C) (Bonnet *et al.*, 1998; Sung and Denmen 1997; Zegzouti *et al.*, 1997; Sanabria and Dubery, 2004). This fact was stated as the main limitation of DDRT-PCR (Sturtevant, 2000). Cho *et al.* (2002) reported that the major effects on the reproducibility of DDRT-PCR are random primers and low dNTP concentration. Some authors indicated that reamplification of DDRT-PCR products may yield such sequences that have arbitrary (forward) primer at both ends instead of T-tail; and proposed that such fragments may be mRNA origin of false positive (Bonnet *et al.*, 1998). However, it should be noted that the conditions especially for DDRT-PCR have quite low stringency to facilitate primer binding and primer targets may be available randomly. Thus, primers can bind any corresponding site. This fact was also observed in this study: for example the ESTs 31 (NLRRfwd/NLRRfwd), 38 (RLRRrev/RLRRrev), 1422a (PtoFenS/PtoFenS), 1528 (P4/P4), 1623 (T7/T7) and 1595 (P8/P8), all of which have the expected size, have the same primers at both ends. It is reported that there may be co-migratory contaminant sequences of the same size with the product band on the sequencing gel and primer dimers can also be cloned (Sanabria and Dubery, 2004). Both of these situations were observed in this study: for example, ESTs 1528 and 1528-D6 have the same size (508 bp) and same band origin, but sequences are different. However, it should be noted that same sized products from different sequences could be obtained by chance in case of random or degenerate primers. Few clones yielded both primer sequences attached to each other with a very short inserts. Few sequences of clones yielded shorter or longer sequences. This may be resulted from concatamer primer sites in the sequences or unknown PCR drawbacks. False positives may be also from other origins not the plant sample itself; such as the pathogen used for infection or any other source. Few clones were observed to be like this. For example; RGA-DDRT-PCR derived EST-2228 is are highly similar to *Pseudomonas* pyruvate decarboxylase; probably a contamination from a source of bacteria.

3.7 Genetic Linkage Analysis

DNA markers that co-segregate with the phenotypic character are neccessary to determine the QTL of the interested character and useful for marker assisted selection (MAS) in breeding. EST markers may map the actual a gene of interest (Cato *et al.*, 2001), which may be important as future possibilities to perform map based cloning and integration of the gene by MAS in another cultivar. To find out gene specific chickpea EST markers that

co-segregate with Ascochyta blight resistance, genetic linkage analysis was performed by using the primers designed for some of the important sequences obtained in the first run of sequencing (Section 3.6). The mapping population of CRIL-7 (Tekeoglu *et al.*, 2000) was described in the Introduction. In addition, parents of CRIL-3 (Tekeoglu *et al.*, 2000) were also used for any possible polymorphism (CRIL-3 parents FLIP84-92C(2), resistant and PI 359075(1), susceptible; CRIL-7 parents FLIP84-92C(3), resistant and PI 599072 (*C. reticulatum*), susceptible). However, since CRIL-7 is an interspecific cross which has a wide range of potential polymorphism then CRIL-3, screening was focused on CRIL-7 rather than CRIL-3.

Firstly, optimization of annealing temperatures of the PCR reactions for the 33 markers (Table 2.23) were performed by using both DNA of CRIL-3 and CRIL-7 parents and ILC195 cDNA from bulked infected (ib) and uninfected (cb) total RNA samples (total RNA of time point samples were bulked; Table 2.3). 27 primers resulted in distinguishable PCR product (examples in Figure 3.7) whereas the remaining gave either no observable product or a smear of various sized products whose polymorphism could not be distinguishable in the agarose gel. This shows that some of the designed EST specific primers were not specific enough, i.e, annealed to many sites of the genome. Only one primer set (EST-1934) gave a clear polymorphic banding pattern between CRIL-7 parents. As stated before, chickpea genome was reported to be quite monomorphic and it shows low polymorphism (Croser *et al.*, 2003) for many type of markers, except STMS (Winter *et al.* 1999; Choumane *et al.* 2000; Hüttel et al., 1999; Pfaff and Kahl, 2003). Therefore, another strategy, CAPS (Cleaved Amplified Polymorphic Sequences), was applied.

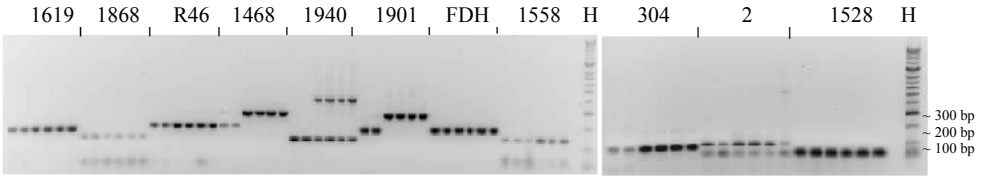

Figure 3.7 An example for PCR optimization of EST specific primers. Numbers are original arbitrary EST names. Sample order is ib/cb/FLIP 84-92C(2)/PI 359075(1)/FLIP 84-92C(3)/PI 599072 as in the text. H, HyperladderII. 1.5 % agarose EtBr gel in TBE.

CAPS analysis: To find a Single Nucleotide Polymorphism (SNP), CAPS methodology was applied. For this purpose, sequence data for the ESTs used to design EST primers were analyzed to find out restriction enzyme (RE) sites by web based Watcut program. These primers were designed to amplify short targets in Real-Time qRT-PCR. REs that showed only one site in the middle of the target region were selected, so that restricted products would be visible in the agarose gel. Trials were done for some of the primers sets that gave PCR products from DNA of CRIL-7 and CRIL-3 parents and from bulked cDNA. PCR products of several EST primer sets were cut by various REs, either with a single RE or with a cocktail of REs as shown in the following Table 3.3. Gel pictures of some examples are shown in Figure 3.8. No polymorphism was detected. This may result from i) the low genetic diversity of chickpea so that corresponding RE sites do not differ in between parents, ii) low possibility to find polymorphic RE site in short sequences, iii) misreadings at the RE site that resulted in wrong annotation of a RE site in the sequence, iv) RE cocktail application might result in indistinguishable products.

Table 3.3 RE digestions of EST amplified product bands.

EST name	Cut with the RE
1990, 1901, 1612, Fdh-3, 179	*Hinf*I
1990, 2161, 1619, 1528	*Mbo*II
2161, 1528, Fdh-3	*Sau*3a
1611, 1619, 1558	*Acs*I
1558, 1611	*Hae*III
1901	*Afl*1
1619	*Acs*I, *Mbo*II, *Hind*III and *Mn*II
1998	cocktails of *Ava*2 +*Acs*I and *Alu*1 + *Hinf*1
1468 and 2161	cocktails of *Hind*III + *Alu*1 + *Hae*III
HRP	cocktail of *Alu*1 + *Pvu*II
1558	cocktail of *Hae*III + *Mn*II
Fdh-3	cocktail of *Hind*III + *Mn*II
1611 and 1940	cocktail of *Ava*II + *Acs*I
2, R50, R13, R46, 1468, FPIP, 1901, 1612, 1934, 1528, HRP	cocktail of *Pvu*II + *Mse*I + *Stu*II + *Xho*1

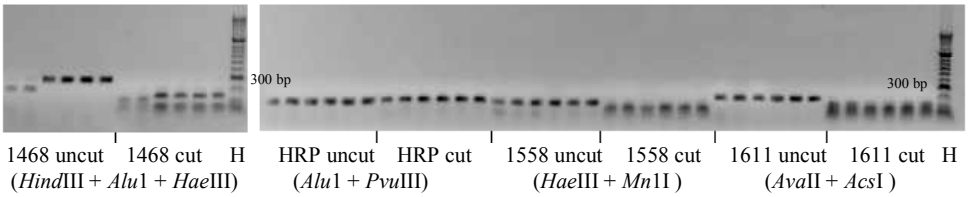

Figure 3.8 Examples of RE restriction of PCR products obtained from EST specific primers. Numbers indicate the original arbitrary EST number. Order of the samples are ib/cb/FLIP 84-92C(2)/PI 359075(1)/FLIP 84-92C(3)/PI 599072 as in the text. H, HyperladderII. 2 % agarose gel in TBE.

So, the solution may be re-sequence the product bands obtained from EST specific primers from CRIL-7 parents, i.e. FLIP84-92C(3) and PI 599072. Relatively important ESTs with respect to BLASTX data were selected. These are: R46, 1468, 1901, FPIP, 1611, Fdh-3, 1619, 1998, 447 and HRP. In order to obtain relatively larger PCR products, some of the ESTs were re-amplified with new sets of primers; i.e., a combination of original GSP/RGA primers (Table 2.8 and 2.16) with the corresponding EST specific primer. For example, a combination of original forward Harford (GSP) and EST specific HRP reverse primer yielded a larger product; so this fragment was sequenced (Figure 3.9).

Sequenced fragments, except one (EST-447), showed similar homologies as the original ESTs (Appendix G). Among 10 pairs of sequenced PCR products (Appendix G) only 2 pairs, EST-R46 and EST-FPIP, showed SNP between CRIL-7 parents. According to dCaps Finder 2.0 EST-R46 primer pair amplified PCR products that have a SNP site which can be cut by *Sma*I (recognition site: CCCGGG). EST-FPIP primer set amplified PCR products that have SNP sites which can be cut *Taq*I (recognition site: TCGA) and TspE1 (isozymes: TSP509i or TAsI or Sse91; recognition site: AATT). PCR products of EST-R46 and EST-FPIP were digested with the corresponding REs. *Sma*I for EST-R46 and *Taq*I for EST-FPIP resulted in polymorphic banding pattern. The available *TSP*509i, which is the isozyme of TspE1, cut both of the parents with the same pattern (Figure 3.10). This contrast might be resulted from wrong base reading at that point of sequence. As a result; 3 polymorphic EST markers created polymorphism in between CRIL-7 parents: EST-1934, EST-FPIP cut with TaqI and EST-R46 cut with SmaI.

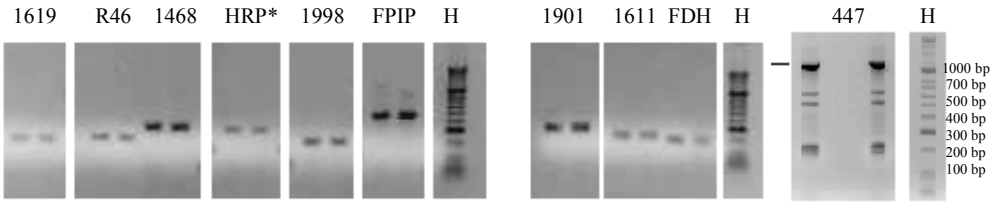

Figure 3.9 Sequenced fragments obtained by PCR amplification of CRIL7 parental DNA by using EST specific primers and some GSP pirmers combined with corresponding EST markers. Numbers indicate the original arbitrary number of ESTs. HRP* is from combination of original HRP-fwd and EST specific reverse primer HRP ASN250 (Table 2.23). The line indicates sequenced fragment of EST-447. Order of the samples are FLIP 84-92C(3)/PI 599072. H, HyperladderII. (2 % agarose gel in TBE buffer).

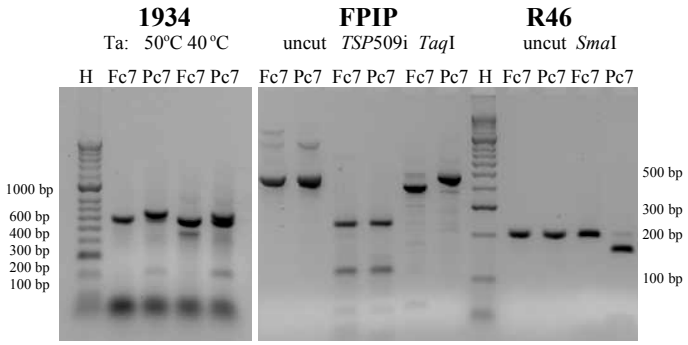

Figure 3.10 Polymorphism in PCR products of EST-1934, EST-FPIP and EST-R46 amplified from DNA of CRIL-7 parents. Fc7 and Pc7 is FLIP 84-92C(3) and PI 599072, respectively. H, HyperladderII. 2 % agarose gel in TBE buffer. H, HyperladderII.

EST-1934 is an DDRT-PCR derived EST which has homology with ubiquitin extension proteins and ubiquitin/ribosomal protein S27a (72.0 bits, $1e^{-11}$) and has a conserved domain Ribosomal_S27 (pfam01599). EST-R46 is an RGA-DDRT-PCR-trial derived EST which has homolgy to unknown proteins (127 bits, $3e^{-28}$) and receptor-like protein kinase (RLK) (120 bits, $4e^{-26}$) having Serine/Threonine protein kinases, catalytic domain (S_TKc cd00189). Finally the GSP-RT-PCR derived EST-FPIP has homology with SLL2-S9-protein (187.0 bits, $1e^{-46}$) with similarity to SLL2-S9-protein, an *S*-adenosyl-L-methionine-dependent methyltransferase (SAMMtase). The importance of these genes was discussed in section 3.8 and 3.11. The sequenced PCR products of CRIL7 parents are larger than EST-FPIP sequence, probably have an intron site. They have homology to the original EST sequence but low similarity.

Analysis of polymorphism in CRIL7 population and mapping: The polymorphic primer set EST-1934 and CAPS primer sets EST-R46 and EST-FPIP were screened in CRIL-7 population. For EST-1934, only PCR was done and products were run on agarose gel and scored. For EST-R46 and EST-FPIP, the population was first PCR amplified, checked on agarose gel and then digested with the corresponding RE and scored (Appendix H). Mapping analysis showed that EST R46 was located on LGVII of Tekeoglu *et al.* (2002) in the upstream of the marker Ta29. This may correspond to the LG7 of the reference map. EST-FPIP was located on LGIII+LGIX of Tekeoglu *et al.* (2002) in the downstream of the markers Ta135/RGA/TR31 and Ta47 and Ta42, respectively. This corresponds to the LG3 of the reference map. EST-1934 showed no linkage. Major QTLs for Ascochyta blight were located on LGIV and LGVIII in Tekeoglu *et al.* (2002) or LG4 and LG2 of the reference map. As mentioned in the Introduction, Bian *et al.*(2007) aligned the reference map to the QTLs of Flandez-Galvez *et al.* (2003b) and found that *QTL1* of Flandez-Galvez *et al.* (2003b) was located on LG3 of the reference map. By taking the combined map of Bian *et al.* (2007) into consideration, markers Ta135 and TR31 are linked tightly to *QTL1* of Flandez-Galvez *et al.* (2003b); so the EST-FPIP marker is located near marker Ta64 but far away from this QTL. It should be also noted that *QTL1* is not a major QTL and was observed only under field conditions (Flandez-Galvez *et al.* 2003b), with different chickpea genotypes and isolates. The exact location of EST-R46 and EST-FPIP markers was disclosed in the poster of Rajesh *et al.* (2008). Consequently, no EST marker linked to QTLs for *A.rabiei* resistance was found (Figure 3.11).

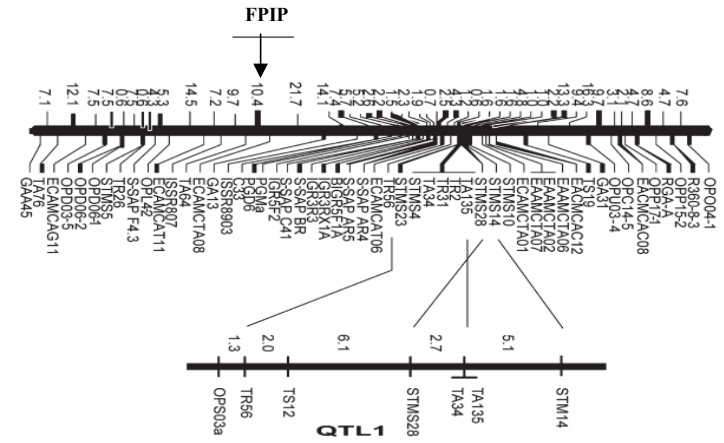

Figure 3.11 LG W-*Ca*- LG3 of Bian *et al.* (2007). EST marker FPIP may be located somewhere shown by the line, above marker Ta64.

3.8 Real-Time qRT-PCR Analysis

Any differential banding pattern in the DDRT-PCR method needs to be confirmed by several other methods such as Real-Time qRT-PCR or Northern Blot analysis. To claim for a differential expression, important ESTs obtained from the differentially expressed bands should be confirmed. In this study, it was aimed to compare differential expression of some of the putative ESTs in between infected ILC195 and a more specified chickpea-*A.rabiei* partnership; i.e., resistant CRIL7 parent FLIP84-92C(3) infected with pathotype I or pathotype II which will be called as PI and PII hereafter. This comparison may help to understand whether or not the expression profile of these ESTs is similar. All of the important ESTs could not be tested for confirmation of expression due to time and cost limitations. Therefore, the remaining ESTs can be tested in a quicker way by constructing a macro or microarray.

New sets of infected/uninfected ILC195 plant materials were prepared by using only the isolate *ank6*. Because; i) there were new findings in the literature (Coram and Pang, 2005b and 2006) which stated that earlier time points are necessary for chickpea's defense responses, i.e., in between 6 to 72h; ii) to obtain discrete results only for one isolate, *ank6*, which is better specified and more aggressive than others; and iii) all these isolates could

not be tested by all time points due to high cost and large number of samples; thus one condition (*ank6*) was chosen. Isolate *ank6* was initially identified being similar to Race 6 (Prof.Dr. Sara Dolar, personnel communication). Race 6 was classified in PII by Cho and Muehlbauer (2004). However, very recently *ank6* was classified into pathotype III (Türkkan, 2008).

The disease reaction of ILC195 against *ank6* was evaluated for this analysis (by Prof.Dr. Sara Dolar, 20 days after infection) and was found to be 3.3 according to 1 – 9 rating scale of Singh *et al.* (1981) scale, which was in between the previously evaluated disease development score of this cultivar against this isolate. No disease symptoms were visible in control plants. The disease symptoms of FLIP84-92C(3) were evaluated (by Tony Chen; USDA-ARS) after 2 weeks post infection and found to be 1.7 against PI and 2.9 against PII. The disease symptoms of other varieties, to confirm infection, was found to be 9 for PI (all death) and 7 for PII in "Spanish white"; 3 for PI and 5.2 for PII for Dwelley. This showed a usual picture of disease development for the given chickpeas and pathotypes.

In Real-Time qRT-PCR analysis, normalization is carried out by using reference genes. These may be housekeeping genes, whose constitutively high expression is not affected considerably upon a given treatment, such as β-actin, tubulin, GAPDH, and 18S ribosomal RNA (18S rRNA) (BioRadiations121, 2007). Actin is widely used in plant-pathogen interaction studies for normalization and it was also used for chickpea by Coram and Pang (2006 and 2007). For that reasons, actin was used firstly as reference gene for normalization. First of all, optimization (annealing temperature) of reactions using actin and other primers were carried out by using cb (control ILC195 bulk) and ib (infected ILC195 bulk) cDNAs in Roche Light®Cycler. Annealing temperatures optimized for linkage analysis PCR amplifications were not suitable for Real-Time PCR reaction. The criterion for deciding of the optimal temperature was decided by observing the shape of the dissociation curve (a single peak without any shoulders) of the amplified product with an acceptable fluorescence. Some examples of melting curves were provided in Appendix C.

Selection of ESTs for time point profile analysis: The total number of ESTs having good quality of primers for this analysis was 18 (Table 3.4). To screen all of the plant samples and time points would give at least 1080 reactions (6 time point's x 5 plant samples (control and *ank6* infected ILC195, control and PI or PII infected FLIP84-92C(3)) x 18 primers x 2 replicates) which would be costly and time ineffective experiment. For that

reason a selection procedure was set up: all of these primers were screened in FLIP84-92C(3) cDNA samples which were constructed from bulked total RNA of all time points (Table 2.4). ESTs which showed more than 2 fold induction were selected. Still this selection procedure is not free from biases; since bulked samples may result in dilution of the message. This effect was called as "dilution effect" throughout this thesis. To get rid of at least some of the artifacts of this effect, a second screening step was conducted. The ESTs selected by FLIP84-92C(3) plants were secondly screened in ILC195 cDNA samples which were constructed from bulked total RNA of all time points (Table 2.3). Finally ESTs showing more than 2 fold induction in both of the chickpea lines were selected for analyzing in time point samples further. Figure 3.12 summarizes the scheme of this procedure. For this purpose, total RNA isolation was performed for each time-point separately. Bulking was carried out after confirming the integrity of the bands from total RNA samples in the gel and by measuring on spectrophotometer, so each time point was represented approximately equally in the bulk (data not shown). FLIP84-92C(3) total RNAs were prepared as Fb as control bulk (calibrator), and PIb and PIIb as infected bulks (samples) and these were used to construct corresponding cDNAs. Fold changes of PIb and PIIb with respect to Fb were calculated by using $2^{-\Delta\Delta C}{}_T$ method (Livak and Schmittgen, 2001). For the second screening, total RNA samples of the ILC195 control plants were bulked as cb (calibrator) and *ank6* infected ILC195 plants were bulked as ib (sample).

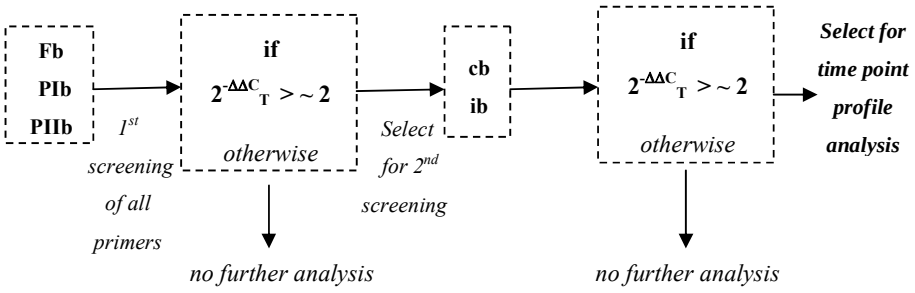

Figure 3.12 Selection procedure for ESTs to be tested in time point profile Real-Time qRT-PCR analysis. Fb (control), PIb (PI infected) and PIIb (PII infected) bulked FLIP84-92C(3) samples; cb (control) and ib (*ank6* infected) bulked ILC195 samples.

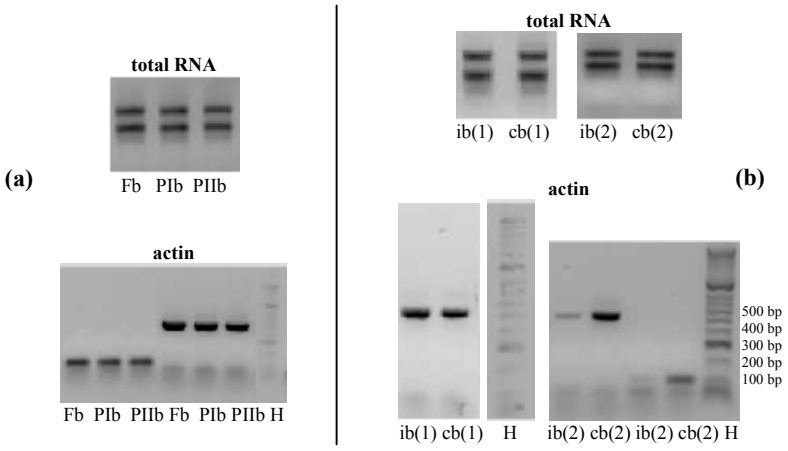

Figure 3.13 Samples for selection procedure for ESTs to be tested in time point profile Real-Time qRT-PCR analysis. (a) DNAse/LiCl treated FLIP84-92C(3) total RNA bulks; Fb, PIb PIIb (see text) and actin on their cDNA. (a) Two sets of DNAse/LiCl treated ILC195 total RNA bulks; ib and cb (see text) and and actin on their cDNA. L, HyperladderII. 1% agarose gel in phosphate buffer for RNA, 1% agarose gel in TBE buffer for DNA. Short actin bands are from actin primers designed for Real-Time qRT-PCR (RT-actSN383 and RT-actASN467; Table 2.23); long actin bands are from actin primers designed for RT-PCR (Act-F and Act-R; Table 2.8).

An observation was the fact that although total RNA samples may be in good quality cDNA preparations may not be qualified enough (Figure 3.13.b). Probably, remained DNAse after DNAse treatment/ethanol or other artifacts inhibit reverse transcriptase activity. So the best quality of cDNA bulks prepared for ib and cb samples were used for experiments. Another observation throughout all the Real-Time qRT-PCR experiments was that, the differences of the traditional PCR band intensity any two cDNAs may not be reflected as the same in Real-Time qRT-PCR. This shows that, in traditional PCR reaction the “end-point” product, (Stratagene, 2007) the amount of which may dependent on the factors of changing environment described by the stationary phase, is observed; however, in Real-Time qRT-PCR, no accumulation occurs but the real amount of cDNA is determined in the early stages of the log phase of PCR; i.e., free from the effect of these

factors. Even for the identical samples, the data related to amount may not be identical; so "end-point PCR" (Stratagene, 2007) is only useful, for qualitative assays, i.e. saying whether a particular target sequence exist or not (Stratagene, 2007). Consequently, using conventional PCR to observe expression differences may result in incorrect levels.

Table 3.4 shows fold changes observed in the first screening with FLIP84-92C(3). 5 ESTs which showed near 2 fold induction (ESTs 1468, 1558, 1619, 1868, Fdh-3) in PI and/or PII infected bulks and one (EST-FPIP) which was found to be polymorphic and also close to 2 fold induction were selected to screen further in ILC195 bulks. As obvious from this table, 3 ESTs (1558, 1868, Fdh-3) were also found to be nearly 2 fold upregulated in ILC195 infected bulk (Table 3.5). So these ESTs were selected as principal ones to check for time-point profile analysis.

Table 3.4 First step of screening. Ta is annealing temperature

Primer set	Ta	Average C_T (Std.Dev C_T)			Fold	
	(^oC)	Fb	PIb	PIIb	PIb	PIIb
Actin	57	22.76 (0.0408)	23.11 (0.0995)	23.42 (0.0836)	-	-
R13	57	21.84 (0.0760)	21.45 (0.0431)	21.70 (0.0670)	1.67	1.74
R46	60	26.49 (0.0250)	26.27 (0.0451)	26.72 (0.0215)	1.48	1.35
R50	61	26.48 (0.0216)	25.95 (0.100)	26.19 (0.132)	1.84	1.93
304	62	27.62 (0.103)	27.51 (0.101)	27.48 (0.207)	1.38	1.74
1468	58	28.34 (0.161)	28.06 (0.0616)	28.00 (0.191)	1.55	**2.00**
1508	59	21.40 (0.111)	20.83 (0.0484)	21.22 (0.0818)	1.89	1.79
1528	61	23.08 (0.0475)	22.74 (0.00154)	23.27 (0.363)	1.61	1.39
1558	61	24.87 (0.0236)	24.04 (0.0801)	24.60 (0.0348)	**2.27**	1.91
1611	61	22.45 (0.084)	21.95 (0.00823)	22.33 (0.00175)	1.80	1.72
1612	63	26.70 (0.00422)	26.28 (0.0718)	26.95 (0.0356)	1.71	1.33
1619	62	24.66 (0.0682)	24.00 (0.0234)	24.50 (0.0306)	**2.01**	1.77
1868	62	24.07 (0.00938)	23.23 (0.0225)	23.87 (0.112)	**2.28**	1.82
1934	58	31.06 (0.0790)	30.90 (0.281)	31.25 (0.406)	1.42	1.39
1940	61	19.90 (0.0468)	19.44 (0.021)	19.65 (0.0130)	1.75	1.88
1990	61	21.11 (0.00883)	20.74 (0.0337)	21.19 (0.0216)	1.65	1.49
2166	63	21.74 (0.0158)	21.32 (0.0344)	21.70 (0.000345)	1.71	1.62
Fdh-3	58	21.29 (0.0119)	20.58 (0.0124)	20.78 (0.00520)	**2.08**	**2.25**
FPIP	61	23.89 (0.00913)	23.60 (0.00668)	23.64 (0.00800)	1.56	1.88

Table 3.5 Second step screening of the selected 6 EST from the first step.

Primer set	Ta (^{o}C)	Ave. C_T (Std.Dev C_T) cb		Ave. C_T (Std.Dev C_T) ib		Fold ib
Actin	57	23.86	(0.108)	23.85	(0.0304)	-
1468	58	27.89	(0.109)	27.24	(0.0370)	1.56
1558	61	26.69	(0.0892)	25.58	(0.0528)	**2.14**
1619	62	25.18	(0.0285)	24.60	(0.0128)	1.48
1868	62	25.52	(0.0752)	23.88	(0.0583)	**3.10**
Fdh-3	58	21.64	(0.0432)	20.47	(0.0229)	**2.23**
FPIP	61	23.89	(0.0441)	23.15	(0.0101)	1.66

Time point profile expression analysis: To analyze the expression profile of selected ESTs in ILC195 and FLIP84-92C(3) plants from 6 to 72 hpi with *A.rabiei*, total RNA from each time-point was DNAse/LiCl cleaned and cDNA was constructed for Real-Time qRT-PCR. The integrity and quality of cDNA was checked with both actin primers (for Real-Time qRT-PCR, RT-actSN383 and RT-actASN467, Table 2.23; for RT-PCR, Act-F and Act-R, Table 2.8). These are shown in Figure 3.14. Firstly, the selected ESTs, namely, 1558, 1868 and Fdh-3, were analyzed. After having some data from these ESTs, more ESTs namely 1468 which was upregulated 2 fold in PII infected FLIP84-92C(3) (Table 3.4) and two polymorphic EST markers found in Section 3.7 FPIP and R46 were analyzed. After calculating the fold changes of infected plants with respect to uninfected ones, expression profiles were drawn as graphs shown in Figures 3.15, 3.16 and 3.17.

With the experiments done with actin as the reference gene and Roche Light®Cycler as equipment; ESTs 1558, 1868 and Fdh-3 showed gradual upregulation starting from 6 h, making a peak at 24 h, followed a sharp decrease at 36 h and stayed almost constant afterwards in both PI and PII infected FLIP84-92C(3); as shown in Figure 3.15.a. This profile is in line with the observations for upregulated genes in the microarray studies (confirmed by Real-Time qRT-PCR) of Coram and Pang (2005b, 2006) and the information that was stated before in the Introduction by many authors, i.e., the timing of the response of chickpea according to the germination of the *A.rabiei* spores 12 hpi followed by penetration after 24 hpi (Pandey *et al.,* 1987; Hohl *et al*., 1990; Coram and Pang, 2005b and 2006).

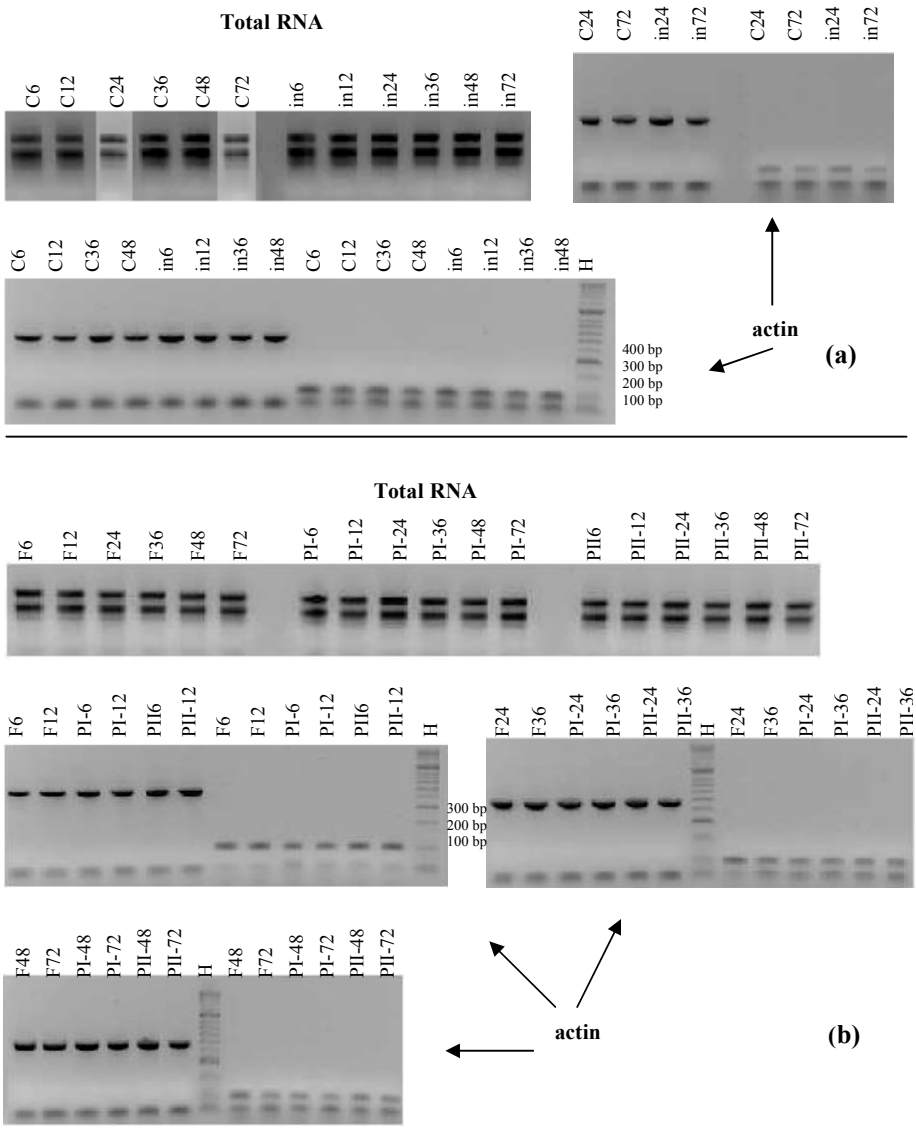

Figure 3.14 Samples for time point profile expression analysis. (a) DNAse\LiCl treated ILC195 total RNA samples and their actin fragments according to time points (Table 2.3). C24 and C72 total RNA were observed in poor quality after treatments and repeated. C, control; in, infected. (b) DNAse\LiCl treated FLIP84-92C(3) total RNA samples and their actin fragments according to time points (Table 2.4). F, control; PI, pathotype I infected; PII, pathotype II infected. H, HyperladderII. 1 % agarose gel in phosphate and TBE buffer for RNA and DNA, respectively. Short and long actin bands are from actin primers designed for Real-Time qRT-PCR and for RT-PCR respectively, as mentioned in the text.

In the small scale study of Coram and Pang (2005b), they stated that in both resistant IC and susceptible LA chickpea genotypes, the upregulation pattern was overpowering than down regulation; and the majority of upregulated at 24 hpi, especially for the IC. On the other hand, in the large microarray study, the situation of expression difference was in favor of downregulation; but the major differences in expression within 24-48 h in all chickpea genotypes, i.e., resistant IC, moderately resistant FL and susceptible LA. This means that, up- or down-regulation pattern depends on the size of the analyzed EST population and on the ESTs selected. For resistant IC, early induced ESTs were reported in between 6-12 hpi and slightly induced ESTs as two clusters in 12-48 and 12-72 hpi in the microarray by Coram and Pang (2006). So, the timing of the differential expression were reflected also by three ESTs for both of the PI and PII infected FLIP84-92C(3) (Figure 3.15.a) in this study.

However, the case of ILC195 infected with the isolate *ank6* was different. Almost no induction was observed in the time points, except a very large peak at 36 hpi (Figure 3.15.a). This may be evaluated as a delayed response within the most expected response time range (i.e. 6-48 hpi) due to relatively less resistance of ILC195 to *ank6* when the disease scores of FLIP84-92C(3) plants to PI and PII are considered. A very large peak lead to idea that, besides the normalization deviation which may be expected by large experimental concentration differences in between two samples, there may be some important biases from the reference gene: Ct of in36 sample was 1.5 fold lower than Ct value C36 sample in actin indicating a decrease in actin amount (data not shown). Nicot *et al.* (2005) noted that depending on the stress some housekeeping genes may vary and they found that actin was the most variable gene for late blight, whereas the most stable genes were *ef*1a and 18S rRNA genes. The role of actin filaments in plant defense response is also under investigation. Upon fungal attack, rapid responses of plants also include rearrangements of the cytoskeleton and allocation of barrier material around fungal penetration site (Schmelzer, 2002) including actin filaments (Kobayashi *et al.* 1994). Four actins were downregulated in *Arabidopsis* upon *A. tumefaciens* at 48 hpi, but Actin 12 gene was upregulated upon inoculation with *P.syringae* (Tao *et al.* 2003, Ditt *et al.*, 2006). Zou *et al.* (2005) noted that during HR-response of soybean (*Glycine max*) against *P. syringae* pv. *glycinea* strains disturbance of cytoskeleton was obvious by decreased abundance of genes related to actin, tubulin, and others; however, actin control was used in qRT-PCR since its expression level did not vary significantly with respect to infections in microarray experiments.

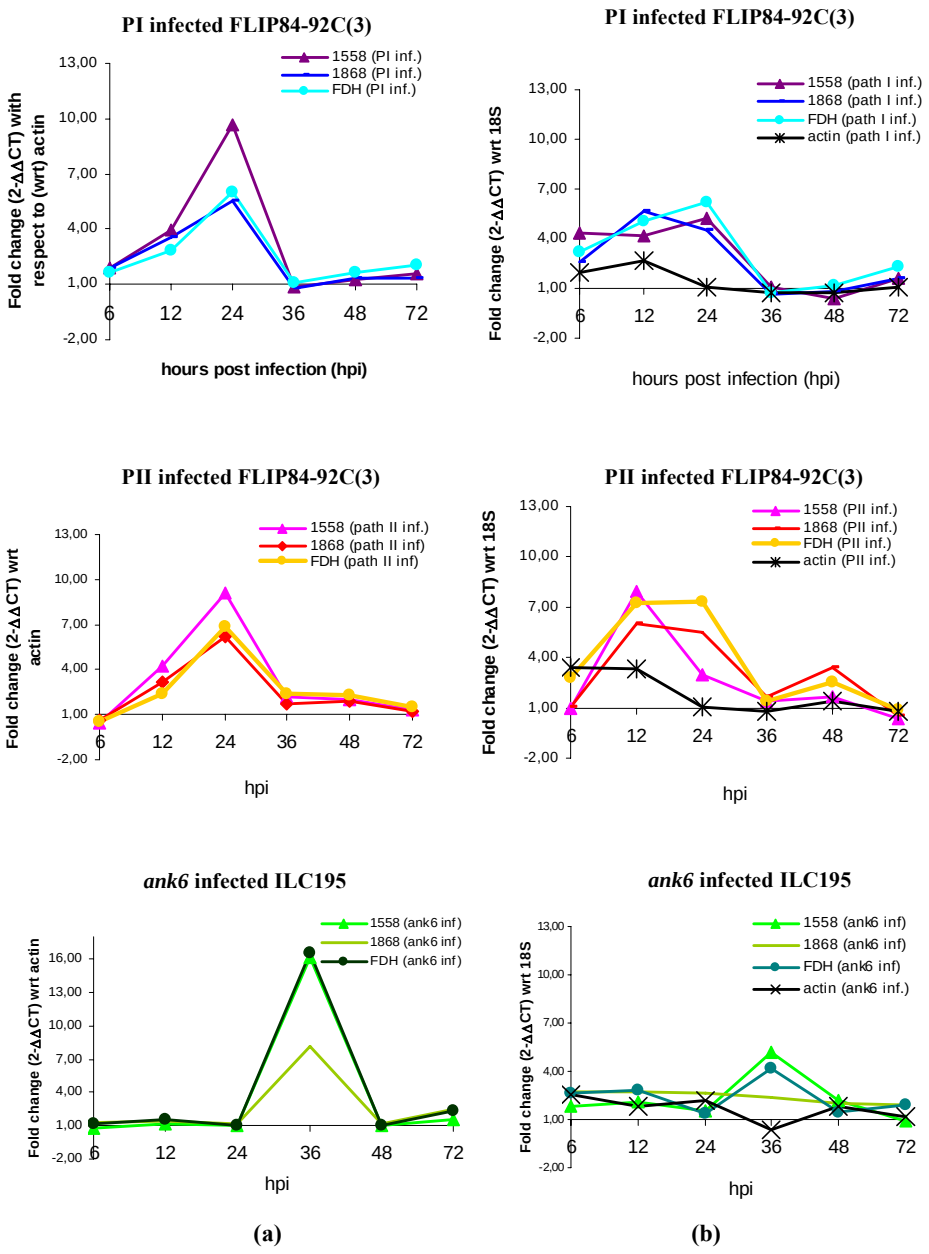

Figure 3.15 Fold changes according to time points. ESTs 1558, 1868 and FDH. (a) is the experiment done with Roche Light®Cycler using actin as reference. (b) is the experiment done with Stratagene MX4000 using 18S as reference.

So, reports indicate varying and controversial situations. There are also some drawbacks to use 18S rRNA gene such as possible variability due to its very high abundance and may not be applicable when poly A(+) RNA is used for cDNA (Nicot *et.al,* 2005; Stratagene, 2007). However, some studies reported the use of 18S rRNA gene as reference (Bozkurt *et al.* 2007). Since it was not aimed to determine to absolute quantity but relative comparison in between samples, 18S rRNA gene was also used here as reference gene and experiments were repeated. The fact that the Ct values of 18S rRNA gene were higher than any other genes leads to decrease in fold changes. Besides, using 18S rRNA gene eliminated the Ct difference in between in36 and c36 samples. However, general induction profile; i.e., 6-24 h for infected FLIP84-92C(3) and 36 h for infected ILC195, was conserved (Figure 3.15.b). For 18S rRNA as reference, actin showed first an increase in 6 and 12 h and then decrease in FLIP84-92C(3) plants infected with PI and PII, respectively. Similarly, in ILC195 infected with *ank6*, a slight increase of actin in 6 and 12 h was detected which was followed by a large decrease (fold values lower than 1 in the graphs) at 36 h. This means that actin may be affected by blight infection also. In general, peaks at 24 h for FLIP84-92C(3) were reduced; but the fact that gene main induction in between 12-24 h upon *A.rabiei* infection was conserved. FLIP84-92C(3) infected with PI showed also higher fold values at 6 h; may be due to its higher resistance against PI. Similarly as before, if 18S rRNA gene as reference, the response of ILC195 plants to *ank6* isolate was observed as a low induction, with a delayed peak at 36 h for two ESTs (FDH and 1558).

Fold changes of three further ESTs; namely, EST-1468, EST-FPIP and EST-R46, were also calculated by using both actin and 18S rRNA genes as reference. Except EST-R46, the fold changes of these ESTs were shown in graphs of Figure 3.17.

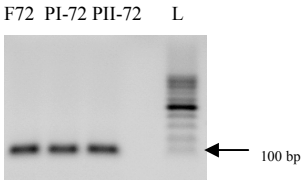

Figure 3.16 Example of 18S rRNA gene amplicons F72, uninfected; PI-72, PI infected, PII-72, PII infected FLIP84-92C(3) 72 hpi samples. L is 100 bp HeliosisLadder.

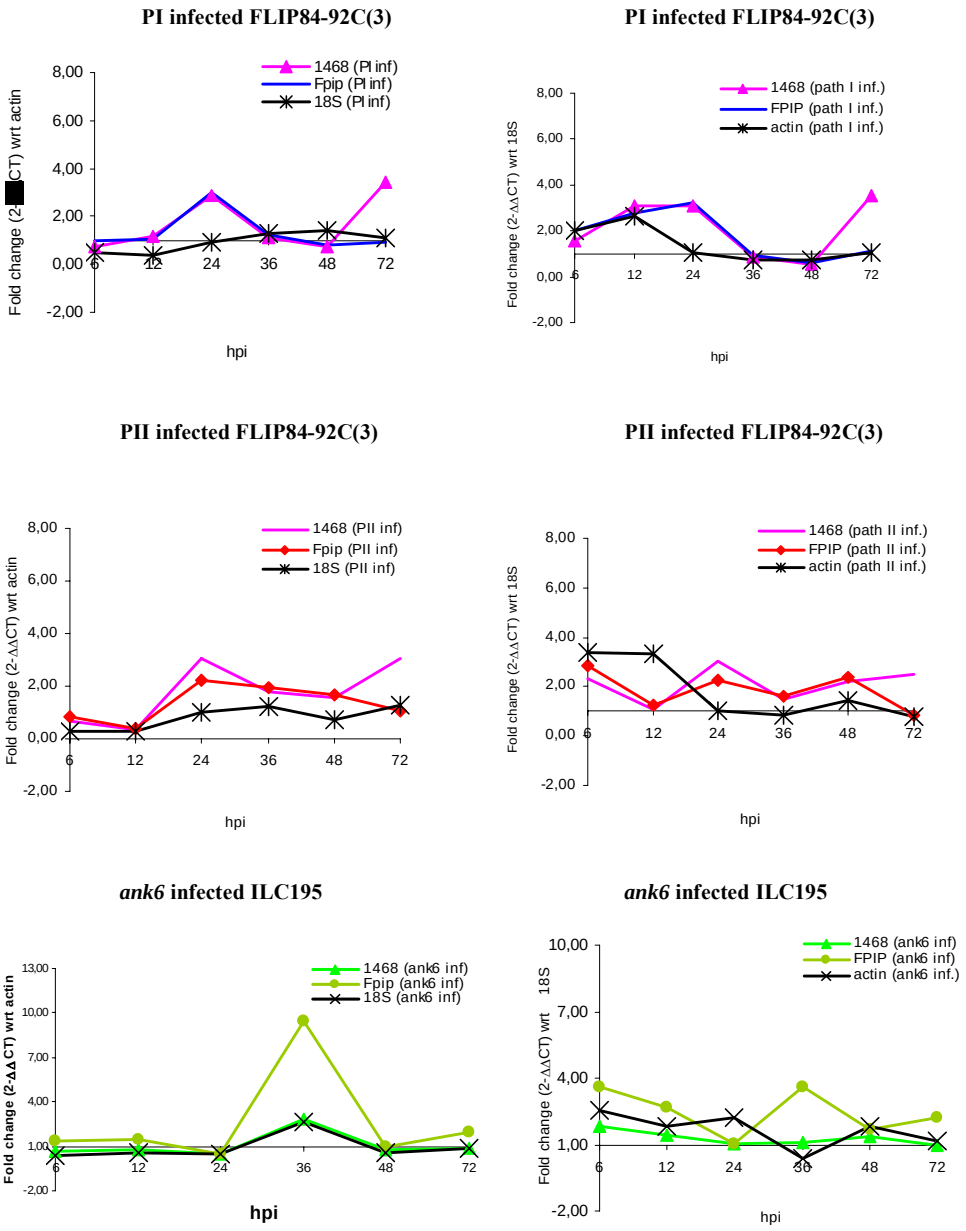

Figure 3.17 Fold changes according to time points. ESTs 1468 and FPIP. (a) actin as reference. (b) 18S rRNA gene as reference.

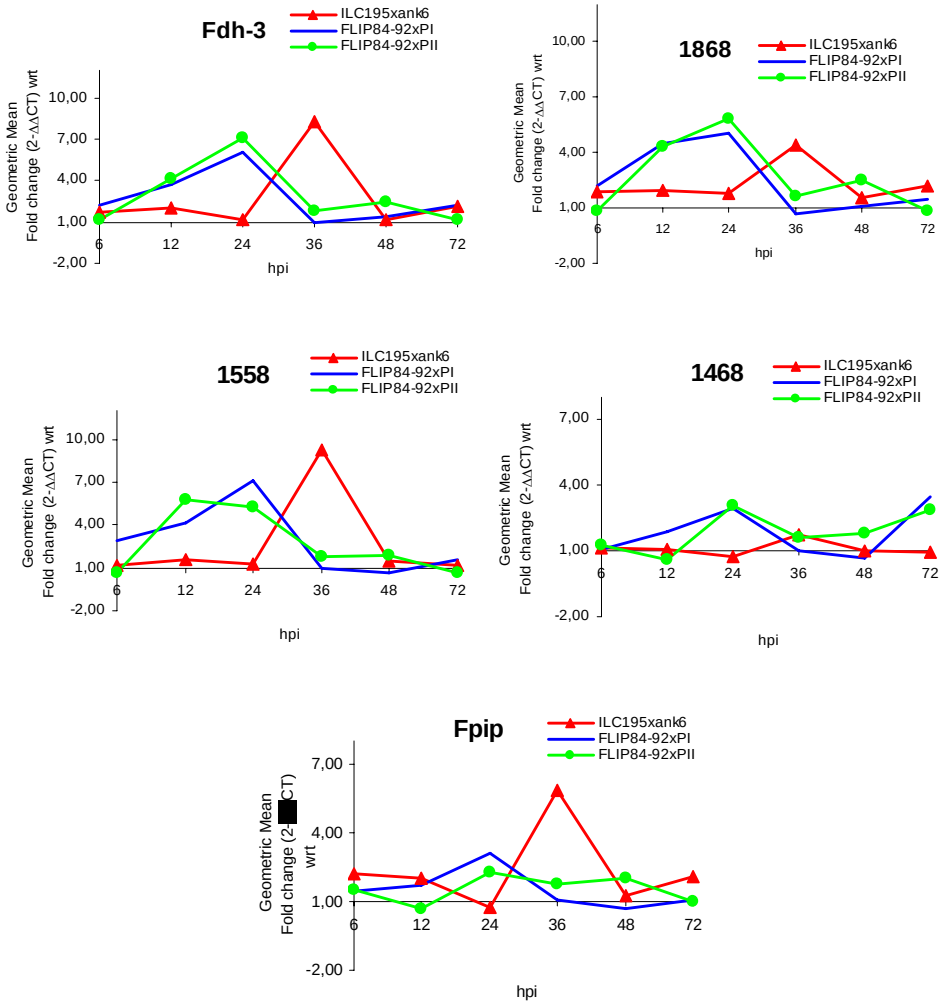

Figure 3.18 Fold changes described by the geometric mean of fold changes calculated separetaly actin or 18S rRNA as reference.

Vandesompele *et al.* (2002) proposed a model of using more than one reference gene in a pair-wise comparison and used geometric mean as a normalization factor. Considering this idea, geometric means of the fold changes obtained for actin and 18S rRNA gene as reference genes were calculated for each EST (Figure 3.18). Geometric means of fold changes conserved the induction profiles. EST- Fdh-3, EST-1868 and EST-1558 were upregulated within 6-24 hpi in FLIP84-92C(3) and at 36 hpi in ILC195. EST-1468 did not show a noteworthy induction. EST-FPIP only induced in ILC195 at 36 hpi considerably. The possible late response of ILC195 may be not only due to its late response to *ank6* but also due to different environmental conditions. It should be also noted that *ank6* may be a more aggressive isolate and classified as pathotype III (Türkkan, 2008) and disease scoring may vary from laboratory to another laboratory which may have resulted different responses of FLIP84-92C(3) and ILC195 plants. Independent from the time of response, it may be concluded that at least three ESTs (Fdh-3, 1868 and 1558) were induced in both of the FLIP84-92C(3) and ILC195 plants which may represent a similar defense response.

EST Fdh-3, cloned by using a GSP pair designed specially for FDH gene using conserved regions of that gene, is very highly similar to plant FDH and has a domain of "2-Hacid_dh_C, D-isomer specific 2-hydroxyacid dehydrogenase, pfam02826" (Appendix D and E). FDH which is located in mitochondria catalyzes oxidation of formate via reducing NAD to NADH leading to production of carbon dioxide (Krüger *et al.* 2004). So that NADH is provided to the respiratory chain (Hourton-Cabassa *et al.* 1998). Formate is produced from various pathways in plants, i.e.; photorespiration, cell wall synthesis and degradation (from methanol), Crebs cycle, glycolysis and tetrahydrofolate pathways in plants (Hourton -Cabassa *et al.* 1998). Formate is a toxic compound. In the stress response, formate is also degradated to produce ATP *via* 10-formyl-THF synthetase into formyl-THF (Hourton-Cabassa *et al.* 1998). Zuo *et al.* (2005) cloned an EST coding NAD-dependent FDH which was upregulated in *Gossypium barbadense* (host)- *Verticillium dahliae* (necrotroph pathogen) system. Another evidence of upregulation of FDH during stress response is its induction in a non-host pathosystem of *A. thaliana* - *Phytophthora infestans* (Huitema *et al.*, 2003). Since FDH was also induced in symbiotic relationship (premycorrhizal stage) in between *Quercus robur* and *Piloderma croceum*, it was classified as stress response protein important for energy metabolism and *"stress-induced signal transduction*" (Krüger *et al.* 2004). Schenk *et al.* (2000) indicated that FDH was upregulated upon necrotrophic fungal pathogen *A. brassicicola* and highly upon SA and MJ, and slightly upon E in *Arabidopsis.* Fungal FDH is also upregulated during infection.

For example, FDH of rice blast fungus hemibiotroph *M. grisea* was induced to adapt the fungal metabolism to the anaerobic growth conditions during the growth in the plant or to detoxify formate released by the breakdown of antifungal toxins of the plant (Soanes and Talbot, 2005). For the above evidences, the induction of FDH in chickpea may be interpreted as this: in chickpea *A.rabiei* attack results in the induction of catabolic pathways such as cell wall related events (fortification/degradation) which lead formation of formate. Degradation of formate by FDH results in alternative production of NADH to meet chickpea's energy requirement during defense response partly. By this way, other energy related resources may be allocated for biosynthesis of defense related compounds. Besides, excess toxic formate is also removed. Early induction of FDH in PI and PII infected FLIP84-92C(3) may provide resistance against PI and PII. However, this response is delayed for ILC195 attacked by *ank6* which maybe the reason for its relatively less resistance as indicated by disease scores.

RGA-DDRT-PCR derived EST-1868 (observed in infected samples H3 and I) is highly similar to a set of plant proteins having serine carboxypeptidase domain (pfam00450, Peptidase_S10; Appendix D, E, and F), probably in membrane. Serine carboxypeptidases can be classified in both MIPS 14 related protein fate and MIPS 30 related to cellular communication. EST 1868 is also highly similar to BRS1, which is a serine carboxypeptidase regulating a cell surface leucine-rich RLK (Brassinosteroid-Insensitive 1 BRI1; Li and Chory, 1997) in Arabidopsis (Li *et al.* 2001). Li *et al.* (2001) suggested that BRS1 releases an active brassinosteroids (BR) binding protein by cleavage of an intermediate one; and the resulting BR–BR binding protein complex binds to the extracellular domain of BRI1 leading to activation of cellular responses. They also suggested that elevated expression of BRS1 would enhance signal transduction pathway. Schenk *et al.* (2003) observed a repressed serine carboxypeptidase precursor transcript at 72 h during systemic responses in Arabidopsis after inoculation with nectrotroph *A. brassicicola*. Lu *et al.* (2004) showed that a putative serine carboxypeptidase was constitutively expressed in compatible and incompatible reaction of rice plants upon hemibiotroph *M.grisea* infection. The same evaluation as FDH could be done for EST-1868. The rapid upregulation of EST-1868, peaking at 24 h and downregulation afterwards, may be interpreted as the increased signal transduction for necessary defense related protein synthesis. However, similarly to FDH, the induction of EST-1868 was delayed in ILC195, accounting its delayed response. Due to role of proteases in signal

transduction and potential role of serine carboxypeptidases in defense responses, further work on EST-1868 is necessary.

Similar induction profile was observed for the EST-1558 which was initially observed in infected bulks (H1, H2 and H3). However, the BLAST hits of this EST did not show any important protein; and the homology was very low (details in Appendix D, E, and F). It is similar to some hypothetical proteins having related to Acyl-CoA synthetases (AMP-forming)/AMP-acid ligases II (Lipid metabolism/Secondary metabolites). So, generally it may be concluded that; *A.rabiei* attack may result in increase in the lipid metabolism and secondary metabolism of chickpea; ranging from production of structural materials to signal transduction, transport and secondary products for defense.

DDRT-PCR derived EST-1468 was observed in infected samples (H1, H2, H3) at a higher intensity then uninfected samples (C1, C2). It is highly similar to metal ion transport proteins having conserved zinc transporter domain ZupT (Appendix K and L) located in membrane. ZupT (Grass *et al.* 2002) is a member of the ZIP family of proteins which are a family of heavy metal ion transporters similar to *Saccharomyces cerevisiae* Zrt1, Zrt2, ZiPI-4 transporters (Zn^{2+} uptake) and *A. thaliana* Irt1 (Fe^{2+} uptake) transporter (Eng *et al.* 1998). Few reports exist about the role of metal transporters in defense response of plants, but the EIN2 (see Introduction) is similar to the disease-related Nramp (Natural resistance associated macrophage proteins) family of metal ion transporters (Alonso, *et al.* 1999) which are suggested to regulate metal ion concentrations during bacterial infection in mice (Supek *et al*. 1997; Hall and Williams, 2003). A zinc transporter, ZIP3, and an iron-regulated transporter were down regulated upon E treatment in Arabidopsis (De Paepe *et al.* 2004). As shown in Figure 3.18, this EST was only significantly induced in FLIP84-92C(3) plants, showing that it is not necessary for the resistance of ILC195 plants to *ank6*.

The GSP derived EST-FPIP, whose primers were polymorphic markers found on LGIII+LGIX of Tekeoglu *et al.* (2002) in Section 3.7, is highly similar to SLL2-S9-protein which has methyltransferase activity (Appendix D, E, and F). If other close hits are also considered, there are two regions overlapping: methyltransferase small (pfam05175) such as methyltransferase C involved in rRNA methylation and methyltransferase (pfam08241) domains. Both are located in SAM (S-adenosyl-L-methionine)-dependent methyltransferases. The initial aim to design the primer set FPIP was to come up with a chickpea copy of the gene *P. sativum* disease resistance response protein, but a different

sequence was amplified (Section 2.5). SAMMtases are involved in the biosynthesis of compounds related to growth, development and disease resistance (Joshi and Chiang, 1998). SLL2 (for *S*-locus linked gene 2) is one of the *S* locus located genes related to self-incompatibility of *Brassica* (Yu *et al*. 1996b; Suzuki *et al*. 1999). However, Takada *et al*. (2001) proposed that the genes in the *SLL2* region might not be related to self-incompatibility. No special report was found related to plant-pathogen interaction during literature search for this study. The genetic mapping of FPIP was discussed in Section 3.7. In all of the chickpea genotypes, this EST was slightly upregulated in varying time points: slightly at 12-24 hpi in PI infected FLIP84-92C(3), very sligthly at 24-48 hpi in PII infected FLIP84-92C(3), and only at 36 hpi in *ank6* infected ILC195. Despite the low induction and unknown role of this EST in plant defense response, future study may cover this transcript.

Protein kinases have important role in signal transduction to trigger defense mechanisms. Veronese *et al*. (2006) identified a putative Ser/Thr kinase (Botrytis-Induced Kinase1, BIK1) gene in *A. thaliana* whose inactivation causes susceptibility to necrotrophic fungal pathogens. In a micoarray study, Coram and Pang (2006) observed down regulation of a Ser/Thr protein kinase (DY475384) in resistant chickpea IC at 24 and 72 hpi, but no change in moderately resistant chickpea FL at 24 hpi. On the other hand, in tolerant genotypes of chickpea, abiotic stress response resulted in repression of two Ser/Thr kinase transcripts (DY475384 and DY396307), which were suggested to repress cell death mechanisms (Mantri *et al*. 2007). Boominathan *et al*. (2004) reported that two putative Ser/Thr protein kinases were upregulated in chickpea upon dehydration stress. EST-R46, which seemed to be down-regulated in RGA-DD-RT-PCR-trial experiments, showed high similarity to unnamed or hypothetical membrane proteins having Ser/Thr protein kinase catalytic domain. The transcript levels of EST-R46 as compared to other ESTs in Real-Time qRT-PCR analysis was very low (Ct levels > 35); such big Ct levels are found to be inaccurate; therefore deviations between parallels were large (up to 1 Ct). However, the fold change of FLIP84-92C(3) samples and ILC195 samples with respect to time-points had similar profile, i.e, decline in 12 and 24 h; for both FLIP84-92C(3) and ILC195 samples (data not shown). The low level of transcripts related to kinases was described for MAPKs which may be true also for general Ser/Thr protein kinases. Germain *et al*. (2005) indicated that: "*because of their capacity to amplify very subtle stimulus, components of signaling modules, such as receptor kinases, mitogen activated protein kinases (MAPKs), and small signaling ligands, are generally expressed at low levels and are weakly*

represented in most EST sequencing projects (Hu *et al.*, 2003)". To make a conclusion, measurements for EST-R46 may be confirmed with higher volumes of cDNA sets.

3.9 BAC Library Hybridization

From the results of Real-Time qRT-PCR analysis, it was decided to select Fdh-3 to perform BAC library hybridization. Finding the physical map position of Fdh-3 could lead to full-length cloning of FDH gene by map-based cloning. BAC library hybridization was performed with the probe prepared PCR product of Fdh-3. Hybridization was carried out with approximately 60 ng of probe. The probe was prepared from FLIP84-92C(3), sequenced to confirm its sequence (Appendix G) and its integrity was checked by agarose electrophoresis (Figure 3.19) before labeling. After hybridization, no positive signal was observed. This may resulted from two reasons: experimental failure (very stringent conditions might affect hybridization) or the colonies on the hybridization filters might not have the clone to represent the an EST for FDH.

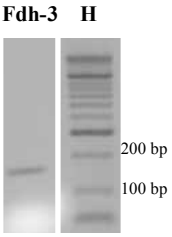

Figure 3.19 FDH probe for Fdh-3 used in BAC library hybridization. H, HyperladderII. 1% agarose gel in TBE buffer.

3.10 Analysis of Sequence Results

In this Section, of all the EST data obtained from RGA-DDRT-PCR trials, RGA-RT-PCR, DDRT-PCR, RGA-DDRT-PCR, and GSP-RT-PCR were evaluated in a collective manner. Sequence data and mainly their BLASTX results used to collect "Molecular Function", "Biological Process", and "Cellular Component" (AmiGO) data were disclosed in

Appendix D and E; audiographs bands for differential ESTs were shown in Appendix F. Altogether, 160 ESTs were evaluated (107 over and 53 under the similarity score of 40 bits). ESTs having BLASTX scores '40 bits' and above were taken as basis to discuss in terms of their possible functions and roles in *C.arietinum-A.rabiei* interaction system. To get the whole view, the distribution profile according to AmiGO (Table 3.6 and 3.7) and MIPS (Munich Information Center For Protein Sequences) Functional Catalogue classes (Table 3.8 and Figures 3.20 and 3.21) of these ESTs were discussed briefly. The number of ESTs of clones considered here are: 6 for RGA-DDRT-PCR-trials, 39 for RGA-PCR, 33 for DDRT-PCR, 21 for RGA-DDRT-PCR and 8 for GSP-RT-PCR. The predictions related to possible "Molecular Function", "Biological Process" and "Cellular Component" (AmiGO) were assigned by considering the information given in the most similar hits of BLASTX result mainly. These information were gathered from AmiGO annotations; Interpro, Pfam, SMART, Uniprot and conserved domain (NCBI) data of the hit sequences. For many of the ESTs, overlapping functions/processes and component data were observed. For such cases, the most emphasized function was used to address the class of the EST in terms of AmiGO and MIPS classes. So, some ESTs could be transferred from one class to another.

Classification based on "Cellular Component" showed that about 25 % of all the ESTs are membrane associated (Table 3.6). This is not surprising, since in a eukaryotic cell most of the proteins related to transport, signal transduction, receptors and cellular interaction are located in a membrane. In addition to this, experiments concerning RGAs (RGA-RT-PCR and RGA-DDRT-PCR) were specifically focused to amplify ESTs, most of which have membrane associated protein products. About 35 % of all of the ESTs have cytoplasmic or organelle protein products: ribosomal and chloroplast proteins are most abundant. About 8 % of ESTs code nucleus located products, whereas only one EST product is located in cell wall (pectin esterase). The cellular component of the protein products coded by 38 % of ESTs is unknown.

Grouping according to "Molecular Function" is not straightforward: many of the proteins have more than one domains having different functional activities. So, the numbers of the activity columns in Table 3.7 are overlapping for some of the ESTs. For example, a protein -like helicase- can have both nucleic acid binding activity and catalytic activity or another one –like chaperon- can have both protein binding activity and ion binding activity.

Table 3.6 Predicted classification according to "Cellular Component" for 107 ESTs having homology above 40 bits.

Experiment	Total # of ESTs	Membrane***	organelles and cytoplasm					nucleus	cell wall	Unknown (including cellular and intracellular)
			cytoskeleton	ribosome	Chloroplast	Mitochondria	Others			
RGA-DDRT-PCR-trial	6	3	0	0	0	0	0	2	0	2
RGA-RT-PCR	39	9/8*	2	4/2*	4	1	4	1	0	18/16*
DDRT-PCR	33	9	0	1	8	1	2	4	0	12
RGA-DDRT-PCR	21	5/4*	0	2	1	3	2	1	0	7
GSP-RT-PCR	8	1	1	0	1	2	0	1	1	2
Total	107	27 25*	3	7/5*	14	7	8	9	1	41 /39*
			39 / 37***							

(*) if similar or homolog ESTs were represented as one within the same experiment; (**) if similar or homolog ESTs were represented as one among experiments, (***) general membrane + organelle membranes if indicated.

Table 3.7 Predicted classification according to "Molecular Function" for 107 ESTs having homology above 40 bits

Experiment	Total # of ESTs	Catalytic act.	Binding					structural	Transporter activity	Motor activity	Unknown
			Ion	Protein	Nucleic acid	Nucleotide	Others				
RGA-DDRT-PCR-trial	6	2	1	0	2	1	0	0	1	0	1
RGA-RT-PCR	39	14/12*	2	5/4*	5	7/6*	5	4/2*	2	2	4
DDRT-PCR	33	17	3	4	3	4	1	1	7	0	4
RGA-DDRT-PCR	21	9	2	5	1	1	3	2	1	0	5/4*
GSP-RT-PCR	8	6	0	1	1	1	1	1	1	0	0
Total	107	48 46*	8	15 14*	12	14 13*	10	8 /6* 4**	12	2	14 13*
			75 or 73*								

(*) if similar or homolog ESTs were represented as one within the same experiment; (**) if similar or homolog ESTs were represented as one among experiments.

However, a general idea about the profile of molecular activity of the ESTs can be obtained. According to Table 3.7, about 45 % of the ESTs products are enzymes; i.e., they have catalytic activity. In all of the five experiments, ESTs coding products with catalytic activity have a considerably higher numbers of representatives. About 70 % of the ESTs may code proteins having various binding activities most of which are protein binding, ion binding or nucleic acid binding activity. ESTs coding proteins of unknown activity are about 13 % whereas structural and transporter activities compromise 7 % and 11 % of total ESTs, respectively.

Grouping of the products of ESTs according to "Biological Process" is also not straightforward: many of the proteins have more than one biological function. Table 3.8 or Figure 3.20 show only one predicted function, which may define the most characteristic role under the view of BLASTX data and literature. The classes in this table are represented as MIPS classes. Matches of functional categories with the MIPS catalog were done by using "Mapping of MIPS Functional Catalogue to GO" (revised by Haris, 2006) and by use of literature data when necessary. Different authors may acknowledge varying biological function to a given gene depending on the study. One example for that is P450 monooxygenases, another example is FDH. So, this classification may vary if other roles are also considered. For that reason, detailed discussion about biological role of ESTs was provided separately for each class in Section 3.11. Class "unclear" indicates ESTs coding proteins of varying functions none of which can be decided as primary role.

When all experiments are considered collectively, the largest groups are "Protein Fate" (20) and "Metabolism" (18). These are followed by "Unknown" (16), "Cell Rescue, Defense and Virulence" (7), "Transcription" (7), "Transport" (7), and "Energy" (6). Protein Fate/synthesis related classes are the largest if taken collectively (23); when "Cell Rescue, Defense and Virulence" and "Cell Fate" groups are combined, they give rise to 12 ESTs. It should be noted that ESTs with "no significant match" were eliminated before for this analysis. So, ESTs having undetermined functions or similar to unknown proteins having no further information were classified under "unclear" and "unknown" respectively. Similar classes of proteins were obtained in SSH study of Ichinose *et al.* (2000) as described in the Introduction (Section 1.5.2.5). The functional distribution of the *A. rabiei* challenged chickpea cDNA library of Coram and Pang (2005a) consisting of 1021 ESTs was as follows: 11% cellular metabolism, 10 % protein synthesis/fate, 9 % energy, 9 % cell rescue/death/ageing (5 %) and defense (4 %), 4 % cellular

communication/signal transduction, 3 % transport, 2 % transcription, 2 % cell cycle and DNA processing. Although it is a library, which is random, the first most abundant coded EST groups are similar (protein synthesis/fate and metabolism) to the ones of this thesis. ESTs collected under "energy" may not be significantly less than the one in Coram and Pang (2005a), since some of the ESTs which belong to both "energy" or "cell rescue/death/ageing" were collected under "cell rescue/death/ageing" (Appendix E). "Transport" and "Transcription" groups were larger than Coram and Pang (2005a). It should be also noted that there may be biases in this study due to collecting the results of four experiments and due to small EST population as compared to the one of Coram and Pang (2005a). However, functional distribution of ESTs in this study shows nearly a random collection. This means that although specific primers were used for RGA-RT-PCR, RGA primers amplified a few RGAs but various transcripts in the genome. Consequently, RGA primers used in this study are quite random.

Table 3.8 Predicted classification according to "Biological Process" and MIPS for 107 ESTs having homology above 40 bits

MIPS CATEGORIES	RGA-DDRT-PCR-trial	RGA-RT-PCR	DDRT-PCR	RGA-DDRT-	GSP-RT-PCR	Total	Total*
Cellular Communication/Signal Transduction Mechanism (MIPS 30)	0	4/3*	1	0	1	6	5
Protein Fate-Folding, Modification, Destination (MIPS 14)	1	8/7*	3	7/6*	1	20	16**
Protein Synthesis (MIPS 12)	0	1	0	2	0	3	3
Transcription (MIPS 11)	0	2	4	0	1	7	7
Cell Rescue, Defense and Virulence (MIPS 32)	0	0	3	3	1	7	7
Energy (MIPS 02)	0	4	2	0	0	6	6
Cell Fate (MIPS 40)	0	4/3*	1	0	0	5	4
Interaction with the Environment (MIPS 36)	0	0	0	1	0	1	1
Biogenesis of Cellular Components (MIPS 42)	0	2	0	0	2	4	4
Transposable Elements, Viral and Plasmid Proteins (MIPS 38)	1	0	2	0	0	3	3
Development (Systemic) (MIPS 41)	0	0	1	0	0	1	1
Cellular Transport, Transport Facilitation and Transport Routes (MIPS 20)	1	2	2	1	1	7	7
Metabolism (MIPS 01)	0	5	8	4	1	18	18
unknown	3	6	4	3	0	16	16
unclear	0	1	2	0	0	3	3
TOTAL	6	39	33	21	8	107	101

(*) if similar or homolog ESTs were represented as one within the same experiment; (**) if similar or homolog ESTs were represented as one among experiments.

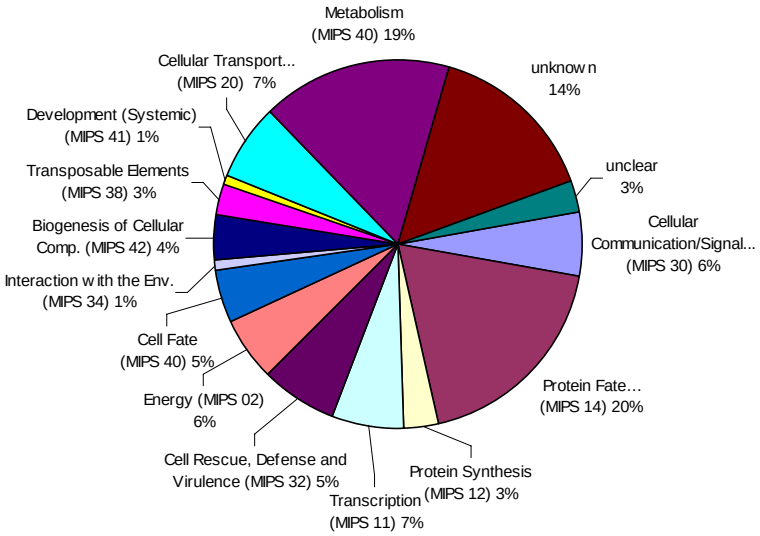

Figure 3.20 Distribution of functional categories for ESTs having similarity score over 40 bits based on MIPS classes, if ESTs from all experiments are taken into consideration.

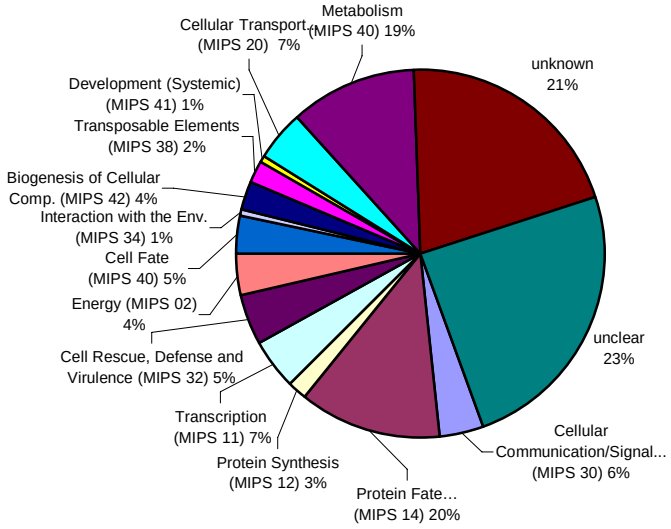

Figure 3.21 Distribution of functional categories for all 160 ESTs based on MIPS classes; if ESTs from all experiments are taken into consideration

14 ESTs with "no significant similarity" are provided in Appendix E. In addition to them, there are a number of ESTs annotated to certain functions with a homology score lower than 40 bits. These ESTs may be also as important as the ones with predicted functions and may be subject to further analysis. Briefly, 39 ESTs showed similarity to some proteins: 3 ESTs derived from RGA-DDRT-PCR trials, 14 ESTs derived from DDRT-PCR, 16 ESTs derived from RGA-RT-PCR, 6 ESTs derived from RGA-DDRT-PCR and none for GSP-RT- PCR experiment (Appendix E, Table E.2). If these 39 ESTs (3 of them are unknown) are assumed to be "unclear" due to low similarity scores and the 14 ESTs without significant similarity are considered as "unknown", the functional distribution for all 160 ESTs may be drawn as Figure 3.21. As a conclusion, nearly 60% of the ESTs (the ones having similarity score above 40 bits) could be annotated with a predicted biological function. This proportion was about 50% in the library of Coram and Pang (2005a).

3.11 Discussion of Functional Groups of ESTs in Terms of Literature

In this section, the possible role of the relatively important ESTs in chickpea defense upon *A.rabiei* attack was discussed in terms of literature. Information from annotations of InterPro, Pfam, AmiGO, NCBI and other databases (Table 2.20) were also used and provided in parenthesis as reference, such as "IPR…" for InterPro, "GO:…" for AmiGo, "smart…" for SMART and "cd…." or "COG…" for NCBI. As explained in the Introduction, the defense responses of chickpea against *A.rabiei* can not be described by generalizations. There are many overlapping and diverging characteristics of this interaction system (Figure 1.6) with other systems, i.e., plant X hemibiotrophic-biotrophic pathogens, wounding response and abiotic stress responses. Literature was considered as the following order of importance and availability: chickpea and *A.rabiei* interaction, chickpea and other necrotrophic pathogen interaction, other plants and necrotrophic-hemibiotrophic-biotrophic pathogens interaction, wounding and abiotic stress responses.

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Since some of the ESTs have multiple functions that can be classified also in other MIPS classes or in different functional groups of other systems, the following grouping should be considered as a broad interpretation. The products of ESTs which could be annotated with a biological process were placed in the model of Coram *et al*. (2007); so that a raw picture of gene products which may have a role in *C.arietinum* - *A.rabiei* host-pathogen system was constructed (Figure 3.22). ESTs obtained in this study may code for the following proteins as shown in Figure 3.22: pectinesterase (PE), cellulose sytnhase (CES), FK506-

binding protein peptidyl prolyl isomerase (FKBP-PPIase), aldolase epimerase (AE), cleft lip and palate transmembrane protein 1 (CLPTM1), methionine synthase (MS), fumarylacetoacetate hydrolase (FAH), 4-coumarate-CoA ligase (4CL), alanine racemase (AR), RAS-like GTP-binding protein (RAS-GTP), SLL2/SAMMtase: SLL2-S9-protein/SAM-dependent methyltransferase, caffeic acid O-methyl- transferase (COMT), 1-deoxy-D-xylulose-5-phosphate reductoisomerase (DXR), terpene cyclases (TC), uroporphyrinogen decarboxylase (UROD), early-responsive to dehydration protein 4 (ERD4), chlorophyll a/b binding protein (CAB), photosystem I subunit VII (PSI-VII), a low molecular weight transmembrane subunit of photosystem II (PsbY), inorganic pyrophosphatase (Ipp), ADP-heptose:LPS heptosyltransferase activity protein (RfaF), elongation factor 1-beta (EF1B), Death Associated Protein-5 (DAP-5), eukaryotic initiation factor 4-gamma (eIF4G), large subunits of ribosomal proteins (L22/17/35), ubiquitin extension protein (UE), ubiquitin conjugating enzyme (E2), ubiquitin 10 (UBQ10), voltage-dependent-gated anion channels (VDAC), formate dehydrogenase (FDH), dicer-like protein (DCL), enhanced silencing phenotype protein 4 (ESP4), SNF2 related protein (SNF2), dihydroorotase (DHOase), reverse transcriptase (RT), hAT-like transposase (hAT), dynein light chain protein (DLC), amino acid permease (AAP), actin related protein (ARP), glucose 6-phosphate/phosphate translocator (GPT), papain cysteine proteinase (PCP), protease-associated domain containing peptidase (PA), cytochrome P450 monooxygenase (P450), quinone oxidoreductase (QR), EIN3-binding F-box protein 1 (EBF1), hyaluronan-binding protein (HABP), RNA-binding protein (RBP), tetratrico peptide repeat having protein (TPR), ubiquitin-associated domain having protein (UBA), UBA-like nascent polypeptide-associated complex chain α (NACα), protein degradation in the ER protein 1 (DER1), enzymatic resistance protein (eR), calcineurin B-like (CBL), mitogen activating protein kinase (MAPK) and receptor-like kinase (RLK). Gene abbreviations given by Coram and Pang (2007) is the same as stated before in the Introduction (Figure 1.6); such as calmodulin-like protein (CAM), catalase (CAT), cationic peroxidase (CP), glutathione *S*-transferase (GS),leucine zipper protein (LZP), protein kinase (PK), translational activator (TA), elicitor-induced receptor protein (EIRP), guanosine triphosphate binding protein (GTP), polymorphic antigen membrane protein (PAMP) and superoxide dismutase (SD).

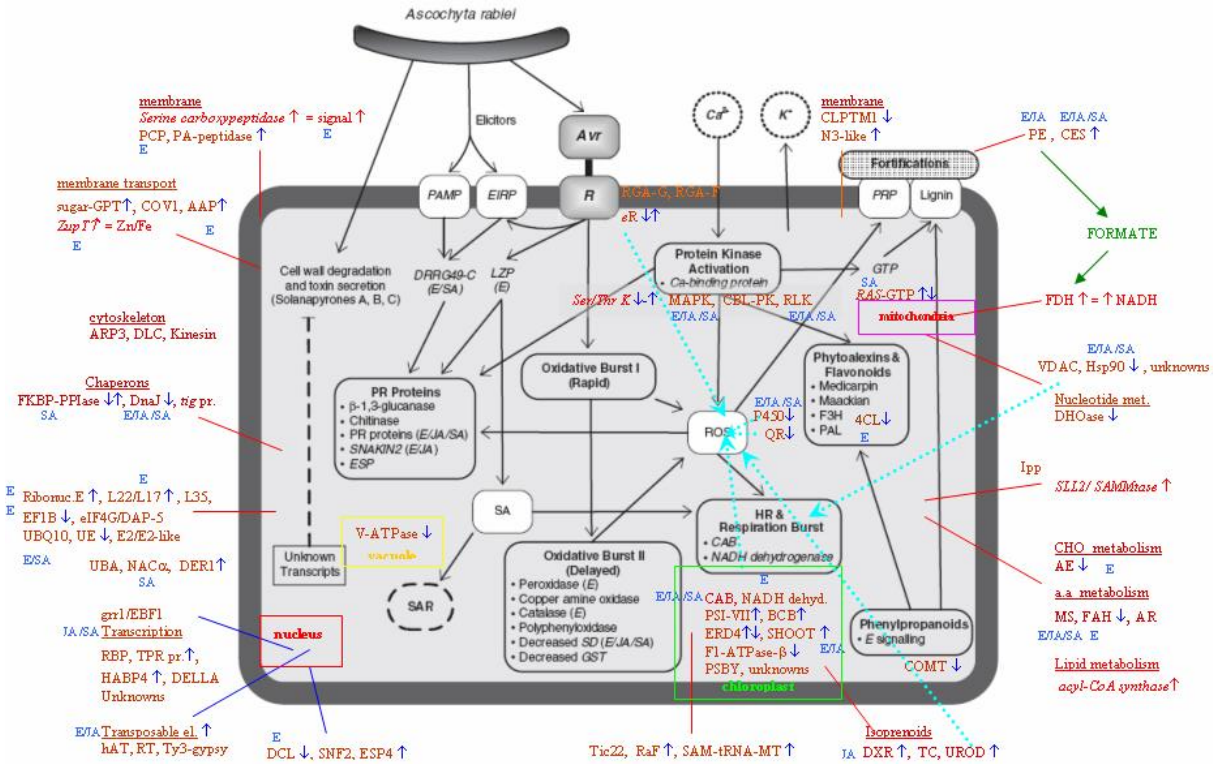


Figure 3.22 ESTs of this study were located on the chickpea's defence model by Coram *et.al.*(2007). Abbreviations were indicated in the text. '↑' or '↓' is confirmed ,'↑' or '↓' is unconfirmed up-or down regulation. 'SA', 'E' or 'JA' represent effect of SA/E/JA disclosed in the literature stated in Section 3.11, dashed blue lines indicate a possible link.

The ESTs located in Figure 3.22 were discussed in the following sub-sections. In this figure, red colored "↑" or "↓" represents confirmed up or down regulation, the blue colored "↑" or "↓" unconfirmed up or down regulation, "↑↓" represents variable data with respect to up or down regulation, SA/E/JA represent effect of SA/E/JA disclosed in the literature on the expression of the gene stated in the subsections of Section 3.11. Dashed blue lines indicate a possible link of the EST with the given event - such as ROS production- or another EST. The real functions of many of the ESTs in the chickpea-*A.rabiei* interaction can be investigated in a further work such as expression analysis with micro/macroarray, full-length cloning or silencing. Therefore, this picture may be a starting point to predict the possible role of these ESTs in this complicated network.

3.11.1 ESTs in MIPS 30: Cellular Communication/Signal Transduction Mechanism

This group contains EST such as RGA-DDRT-PCR derived R46; DDRT-PCR derived 1940; GSP-RT-PCR derived HRP; RGA-RT-PCR derived 2, 402, 863 and undetermined B20 (Appendix D, E, and F). Ser/Thr kinase like EST-R46 was discussed in Section 3.8. Other EST which showed significant similarity to CBL (calcineurin B–like)-interacting protein kinase having Ser/Thr protein kinase domain is EST-B20. A chickpea mRNA for protein kinase sequence (AJ131048) is available having a molecular function of protein Ser/Thr kinase activity (UniProtKB:Q9ZRU3_CICAR). This sequence, Ser/Thr protein kinase (DY475384, Mantri *et al.* 2007), ESTs B20 and R46 are not similar to each other showing that these may code for different proteins having Ser/Thr protein kinase domain.

EST-HRP has homology with some receptor protein kinases (such as RLKs) although HRP primers were designed to target HR-induced protein ESTs (Section 3.5). It is known that, a RLK (ERECTA)-mediated signaling is required in Arabidopsis for resistance to necrotrophic fungus *Plectosphaerella cucumerina* (Llorente *et al.* 2005). Schenk *et al.* (2000) indicated that except one receptor protein kinase (upregulated upon SA), other receptor protein/receptor-like kinases were downregulated upon SA, MJ, E or incompatible fungal pathogen *A. brassicicola*. Two RGA ESTs, EST-2 (derived from WIPK primers) and EST-863, were found to be similar to protein kinases having Ser/Thr protein kinase domain: 863 is 69 % similar whereas EST-2 is 91 % similar to CaMPK1 of chickpea (ABF82263.1 or DQ659098.1). So most likely RGA-RT-PCR analysis resulted in derivation of a known chickpea MAPK; by keeping in mind that they may be different despite the high homology. MAPKs are reported to be involved in defense responses.

Arabidopsis AtMPK6 was induced by elicitors from bacteria and fungi (Nuhse *et al*., 2000). Schenk *et al.* (2003) observed induction of AtMAPK3 at 24 and 48 h and than repression at 72 h in local and distal tissues, respectively; after inoculation of Arabidopsis with incompatible necrotrophic pathogen *A. brassicicola*. Some MAPKs was shown to be upregulated upon SA and MJ (AtMAPK3; Schenk *et al*., 2000) and E treatments (De Paepe *et al*., 2004) in Arabidopsis. A constitutively active MAPK, called NtMEK2DD, elicits HR-linked cell death in tobacco (Kuhurana *et.al*., 2005). In this study, the amplicon of EST-2 existed in all of the bulks B1, B2 and B3. As mentioned before, B1 represented plants infected with various isolates separately (aggressive and mild isolates), B2 represented mild isolate infected plants and B3 as uninfected plants (Table 2.10). Observation of EST-2 in all of these bulks should not be directly interpreted such that it is expressed constitutively since different MAPKs may have varying levels of expression upon stimuli. To confirm the MAPK expression in chickpea upon *A.rabiei* infection, comparison of the infected/uninfected time point samples separately with Real-Time qRT-PCR or with Northern Blot analysis is required. The expression may change with respect to time but this may not be obvious due to bulking by taking the dilution effect and end-product observation with PCR.

EST-402 is highly similar to DELLA proteins, one of the group of GRAS (GAI, RGA and SCR) which are plant-specific transcriptional regulatory proteins having important roles in signal transduction (Bolle, 2004). Since they are transcription factors, they could also be collected under MIPS 11, i.e. transcription. They are involved in hormone (ex: giberellic acid) mediated signaling upon stimuli, as stated by "Mapping of MIPS Functional Catalogue to GO" assignments (revised by Haris, 2006). *Solanium lycopersicum* GRAS transcripts was shown to be accumulated upon *P. syringae* pv. tomato infection and silencing of one of them -SlGRAS6- impaired tomato resistance (Mayrose *et al.* 2006). On the other hand, Venu *et al.* (2007) observed 11 copies of a GRAS transcripts only in uninfected rice plants after fungus *Rhizoctonia solani* infection (sheath blight). So, these data suggest that expression of GRAS transcripts is variable and unique to each situation. In this study, EST-402, which may have a role in gene expression, was observed in all the bulks B1, B2 and B3. Therefore, the same reasons stated for EST-2 would be applicable for EST-402 also.

The final EST under this class is EST-1940 which was observed in uninfected sample C1 (10 h) but less in infected sample (H3) and is similar to GTP-binding proteins or RAS

GTPases. Ichinose *et al.* (1999) reported that a rac type (ELR26) small GTP-binding protein showed enhanced expression in *A. rabiei* inoculated leaves. Coram and Pang (2006) observed very slight down regulation of a small GTP-binding protein (DY396367) at 24 h and then a recovery at 72 h in moderately resistant chickpea (FL) after inoculation with *A.rabiei*. The same GTP-binding protein was upregulated in chickpea FL upon SA treatment (Coram and Pang , 2007). The EST 1940 did not show any significant similarity to either chickpea GTP-binding proteins (10334502) or chickpea partial mRNA for RAS-like protein (6469128). This EST was analyzed in the first step of screening of Real-Time qRT-PCR analysis and showed 1.75 and 1.88 fold upregulation in bulked samples of FLIP84-92C(3) plants infected with PI and PII. Since these were under 2.00 fold no further analysis was done. The contradiction that this EST was obtained initially in control plant and then later time point in infected sample of ILC195 in DDRT-PCR; but upregulation in bulked samples of FLIP84-92C(3) can be due to i) bulking of samples (dilution effect) which hides the changes in time-point expressions in Real Time qRT-PCR analysis ii) different chickpea line infected with different pathotypes (different infection conditions and genotypes) in DDRT-PCR and Real-Time qRT-PCR analysis or, iii) EST-1940 being as a false positive band (which was only observed in one of the control and one of the infected samples)- a possible problem of DDRT-PCR.

3.11.2 ESTs in MIPS 14: Related to Protein Fate- Folding, Modification, Destination

This group contains EST such as RGA-DDRT-PCR derived 1868, 1901, 2132, 1793, 1758; DDRT-PCR derived 1508, 1611, 1990; 1934; GSP derived EDS1-4; RGA-RT-PCR derived 645, 937 and 997 and undetermined ESTs 350, B20-2 and D8-4 (Appendix D, E, and F). Serine carboxypeptidase like EST 1868 was discussed in Section 3.8. EST-1508 (in infected samples H1, H2, H3) can also be grouped under MIPS class 30. It is similar to a set of proteins having "Protease-associated (PA) domain C_RZF-like" domain. This domain is associated with family of M80/M33 amino- and carboxypeptidases and pyrolysin family of subtilases (Siezen and Leunissen, 1997)-including plant subtilases (cucumusin) involved in plant pathogen defense and development (Mahon and Bateman, 2000). This domain may have role in substrate determination of peptidases or protein–protein interactions (Mahon and Bateman 2000). The Real-Time qRT-PCR analysis of EST-1508 with bulked samples resulted in a upregulation of 1.89 and 1.79 fold in PI and PII in FLIP84-92C(3) plants as mentioned before, for ESTs having upregulation under 2.0 fold were not analyzed further. To understand the expression profile of this important EST,

time-point based analysis should be performed since it may be different as compared to bulked samples.

Some ESTs show homology to genes of proteins related to ubiquitin. EST-D8-4 whose, expression type is not known, is highly similar to a set of plant proteins having F-box domain and similar to EIN3-binding F-box proteins which were indicated as SCF ubiquitin ligase complex and ubiquitin-protein ligase activity. This EST covers only the LRR region. It is also similar to F box proteins EBF1 and EBF2; these proteins interact with EIN3 and EBF1 overexpression resulted in insensitivity to E in Arabidopsis (Potuschak *et al.*, 2003). Under the view of BLASTX data, this EST may have E3 ligase activity. Other two ESTs B20-2 and 350 were highly similar to each other (92%, considering aligned parts) and highly similar to ubiquitin and UBQ10. Both of them have two ubiquitin (cd01803) domains; namely, Ubiquitin- Ribosomal L40 and Ubiquitin-Ribosomal S27. Three other ESTs (645, 2132 and 1934) are similar to ubiquitin, especially to ubiquitin extension proteins. These can be also put under MIPS 12. Ubiquitin extension proteins consist of ubiquitin fused to one of the ribosomal proteins (Ribosomal L40 or Ribosomal S27) (Bachmair *et al.*, 2001). The last EST related to ubiquitin is EST-937 which has homology to a set of proteins having a domain of unknown function called as DUF1782 or pfam08694, UFC1, ubiquitin-fold modifier-conjugating enzyme 1. UFC1 is an E2-like enzyme forming an intermediate complex with activated ubiquitin-fold modifier 1 (Ufm1), a ubiquitin-like (UBL) post-translational modifier (Komatsu *et al.*, 2004). Ubiquitin, ubiquitin-like and polyubiquitins were also reported to have altered expression upon biotic stimuli: In non-host pathosystem of *A. thaliana* and oomycete pathogen *P. infestans*, Huitema *et al.* (2003) observed upregulation in a transcript similar to ubiquitin by microarray analysis. Although UBQ10 (or SEN3, senescence-associated protein 3; Park *et al.*, 1998) is a widely used reference gene in normalization (Karen *et al.*, 2006), Blanco *et al.* (2005) indicated that UBQ10 was induced by auxin, E and fungus infection and was one of the genes that were early up-regulated by SA. Coram and Pang (2007) reported that several numbers of polyubiquitin and ubiquitin transcripts were upregulated only in resistant IC genotype upon ACC treatment (E precursor). Ditt *et al.* (2006) indicated that two ubiquitin extension proteins were down regulated in *Arabidopsis* upon *Agrobacterium tumefaciens* inoculation after 48 h. Jones *et al.* (2002) indicated that a ubiquitin extension protein related to defense was one of the most abundant transcripts in cacao (*Theobroma cacao*). The expression type of EST-B20-2 and EST-350 (ubiquitin/UBQ10) is (up or down) not known. Although RGA-RT-PCR derived EST-645 is the same as RGA-DDRT-

PCR derived EST-2132 (ubiquitin extension protein), the former was observed in all sample bulks (B1, B2, B3) and the latter was observed in uninfected bulks (C3, u). Despite this contradiction, it should be noted that bulks B1 and B2 also contain additional samples of different time points that might contribute to the difference in expression pattern. EST-1934 (ubiquitin extension protein), which was a polymorphic marker found in Section 3.7, was observed in uninfected bulks (C1 and C2). In Real-Time qRT-PCR analysis for bulked samples of FLIP84-92C(3) plants, this EST revealed not a significant induction (lower than 2.00 fold) and no further analysis was done. The contradiction that this EST was obtained initially in control plant but then observed upregulation in bulked plants can be explained due to i) different chickpea genotypes infected with different pathotypes, ii) dilution effect in Real-Time qRT-PCR analysis as explained in Section 3.11.1. EST-937 (UFC1) was observed in bulks B2 (mild isolate infected) but not in B1 (various isolates infected, including aggressive ones); so this profile may be a differential expression in between these bulks. To understand whether expression of ubiquitinilation elements significantly change in *C.arietinum-A.rabiei* interaction or not, further expression analysis is required.

Two clones from the same RGA-DDRT-PCR band (EST-1793 in infected samples H3 and I; and EST-1758, latter probably co-migrated) are found to be 87 % similar to each other and have ubiquitin-associated (UBA) domains. EST-1793 has also another region called DER1 (protein Degradation in the ER in yeast; Knop *et al.*1996). Although, their function are not well-known, UBA domains, which have a ubiquitin binding site, are located in some kinases, enzymes of ubiquitination, nucleotide excision repair (NER) protein Rad23 and DDI1 (DNA Damage-Inducible) protein (Bertolaet *et al.*, 2001). Another one is EST-997 having high homology to UBA-like nascent polypeptide-associated complex (NAC), α-chain (α-NAC) and a NAC domain (pfam01849). The function of NAC is still not clear but eukaryotic NAC protein (α-NAC and β-NAC heteromer) has been suggested to be involved in sorting and translocation of nascent polypeptide chains into endoplasmatic reticulum (Rospert *et al.*, 2002, Yan *et al.* 2005). NAC was proposed also to be involved the in ubiquitination pathway (Spreter *et al.*, 2005). α-NAC, over expressed or free from dimerization with β-NAC, could function also as a transcriptional co-activator (Yotov *et al.*, 1998, Spreter *et al.* 2005). Inoculation of Arabidopsis cell cultures with an oncogenic Agrobacterium resulted in downregulation of α-NAC at 48 hpi (Ditt *et al.*, 2006). Morel *et al.* (2005) observed that a transcript similar to α-NAC*e* protein was up-regulated during symbiotic relationship between the fungus *Paxillus involutus* and birch tree (*Betula pendula*). EST-997 was observed in bulked sample B1 (various isolates infected, including

aggressive ones) but not in B2 (mild isolate infected). This difference may be confirmed by further expression analysis.

Some ESTs similar to molecular chaperons were also collected in this class: EST-1990 is highly similar to peptidylprolyl isomerases (PPIases) and has FKBP_C, FKBP-type peptidyl-prolyl cis-trans isomerase conserved domain (pfam00254). PPIases are chaperons that catalyze "*cis–trans isomerisation of peptide bonds N-terminal to proline residues in polypeptide chains"* (Shaw, 2002). Two of them are cyclophilins and FK506 (immuno suppressant drug, Stoller *et al*., 1995) binding proteins (FKBPs) (Fanghänel and Fischer, 2004). Most of the reports are about the involment of cyclophilin type PPIases in defense responses of plants but not about FKBPs. The study of Krüger *et al.* (2004) was the only report found in the literature search indicating the role of a FKBP in plant-microorganism relationship: an EST similar to FKBP-type PPIase was slightly upregulated in a symbiotic interaction between *Q. robur* and *Piloderma croceum* (Krüger *et al.* 2004). A FKBP type PPIase was upregulated upon BTH at 24 hpt in the study of Pasquer (2005). Schulz (2004) suggested a role for a FKBP (*TWD1*; twisted dwarf1) in BR signaling in *A. thaliana*. EST-1990 was observed in uninfected plant samples (C3 and u); however Real-Time qRT-PCR analysis with bulked samples resulted in a upregulation of 1.89 and 1.79 fold in PI and PII infected bulked FLIP84-92C(3) samples. This contradictory result can be explained by similar reasons as explained before for EST-1934 in Section 3.11.2. Another FKBP-type EST is EST-Eds1-4 that is not similar to EDS1 (see Section 3.5) but similar to ribosome-associated molecular chaperone trigger factor (*tig*) which has low homology to other FKBPs and no known binding affinity to FK506 or rapamycin as other FKBPs (Stoller *et al*., 1995, Maruyama *et al.,* 2004). Kokkinos (2006) indicated a *tig* type chaperone family protein differentially expressed upon virus infection in sweet potato (*Ipomoea batatas* L.). Since two FKBP-type ESTs were derived in this study, the unknown role of FKBPs in plant defense should be investigated further. The last chaperon-like EST, EST-1901 (in uninfected samples C3 and u), is similar to a set of plant proteins having two domains; namely, DnaJ central domain (pfam00684) and DnaJ C terminal region (pfam01556). DnaJ (*Escherichia coli* ortholog of heat-shock protein 40, HSP40) is a co-chaperone acting with molecular chaperon DnaK (*E. coli* ortholog of heat-shock protein 70, HSP70) in protein folding, assembly, translocation across membranes and preventing aggregation of unfolded polypeptide chains (Greene *et al.*, 1998; IPR008971, Han and Christen, 2004). NAC, HSP70, HSP40 and related chaperonins work cooperatively (Hartl and Hayer-Hartl, 2002). Coram and Pang (2007) reported that a DNAJ-like protein (DY475488) was upregulated

only in moderately resistant FL chickpea upon MJ treatment. Alfano *et al.* (2007) showed that infection with *Trichoderma hamatum* 382 which is a biocontrol fungus against bacterial spot (*Xanthomonas euvesicatoria*) of tomato (*S. lycopersicum* L.) resulted in up- and downregulation of two DnaJ chaperones, respectively. However, Asselbergh *et al.* (2007) showed that a DnaJ domain-containing protein was upregulated in Sitiens (ABA deficient tomato, see Introduction) at 8 hpi with *B. cinerea* as compared to 0 hpi. Therefore, it may be predicted that attack of *A.rabiei* isolates may resulted in suppression of some of the necessary protein folding for defense. As a result, three different chaperon/co-chaperon-like EST were obtained from chickpea responding to *A.rabiei* which should be confirmed in the future.

The last EST under this class is EST-1611 (in infected samples H1 and H2) which is similar to *G.max* SHOOT1 protein whose function is not known but it has a tetratrico peptide repeat (TPR) region important for protein-protein interactions. This EST also has a domain called PDZ_signaling (cd00992) which is found in signaling proteins and probably mediating specific protein-protein interactions. This EST could not be assigned to a certain biological function. Considering the definition of TPRs, PDZs and GO annotations of other hits it maybe related to various processes such transcriptional control, signaling, protein transport, protein folding. Besides observing it in infected samples, an upregulation of 1.80 and 1.72 fold in PI and PII infected FLIP84-92C(3) bulked samples was measured, respectively. It was not analyzed further (<2.00 fold). The unknown role of SHOOT1 protein in chickpea/*A.rabiei* interaction may be studied further.

3.11.3 ESTs in MIPS 12: Related to Protein Synthesis

Class MIPS 12 is related to protein synthesis (ribosomal, translation and translational control proteins). Several ESTs were similar to ribosomal proteins of large subunit (L1 to L44) and similar to eukaryotic translation factors. Under this class, ESTs such as RGA-DDRT-PCR derived 1806, 1881 and 2325; RGA-RT-PCR derived 38, 154, 405, 417 and DDRT-PCR derived 1619 were collected (Appendix D, E, and F). EST-1881 is similar to a set of proteins having SAM-dependent tRNA (guanine-N(7)-)-methyltransferase activity, located probably in chloroplasts. These enzymes transfer methyl group from SAM to a guanine residue in a tRNA molecule (GO:0016423). No reports were available on the role of tRNA (guanine) methyltransferases in biotic or abiotic stress responses of plants.

However, isolating this EST in infected samples (H3, I) may show that tRNA (guanine) methyltransferases have role in protein turnover during plant defense response.

Some ESTs may code proteins related to translation. EST-1806 is similar to a set of proteins having a conserved domain called ribosomal protein L22/L17e (cd00336). ESTs 405 and 417 have a conserved domain ribosomal_L35Ae (pfam01247). EST-154 is similar to both ribosomal protein L33 and L35a. L22 helps folding and stabilizing of 23S rRNA and in legumes it is encoded in the nucleus (IPR001063). EST-2325 is similar to putative elongation factor 1-beta (EF1B) and has EF1_GNE, EF-1 guanine nucleotide exchange domain (pfam00736). EST-38 is similar to translation initiation factor proteins having MA3 domain (pfam02847) and its size is larger than expected having RLLRrev primers on both ends. Such sequences were discussed in Section 3.6. MA3 domain exists in DAP-5 (Death Associated Protein-5), eIF4G (eukaryotic initiation factor 4-gamma) and in MA-3 protein (Shibahara *et al.*, 1995; IPR003891; Pontig, 2000). DAP-5, a homologue of the eIF4G and PCD regulator (Levy-Strumpf *et al.*, 1997) and MA-3 (Shibahara *et al.*, 1995) are involved in apoptosis (IPR003891). In translation, mRNA binding is mediated by eIF4F complex composed of eIF4E, eIF4A and eIF4G; and this step is regulated during apoptosis (Morley *et al.*, 2005).

There are many reports on altered expression of ribosomal proteins, translation initiation factors and elongation factors upon various biotic and abiotic challenges. Coram and Pang (2005b) observed that pathogen-induced translation initiation factor nps45 was non-differentially expressed by resistant chickpea accession IC and susceptible LA upon *A. rabiei*. Ditt *et al.* (2006) observed a downregulation of a large number of the ribosomal proteins (including L22 and L35a) and an elongation factor related protein upon *A. tumefaciens* infection in *A.thaliana*. They concluded that downregulation of a large number of ribosomal proteins indicated slowing down the productive cell metabolism. Zou *et al.* (2005) showed that during HR–associated resistance of soybean (*Glycine max*) against *P. syringae* pv. *glycinea* strains (*avrB)* chloroplast-associated genes were downregulated at 8 hpi, including the several ribosomal proteins (including L35), translation elongation and translation initiation factors. Expression of some ribosomal proteins (ex: L33) and initiation/elongation factors (ex: eIF4) were reported to be differentially regulated by E in *A. thalina*. (Van Zhong and Burns, 2003). Schafleitner and Wilhelm (2002) showed that in *Castanea sativa*, elongation factor 1 alpha-like protein mRNA, L18a and L17 mRNA were wound-responsive. Under the view of literature, translation plays one of the key roles in

plant-pathogen interaction and its components are subject to regulation upon pathogen challenge. There are a wide variety of situations with respect to expression, even under similar conditions; i.e., same plant and same pathogen, contrasting data may exist. ESTs 405 and 417 were observed in all of the bulks, B1, B2 and B3; however, 154 was observed in B2 (mild isolate infected). Although 154, 405 and 417 are about 80 % similar to each other and ribosomal L proteins, the lack of this band in B1 might be taken a difference as explained for the case of EST-2. EST-1806 was observed with higher intensity in infected bulks, H3 and I, indicating a possible L22 upregulation. EST-38 (observed in bulks B1, B2, B3) is a potential EST for the future work due to having MA3 domain observed in some apoptosis related proteins as well as EIF4G. Another elongation factor EF1B like EST-2325 was observed in uninfected samples (C3 and u). As a general rule, no idea can be constructed about expression of these ESTs unless evaluating time points separately with respect to mock infection as indicated for other differentially expressed transcripts.

EST-1619 is similar to proteins having CafA- Ribonucleases G- and E-domain, involved in translation, ribosomal structure and biogenesis (COG1530). Most of the reports on these proteins are related to *E. coli*. Endoribonuclease RNase E degrades and processes mRNAs and stable RNAs (Morita *et al.*, 2005). RNase E and its smaller homolog Ribonuclease G (CafA) cooperate in processing of the 5' end of 16S rRNA (Li *et al.,* 1999). Some ribonucleases are known to be involved in defense and stress responses. For example legumes have some PR-10 proteins having ribonuclease activity (Bantignies *et al*., 2000). Liu *et al.* (2006) reported a stress- and pathogen-inducible PR10 having ribonucleolytic and antimicrobial activity against *Pyricularia oryzae*, from *Solanum surattense.* Although this report is not directly related to CafA- Ribonucleases G- and E-domain containing proteins, it is clear that ribonucleases are involved in defense and stress responses. Therefore, the upregulation of EST-1619 in DDRT-PCR analysis (in infected samples H1, H2 and H3) and in Real-Time qRT-PCR analysis in bulked samples (2.01 and 1.91 fold upregulation in PI and PII infected FLIP84-92C(3) bulks respectively; and 1.48 fold upregulation in *ank6* infected ILC195 bulk) may indicate an increased mRNA turnover process to synthesize the necessary proteins for defense response upon *A.rabiei* attack.

3.11.4 ESTs in MIPS 11: Transcription

This group contains ESTs such as DDRT-PCR derived 1536, 1555, 1595, GSP-RT-PCR derived Rar1-4 and RGA-RT-PCR derived 31, 353 and 826 (details of ESTs in Appendix

D, E, and F). Some ESTs were observed to be similar to proteins having RNA-binding motifs: EST-31 (observed in B1, B2 and B3) and 826 (observed in B1 but not in B2) are not similar to each other but both have RNA recognition motif domain (smart00360, RRM). EST-353 (in B2 but not in B1) is similar to an unnamed protein product having RNA recognition motif. EST-31 has very high homology and is exactly similar to many other clones; but the primers observed in the sequence were useful to determine its identity. In general, there are several conserved RNA-binding motifs in RNA-binding proteins (RBPs) such as RNA-recognition motif (RRM or consensus-sequence-type RNA-binding domain - CS-RBD or RNP motif-), Zn-finger motif and the double-stranded-RNA-binding motif (DSMR) (Burd and Dreyfuss, 1994; Alba and Pages, 1998). RRM is found in proteins involved in post-transcriptional processes (such as splicing factors, poly(A)-binding proteins, GRPs, etc.) which bind pre-mRNA, mRNA, pre-rRNA, small nuclear RNAs or chloroplast RNAs (Alba and Pages, 1998). RBPs respond to internal and external signals such as wounding, ABA, dehydration and SA (Sturm, 1992; Gomez *et al.*, 1988, Naqvi *et al.*, 1998). A RRM-containing protein was reported to be downregulated upon inoculation with *P. syringae* (Tao *et al.*, 2003; Ditt *et al*., 2006). Huitema *et al.* (2003) observed an induction in a glycine-rich RBP upon non-host *P. infestans*. Schenk *et al.* (2000) indicated that in Arabidopsis an RNA-binding protein cP29 was upregulated upon SA; but not upon MJ or incompatible fungal pathogen *A. brassicicola*. Another EST similar RBPs is EST-1595 (in infected samples H2 and H3) which is very highly similar to a set of functionally variable proteins having a conserved HABP4_PAI-RBPI (pfam04774) domain (HABP4, hyaluronan-binding protein 4 and PAI-RBPI, type-1 plasminogen activator inhibitor mRNA-binding protein; IPR006861). One of the hits is a transcription factor of *Vicia* sp. HABP4 binds to RNA with a lower affinity then hyaluronan (Huang *et al.*, 2000; IPR006861). Heaton *et al.* (2001) reported that PAI-RBPI may be involved in regulation of mRNA stability. Kottapalli *et al.* (2006) reported that hyaluronan/mRNA binding protein gene is located in rice chromosome 5 (Region 2) which has a minor QTL for submergence tolerance together with genes for bacterial blight resistance. Due to the importance of RPBs in plant defense responses and possible involment in transcription, especially EST-31 and EST-1595 may be included in future work for confirmation of expression profiles and full-length cloning.

ESTs 1536, Rar1-4 and 1555 can be discussed together due to their homology to related proteins collected under nucleic acid metabolic process. EST-1536 is highly similar to helicases (C-terminal), argonaute (AGO), dicer proteins and Ribonuclease III. It may code

for probably a dicer-like (DCL) protein. Dicer and AGO proteins are key components of RNAi. Reports of RNAi response against bacterial and fungal pathogens are limited. Dunoyer *et al.* (2006) indicated that Arabidopsis *dcl1* mutants immunity to *A. tumefaciens* infection. Arabidopsis mutants *dcl4-4* and *ago1-36* have a cluster of disease resistant genes regulated by transcriptional activation and RNAi for fungi resistance (Yi and Richard, 2007). *P. syringae* flagellin-derived peptide elicitor induces a microRNA, miR393, which suppresses F-box auxin receptors in a DCL1-dependent manner, resulting in restriction of bacterial growth in Arabidopsis (Navarro *et al.*, 2006). Some of the RNA helicases are upregulated (De Paepe *et al.*, 2004) but some of them are downregulated (Van Zhong and Burns, 2003) in *ctr1-1* mutants or E treatment of wild type Arabidopsis. EST-1536 was observed at higher intensity in uninfected samples (C1, C2, C3). So it could be predicted that dicer-like transcripts may be suppressed upon *A.rabiei* attack. EST-1555 (in infected samples H2 and H3) is similar to a plant protein product of unknown function. However, it has a low similarity to ESP4 (Enhanced Silencing Phenotype 4) which is an Arabidopsis protein involved in mRNA cleavage and polyadenylation specificity factor complex. Defects in ESP proteins affect RNAi based processing of FCA (a RBP controlling flowering time in Arabidopsis) (Macknight *et al.*, 1997; Herr *et al.*, 2006). Rar1-4 has homology to helicases and SNF2 related proteins (e.g. SNF2/SWI and SWI2/SNF2) related to transcription regulation, DNA repair-recombination-replication and chromatin remodeling (Eisen *et al.*, 1995; Linder *et al.*, 2004, Havas *et al.*, 2001, Boyko and Kovalchuk, 2008; IPR000330). For example, SWI2/SNF2 controls gene expression through DNA methylation: ddm1-induced hypomethylation (mutant allele of a SWI2/SNF2 DNA helicase DDM1: Decreased DNA Methylation 1) resulted in activation of disease resistance genes (Stokes, 2002) and retrotransposons (Kato *et al.*, 2004; Boyko and Kovalchuk, 2008). Smith *et al.* (2007) identified a nuclear SNF2 domain–containing protein acting in the upstream process of siRNA production. Isolation of these ESTs shows that RNAi may be also important for gene activation/repression in chickpea/*A.rabiei* interaction, and, therefore especially ESTs 1536 and Rar1-4 may be investigated further.

3.11.5 ESTs in MIPS 32: Related to Cell Rescue, Defense and Virulence

This group contains ESTs such as RGA-DDRT-PCR derived R48, 2191, 2296 and 1804; DDRT-PCR derived 1606 and 1571 and GSP-RT-PCR derived FDH which was discussed in Sections 3.8 and 3.9 (Appendix D, E, and F). Most of them (1751, 1606, 2191, 1804 and FDH) can be sub-classified under detoxification MIPS 32.07. EST 1571, 1606, 1804 and

2191 can also be put under MIPS 02, energy. The first EST to be discussed is EST-2296 which is similar to proteins having Hsp90 domain (pfam00183). The role of Hsp90 chaperons in plant defense was stated in the Introduction. *P. syringae* infection causes upregulation of HSP90.1, which interacts with RAR1 and SGT1 for RPS2-mediated disease resistance in Arabidopsis, but not of other HSP90s (Takahashi *et.al*., 2003) meaning that almost identical cytosolic HSP90s might have different functions (Sangster and Queitsch, 2005). Mantri *et al.* (2007) noted that an HSP protein was repressed in salt-tolerant chickpeas at 24 hpt upon salt treatment. EST-2296 was observed in uninfected bulks (C3, u) indicating a possible downregulation of this EST in *A.rabiei* response. Up or downregulation of HSP90s upon biotic and abiotic stress may depend on different cell compartments (one of the hits of EST-2296 is a mitochondrial HSP90) and treatments.

Under the sub-group '*detoxification*", the first EST is 1571 which is similar to a set of plant proteins having NADP-dependent quinone oxidoreductase, QR, (NADPH2 + quinone = NADP + semiquinone) activity which is a part of the zinc-containing alcohol dehydrogenase family of enzymes (IPR002364). QRs scavenge toxic radical semiquinones (Mano *et al.*, 2002). ROS triggered lipid peroxidation end-products (such as 4-hydroxy-2-nonenal, HNE, and 4-hydroxy-2-hexanal, HHE) are reduced by an NADPH:QR called PI-z-crystallin in Arabidopsis (Mano *et al.*, 2002; Taylor *et al.*, 2003). This EST is similar to ripening-induced auxin-dependent putative QR (Raab *et al.*, 2006). On the contrary to most of the literature information and the general role of ROS mediating for some necrotrophic pathogens (in Section 1.5.1), the possible downregulation of EST-1571 (intensity more in uninfected samples C1, C2, C3) may lead to idea that toxic radical semiquinones mediating cell death may be necessary for *C.arietinum-A.rabiei* interaction system.

EST-2191, EST-1804, and EST-R48 are all observed in uninfected samples. All of them are highly similar to cytochrome P450 monooxygenases or related unknown/ hypothetical proteins; but not to each other. In addition, ESTs 2191 and 1804 have a conserved domain called Cytochrome P450 (pfam00067, p450). However, BLASTN hit of R48 is similar to ribosomal RNA. Haem-thiolate containing cytochrome P450s (P450s) catalyze monooxygenase reactions (Graham-Lorence *et al.*, 1995; pfam00067). P450s act in plant biochemical pathways including defense compounds (isoflavonoids and phytoalexins), hormone signaling (gibberellins, GB; BR; SA and JA), and lignin (Werck-Reichhart 1995; Li *et al*., 2007). A known P450 involved in camalexin biosynthesis is Arabidopsis PAD-3, effective against necrotrophic pathogens (Thomma *et al.*, 1999; Zhou *et al.*, 1999; Ferrari

et al., 2003; Glazebrook, 2005). Cytochrome-like proteins were the most common ESTs in the category "cellular metabolism" *A.rabiei* challenged chickpea library (Coram and Pang, 2005a). Coram and Pang (2007) found that a P450 (DY475136) was highly upregulated at 27 hpt in all chickpea genotypes (resistant, moderately resistant, susceptible) upon ACC treatment. Cyp83B1, a P450, was induced upon *A. brassicicola* (Schenk *et al.*, 2003), *P. infestans*, SA and MJ treatments (Mikkelsen *et al.*, 2003, Huitema *et al.*, 2003). Kottapalli *et al.* (2007) noted that JA-inducible cytochrome P450s were downregulated under bacterial leaf blight infection (*Xanthomonas oryzae* pv. *oryzae* or *Xoo*, shortly) in resistant rice cultivar with respect to susceptible one. They proposed that the resistance against *Xoo* might be due to oxidative burst resulting from down regulation of ROS scavenging genes. Similarly, P450s were downregulated upon E in Arabidopsis; so De Paepe *et al.* (2004) suggested that this increased cell death signal by inhibiting ROS scavenging. Observing downregulation of two differently prepared samples, i.e.; ESTs 2191 or 1804, and EST-R48, may show a situation as explained in the last three examples on the contrary to the other studies for chickpea (Coram and Pang, 2005a). It should be kept in mind that P450s are involved in diverse reactions and also very abundant in chickpea (Coram and Pang, 2005a). Consequently, expression pattern of each should be variable in defense responses.

EST-1606 similar to plastocynanine-like blue copper proteins having Cu_bind_like plastocyanin-like domain (pfam02298) is probably bound to membrane. Both of the terms "Blue copper protein precursor" or "blue copper-binding protein" (BCB) are used in the literature. The first group of blue (type 1) copper proteins are plastocyanins (PC) that transfer electrons from the cytochrome *b6/f* complex of photosystem II (PSII) and to the P700+ photoreaction centre of photosystem I (PSI) (Sato *et al.*, 2003). In this system, excess electrons, not used by the carbon reactions, result in the production of ROS, leading to disruption of photosynthesis and leaf senescence (Desikan *et al.*, 2001; Schöttler *et al.*, 2004). ROS formation repress genes such as PSII, PSI, cytochrome (cyt)-bf and PC (Foyer and Noctor, 2003; Gray *et al.*, 2003; Schöttler *et al.*, 2004). Other members of BCB proteins are uclacyanins, stellacyanins, and plantacyanins (Nersissian *et al.*, 1998). ToxA, a cell-death inducing proteinaceous toxin of the necrotrophic fungus of *Pyrenophora tritici-repentis* was shown to target a plastocyanin in wheat (Tai *et al.*, 2007). A plastocyanin-phytocyanin like protein was induced by H_2O_2, wilting, UV, hairpin, senescence (Desikan *et al.*, 2001) and by MJ but not significantly upon *A. brassicicola* infection or SA or E (Schenk *et al.,* 2000). De Paepe *et al.* (2004) described a phytocyanin or uclacyanin downregulated upon E treatment in wild type and in *ein2-1* mutant (E-

insensitive) of Arabidopsis and they suggested that it is involved in redox reactions during primary defense responses of plants. Consistent with most of the literature mentioned, the EST-1606 was observed in infected samples (H1, H2, H3) which may indicate ROS production as a defense response.

On the contrary to most of the literature information and the general role of ROS mediating necrotrophic pathogens (Section 1.5.1), the possible downregulation of the EST-1571 (QR), possible downregulation of ROS scavenging ESTs (p450; 2191, R48 and 1804) and possible upregulation of ROS producing EST-1606 (BCB) may be interpreted such that toxic radical semiquinones and ROS mediating cell death may be necessary for *A.rabiei* resistance in chickpea. Due to the fact that no complete resistant chickpea plants against *A.rabiei* exist, this situation may be also interpreted like that: in order to survive on chickpea, the necrotroph *A.rabiei* mediates host cell death by suppressing enzymes for scavenging toxic compounds and ROS. To understand the true picture, this expression profiles of these enzymes should be confirmed.

3.11.6 ESTs in MIPS 02: Related to Energy

This group contains ESTs DDRT-PCR derived 1623 and 1601; and RGA-RT-PCR derived 253, 853, 892 and 955 (Appendix D, E, and F). ESTs, namely DDRT-PCR derived 1623 and 1601, RGA-PCR derived 253, 892 and 955, can be grouped under MIPS class energy, specifically under photosynthesis. EST-1623 is highly similar to a set of proteins concerning photosystem I (PSI) subunit VII and it has two conserved domains: cd01916 or ACS_1 (Acetyl-CoA synthase, ACS), and CHL00065 or psaC (PSI subunit VII). EST-253 is highly similar to a chlorophyll a/b binding (CAB) proteins, one of which is from chickpea (CAA10284.1). EST-892 and EST-955 are both similar to PSBY protein. EST-1601 is highly similar to a set of ATP synthases (ATPases) with a conserved domain F1-ATPase_beta (cd01133) located in chloroplast.

In photosynthesis, NADPH (by ferredoxin-$NADP^+$ reductase) and ATP (by ATP synthase) are produced *via* light-harvesting and electron transport mechanism carried out by photochemical reaction centers PSI and PSII with the help of two other major complexes cytochrome *b6f* and LHC (a light-harvesting protein complex) in chloroplast thylakoid (Eckardt, 2001). LHC, which transfers light energy to PSI and PSII, consists of non-covalently bound chlorophylls a, b and CAB proteins (Liu and Shen 2004, IPR001344).

PSI subunit VII is coded by the gene psaC (Chitnis and Nelson, 1992; Buchanan *et al.*, 2000). PsbY, whose function is unknown (Kawakamia *et al.*, 2007), is a low molecular weight transmembrane subunit of PSII (Gau *et al.*, 1998). The membrane-bound ATPases synthesize ATP *via* ion flux across the membrane or work in reverse by hydrolyzing ATP to create a proton gradient across a membrane. There are three main types, namely F-, V- and A-ATPases (Cross and Muller, 2004; IPR005722). In plants, F-ATPases (F1F0-ATPase: catalytic F1- and membrane-embedded proton channel F0-ATPase) produce ATP using the proton gradient generated in mitochondrial membrane by means of oxidative phosphorylation or in chloroplast membrane by means of photosynthesis (IPR004100, IPR005722).

Most of the studies reported downregulation of photosynthesis related genes in biotic stress, an example for an exception may be the induced resistance for rust inoculation in broad bean (*Vicia faba* L.) (Murray and Walters, 1992). Matsumura *et al.* (2003) indicated that *P.infestans* elicitor (INF1)-treatment to *Nicotiana benthamiana* resulted in downregulation of photosynthesis related genes such as CAB protein, PSII protein and ATPase. Similarly, Zou *et al.* (2005) observed that HR-inducing strain *P. syringae* pv. *glycinea* resulted in downregulation of chloroplast-associated genes in soybean (*G. max*) at 8 hpi, including several CAB proteins, PSI subunits and PSII proteins including psbY and ATPase β chain. These studies proposed that pathogen attack damaged PSII reaction centers and this in turn resulted in HR-associated oxidative burst which restricted pathogen growth (Matsumura *et al.*, 2003, Zou *et al.*, 2005). Some other studies reported that SA and JA (Walters and Heil, 2007), MJ (De Rosa *et al.*, 2005), H_2O_2 (Desikan *et al.*, 2001) and E (Van Zhong and Burns, 2003) repressed photosynthesis-related gene expression as well. However, a CAB protein was found to be upregulated upon SA and MJ treatment (Schenk *et al.*, 2000). Coram and Pang (2005b) used CAB protein as a control in their microarray analysis due to its housekeeping activity and they noted no significant fold change in resistant IC and susceptible LA chickpea genotypes after *A. rabiei* inoculation. However, Coram and Pang (2006) noted that *A.rabiei* infection of chickpea resulted in upregulation of CAB (at 6 and 12 hpi) but down regulation of an ATPase and PSII reaction centre I protein (at 48 hpi) in resistant IC genotype and downregulation of CAB and ATPase C chain in susceptible LA genotype. In a proteomics assay, Subramanian *et al.* (2005) identified a differentially regulated protein similar to ATPase β subunit from necrotrophic pathogen *Leptosphaeria maculans* inoculated resistant *Brassica carinata* at 48 hpi with respect to susceptible *B. napus* (canola).

As a result, most plants response to bacterial or fungal pathogens by decreasing photosynthesis related gene expression. The situation of EST-1601 (F1-ATPase-β) which was observed in uninfected samples (C1, C2, C3) is inline with these data. However, in the case of EST-1623 (PSI subunit VII) the situation seems to be reverse (observed in infected samples H1, H2, H3). On the other hand, it is not easy to conclude that the expression levels of ESTs 253, 892 and 955 are constitutive (observed in all of the bulks B1, B2, B3) without confirming their expression level by comparing infected and uninfected samples taken at the same time points. Besides, the above microarray data of Coram and Pang (2006) showed that downregulation of photosynthesis related transcripts may not be related to resistant chickpea genotypes only. Thus, further evidences are needed to conclude whether or not the repression of photosynthesis is required for resistance response of chickpea upon *A.rabiei* attack. For that reason, besides confirmation of expression levels of ESTs 1601, 1623 and 253, expression analysis of photosynthesis related genes such as cytochrome *b6f* and ferredoxin-$NADP^+$ reductase have to be investigated.

The last EST under this section is EST-853 (observed in B1, B2, B3) which is highly similar to alternative NAD(P)H dehydrogenase-2 and putative internal rotenone-insensitive NADH dehydrogenase with a conserved domain (COG1252, Ndh). In the aerobic respiration, inner mitochondrial membrane harboring complexes (complex I, II, II, IV) catalyze electron movement from NADH and $FADH_2$ to oxygen creating proton gradient across this membrane which drives ATP synthesis (*via* ATPase; complex V) (Buchanan *et al.*, 2000). As the electron donator several NAD(P)H dehydrogenases are one of the main sources of ROS production in mitochondria (Moller, 2001; Rasmusson *et al.*, 2004). However, the contribution of rotenone-insensitive type 2 NAD(P)H dehydrogenases is unclear (Moller and Kristensen, 2004). Some reports show altered expression of NADH dehydrogenases in plant-pathogen system. Coram and Pang (2006) reported that a NADH dehydrogenase was upregulated in resistant IC but downregulated in moderately resistant IL and susceptible LA genotypes of chickpea upon *A.rabiei* infection. Gibly *et al.* (2004) reported that a NADH dehydrogenase was downregulated in resistant tomato cultivar inoculated with *Xanthomonas campestris pv vesicatoria* (AvrXv3). Van Zhong and Burns (2003) showed that in *A.thalina*, a NADH dehydrogenase ND4 was negatively regulated by E. Under the view of the literature, NADH dehydrogenases are one of crucial proteins located in a regulatory point in biotic stress. Due to importance of NADH dehydrogenases

in the ROS production, further analysis is required to find out its expression profile upon *A.rabiei* attack.

3.11.7 ESTs in MIPS 40: Cell Fate

This group contains ESTs related to apoptosis; such as DDRT-PCR derived EST-1937 and RGA-RT-PCR derived 104, 860, 1072 and 860 (details of ESTs in Appendix D, E, and F). DDRT-PCR derived EST-1937 observed in uninfected samples (C1, C2, C3) is similar to several plant membrane proteins having the conserved domain Cleft Lip and Palate Transmembrane Protein 1 (CLPTM1, pfam05602). This domain was also found in a human protein CRR9 (Cisplatin Resistance Related gene 9) which is associated with CDDP- induced apoptosis (Cisplatin, anticancer agent) (Yamamoto *et al.* 2001). The biological role in plants and molecular function of CLPTM1 domain containing proteins are unknown.

RGA-RT-PCR derived ESTs 104 and 1072 are similar to two chickpea R genes, which were determined in Fusarium resistant chickpea lines by Hüttel *et al.* (2002), RGA-F (CC-NB-LRR) and RGA-G (TIR-NB-LRR), respectively. EST-1072 is also similar to NBS-LRR type disease resistance proteins of *Vicia faba* and chickpea (Palomino *et al.* 2006). Both of them have NB-ARC domain (APAF-1, R gene products and CED-4) which is a signaling motif of both plant R gene products and regulators of cell death in animals, i.e. caspases CED-4 and APAF-1 (Khurana *et al.*, 2005; Chinnaiyan, 1997, Zou *et al.*, 1997, Nicholson and Thornberry, 1997; Van der Biezen and Jones, 1998). NB domain consists of kinase 1a, 2a and 3a motifs (Van der Biezen and Jones, 1998). Hüttel *et al.* (2002) obtained RGA-F and RGA-G by combination of primers P-loop (S1) and conserved GLPL(T/A) amino acid motif from genomic DNA. Palomino *et al.* (2006) obtained NBS-LRR type RGA similar to RGA-G by similar primer sets from DNA. In this study, EST-104 was obtained by P-loop (S2) / NBS domain primers and EST-1072 was obtained by P-loop / GLPL(T/A) motif (AS) primers from cDNA. This shows that, these RGA primers are specific enough to obtain putative R genes. RGA-F and RGA-G may also be important for Ascochyta blight; i.e., if RGA-F and RGA-G are functional, they may recognize common elicitors (maybe plant origin) upon *A.rabiei* or *F.oxysporum* attack, both of which are necrotrophs. Since they were derived from cDNA in this thesis as compared to other studies (Hüttel *et al.,* 2002; Palomino *et al.,* 2006), they may be also functional. Although these ESTs were observed in all of the bulked samples (B1, B2, B3) and no differential

expression of chickpea RGAs were studied in the literature, differential expression of RGAs in chickpea is still a question and may be investigated further.

ESTs 860 and 1158 are similar to papain cysteine proteinase (CAA08906) obtained from chickpea at 4 dpi with *A.rabei* (Gitmans, 1998). EST-860 is a longer sequence than EST-1158. Their aligned parts are nearly 94 % similar to each other. Both of them are observed in all samples (B1, B2, B3). So this means that RLLRfwd primer can also target the largest group of papain family cysteine proteases. The role of cysteine proteases in plant defense responses were stated in the Introduction. Coram and Pang (2007) reported two cysteine proteases (DY475458 and DY475066) upregulated in resistant chickpea IC genotype by ACC. Giri *et al.* (1997) reported an induction of protease activity in resistant chickpea cultivar roots upon challenge with *F. oxysporum* f.sp. *ciceris.* Two papain cysteine proteinases of *Nicotiana benthamiana* were upregulated upon hemibiotrophic fungus *Colletotrichum destructivum* infection at the switching point from biotrophic to necrotrophic growth (Hao *et al*., 2006). From this literature, it is obvious that papain cysteine proteinases and therefore protein degradation are important especially for necrotrophs, and this maybe the reason for the observation of this ESTs in all samples. However, the expression study based on time profile analysis is required. Since this sequence was reported to be isolated form *A.rabiei* infected chickpea plants by another study also, papain cysteine proteinases may have a role in defense responses in chickpea such as processing defense related proteins and degradation of possible pathogen effectors.

3.11.8 ESTs in MIPS 34: Related to Interaction with the Environment

The only EST to be mentioned under this category is RGA-DDRT-PCR derived EST-2166 (in uninfected samples, C3 and u) similar to several plant proteins having DUF221 domain (pfam02714, domain of unknown function). It is highly similar to an unknown protein and several ERD4 (Early-Responsive to Dehydration 4) proteins (Appendix D, E, and F). ERD genes are induced by dehydration stress (Kiyosue *et al.*, 1994) and ERD4 is over expressed in plants having 35S:DREB1A (Seki *et al.*, 2001) which is an ERF family of transcription factor acting on drought responsive *cis*-acting elements and involved in plant stress signaling (Agarwal *et al.* 2006). No information related to involment of ERD4 in plant defense responses was found in the literature. Although EST-2166 was observed in uninfected samples before, Real-Time qRT-PCR analysis revealed that 1.71 and 1.62 fold upregulation in PI and PII infected FLIP84-92C(3) bulks, respectively. Upregulation may

be evaluated as increased stress signaling for related gene expression. Yang *et al.* (2007) observed induction of another ERD gene, ERD6, in canola (*Brassica napus* L.) upon necrotrophic fungal pathogen *S. sclerotiorum* at 12 hpi, and they discussed this gene under ROS defenses. This observation may be useful to predict a possible role of ERD4 in *A.rabiei*-chickpea interaction which could be investigated in the future work.

3.11.9 ESTs in MIPS 32: Biogenesis of Cellular Components

This group contains ESTs namely, RGA-RT-PCR derived 179, 447; and GSP-RT-PCR derived Eds1-1 and PE (details of ESTs in Appendix D, E, and F). EST-179 (observed in B2 but not in B1 bulk, Table 2.10) is similar to dynein light chain proteins having a conserved domain, Dynein_light, Dynein light chain type 1 (pfam01221). Dynein light chains are located in microtubule motor proteins dyneins, which transport specific cargo along the microtubules by means of ATP (Buchanan, *et al.* 2000). Although, their role is not known exactly, dynein light chains interact with various proteins in fungi and animals (Emi *et al.* 2005). EST-447 (observed in all B1, B2, B3) shows low similarity to a putative chromosome-associated kinesin protein (like EST-304). These proteins have role in microtubule-based movement, organelle transport, and formation of mitotic spindles (IPR001752, Buchanan *et al.*, 2000). No reports were found related to kinesins in plant defense responses. However, isolation of two motor proteins which move in the opposite directions during transporting cargo along the microtubules (Buchanan *et al.,* 2000) may be regarded as an indication of normal cellular traffic. Another structural EST is EST-Eds1-1 is very highly similar to proteins unnamed/actin related 3 (ARP3)/actin-like proteins having the conserved domain of ACTIN (cd00012). Since it is not similar to the only disclosed *C. arietinum* mRNA for actin (AJ012685), this EST maybe a novel actin related/actin-like sequence for chickpea. ARP3 (or DIS1, Distorted Trichomes 1; trichome: plant epidermal hair) is a subunit of ARPII/3 complexes (Robinson, 2001), which may be target of bacterial virulence factors directly or indirectly (Boquet and Lemichez, 2003).

The GSP-RT-PCR derived EST Pe-3 is similar to proteins having pectin esterase (or pectin methylesterase, PME) domain (pfam01095). This shows that the GSPs which were designed for the conserved regions of some legume species (Table 2.16) were successful to derive an EST from PME family but less similar to the known chickpea PME (CAE76633.2). PME esterifies pectin, one of the main components of plant cell wall, into pectate and methanol. Plant PME takes role in fruit ripening (Di Matteo *et al.*, 2005;

IPR000070). Pelloux *et al.* (2007) stated that PMEs might directly interact with virulence factors or catalyze reactions to give rise to products important in plant defense such as methanol (signal in plant–herbivore interactions; Von Dahl *et al.*, 2006) which is one of the precursors of formate (Hourton-Cabassa *et al.*, 1998) and pectin-derived compounds that elicit plant defenses or modify the cell wall charge and pH of apoplasmic environment. A PME was upregulated upon E, JA and pathogen attack (Gachomo *et al.* 2003), but another putative one was downregulated upon E treatment in Arabidopsis (De Paepe *et al.* 2004). A putative PME together with other several cell wall modification enzymes, was induced in *Arabidopsis* during systemic defense responses against *A. brassicicola* (Schenk *et al.*, 2003). Two PME-related proteins were downregulated in *Arabidopsis* cell cultures upon an oncogenic *A. tumefaciens*, but their cDNA were upregulated upon *P. syringae* (Tao *et al.*, 2003; Ditt *et al.*, 2006). Pfaff and Kahl (2003) mapped a set of PE markers in the LG5 of chickpea. These findings show that although most of the time PME is upregulated in plant defense responses there are still some controversial situations. Since EST-Fdh-3 was upregulated in this thesis, EST-PE which is involved in formate biosynthesis from methanol, may be predicted to have role in the upstream of FDH. The expression profile of the EST Pe-3 was not studied in this study and any other literature related to *A.rabiei*-chickpea interaction. For that reason and due to potential function in the defense reactions, PE should be considered for further analysis.

3.11.10 ESTs in MIPS 38: Transposable Elements, Viral and Plasmid Proteins

Under this group, three ESTs will be covered in this section all of which were observed in infected plant samples, namely, RGA-DDRT-PCR-trials derived EST-R50, DDRT-PCR derived EST-1479 (in H2 and H3) and EST-1528 (in H2 and H3) (Appendix D, E, and F). EST-R50 is similar to the putative chickpea polyprotein (CAC44142.1) of Ty3-gypsy like retrotransposon (AJ411810.1) having an integrase core domain region (rve). Long terminal repeats (LTR) of these retrotransposons of chickpea (Staginnus *et al.*, 1999) were used by Pfaff and Kahl (2003) for mapping of chickpea genome (Section 1.5.2.3). Rajput and Upadhyay (2007) also disclosed a *Ty*3-*gypsy* LTR retrotransposon *CARE*1 (*C. arietinum* retro-element 1) in chickpea (DQ239702). EST-1479 is similar to a peptidase aspartic, active site of *Medicago trunculata*, i.e., a retro element having reverse transcriptase (RT_LTR) and retroviral aspartyl protease (RVP_2) regions. EST-1528, on the other hand; is highly similar a set of proteins concerning hAT-like transposases. *hAT* transposons (hobo, Activator, Tam3) are mobile genetic elements common to eukaryotes, including

plants (Kempken and Windhofer, 2001) and they found to be essential for normal growth and global gene expression in *A. thaliana* (Bundock and Hooykaas, 2005).

Hüttel *et al.*(2002) reported that because of degenerate character of RGA primers, obtaining retro-transposable elements is often for chickpea. Besides this, Boyko and Kovalchuk (2008) stated that "*activation of transposons in response to stress is common phenomenon*"; such as tobacco Tntl retrotransposon activated by HR triggering elicitors of *Trichoderma viride, Phytophthora* sp. and bacterium *Erwinia chrysanthemi* (Pouteau, 1994). Transposable elements and their derivatives within the R-genes such as *Xa21* family (Song *et al.*, 1997) and retrotransposon-related sequences such as *Pto* paralogs are important for the evolution of R genes (Michelmore and Meyers, 1998). Nimbalkara *et al.*(2006) showed that root infection by *F. oxysporum* f sp. *ciceri* in chickpea resulted in induction of several retro elements (Ty-1 copia-type retrotransposon, non-LTR retro element and GAG-POL precursor gene) in resistant lines with respect to control and susceptible lines. Schenk *et al.* (2000) indicated that a copia-like transposable element was upregulated upon incompatible *A. brassicicola* and MJ in *Arabidopsis*. One interesting report is that an unknown protein from MuDR (mutator) transposase family protein was repressed upon E treatment in Arabidopsis (Rudenko and Walbot, 2001; De Paepe *et al.*, 2004). Like in most of the above mentioned literature, these three ESTs, observed in infected samples, may code *A.rabiei* responsive retro/transposable elements. In addition, the Real-Time qRT-PCR data in the bulked PI and PII infected FLIP84-92C(3) samples were 1.84 and 1.93 for EST-R50; and 1.61 and 1.39 for EST-1528, respectively. Although these are lower than 2 fold conservation of this expression pattern in a different chickpea genotype-pathotype systems shows that upregulation of transposable elements in chickpea defense may be necessary. Due to importance of *hAT* transposons in plant gene expression, EST-1528 may be studied further.

3.11.11 ESTs in MIPS 41: Related to Development (Systemic)

The only EST under this topic is DDRT-PCR derived EST-1490 (observed in infected samples H1 and H2) similar to N3-like proteins which are transmembrane proteins of unknown function (IPR004316) (Appendix D, E, and F). One of them (MtN3) was reported in root nodule development in Medicago truncatula (Gamas et al., 1996). An unusual R gene rice Xa13 against *Xanthomona oryzae* was shown to be homologous MtN3 (Chu et al., 2006; Liu et al. 2007). De Paepe et al. (2004) recorded downregulation of a

nodulin MtN3 family protein upon E treatment in Arabidopsis. Since these reports point a possible function of this EST in plant-pathogen interaction, EST-1490 may have role in chickpea defense against *A.rabiei*.

3.11.12 ESTs in MIPS 20: Cellular Transport, Transport Facilitation and Transport Routes

This group contains ESTs such as RGA-DDRT-PCR derived R13 and 1749; DDRT-PCR derived 1468 and 1560; and RGA-RT-PCR derived 19, 384 and 1089 and GSP-RT-PCR derived Rar1-1 (Appendix D, E, and F). EST-1468 was discussed in Section 3.8. EST-1089 is highly similar to ATP-binding cassette (ABC) transporter of Arabidopsis pathogen *P. syringae* pv. *tomato* str. DC3000. Keeping in mind that ABC transporters of plants and pathogens may be highly similar to each other (Panabières *et al.*, 2005), this EST is may be a contamination. Therefore this EST was not accounted for further analysis.

EST-R13 (in infected sample) is similar to a plastid glucose-6-phosphate/phosphate translocator (GPT). Kammerer *et al.* (1998) stated that sucrose and hexoses are converted to glucose 6-phosphate (Glc6P) which can be imported into the nongreen plastids *via* the GPT using triose phosphate (trioseP) and inorganic phosphate as counter substrates. This Glc6P is used for starch biosynthesis or transformed into trioseP *via* the oxidative pentose phosphate pathway resulting in reduction of nitrite and synthesis of glutamate (Kammerer *et al.*, 1998) which in turn may be converted into other amino acids (arginine, proline, glutamine, histidine). So, GPT action is indirectly important for synthesis of some amino acids. No special report was found for GPT activity in defense responses of plants. However, Truernit *et al.* (1996) reported induction of a sugar transporter gene after microbial challenge, elicitor treatment and wounding. Real-Time qRT-PCR data in the PI and PII infected bulked FLIP84-92C(3) samples were 1.67 and 1.74 for EST-R13. These results show that altered gene expression of carbohydrate metabolism in chickpea may also be necessary to counteract with *A.rabiei.*

EST-1560 (in infected samples H2 and H3) is highly similar to proteins having transmembrane amino acid transporter protein domain, Aa_trans (pfam01490) such as amino acid permease 7 (AAP7) of *A. thaliana*. There are many reports on amino acid transporters (AAT) or permease (AAP) especially in biotrophic fungus pathogen-plant host interactions specific for haustoria such as UfAAT (Struck *et al.*, 2004), AAT1 (Struck *et*

al., 2002) and AAT2 (Hahn *et al.*, 1997) of rust fungus *Uromyces fabae*; but examples for necrotrophs are limited. Lu *et al.* (2004) showed that a peptide/amino acid transporter was constitutively expressed in resistant and susceptible rice plants upon hemibiotroph *M. grisea*. De Paepe *et al.* (2004) indicated E-regulated AAP genes in Arabidopsis. Wounding rapidly upregulated proline transporter in *Arabidopsis* (Cheong *et al.*, 2002). These findings show that EST-1560 may have a role in *A.rabiei*-chickpea interaction and the expression profile should be confirmed.

EST-19 (observed in B1, B2 and B3) is highly similar to a protein of unknown protein function. It has two overlapping regions; namely, branched-chain alpha-keto acid dehydrogenase subunit E2 (PRK11856) and Tic22 (pfam04278). Tic22 belongs to Tic complex located at the chloroplast inner membrane and takes role in preprotein translocation at the inner envelope membrane of chloroplasts (Heins *et al.* 2002). No special report was found concerning these hits. However, in general protein transport may be affected due to defense related changes in protein traffic in plants which may be also crucial for chickpea - *A.rabiei* interaction.

Voltage-dependent-gated anion channels (VDACs) are known to realize transport of small hydrophilic molecules across the mitochondrial outer membrane (IPR001925). Hofiusa *et al.* (2007) stressed that VDACs are included in cell death in both plants and animals, supported by increased VDAC expression not only during HR-associated cell death (Lacomme and Roby, 1999). Khurana *et al.* (2005) summarized that in the early signal transduction in HR processes resulting in PCD, plasma membrane depolarization by means of anion channels may activate Ca^{2+}-VDACs or K+ channels. Increased expression of mitochondria VDACs facilitate the release of cytochrome *c* during apoptosis in mammals (Takagi-Morishita *et al.* 2002) which may be also similar in early stages of HR in plants (Lacomme and Roby, 1999). The EST-Rar1-1 was very highly similar to legume mitochondrial voltage-dependent anion-selective channel, VDAC or Porin 3. No expression profile was studied for this EST in this work. It should be kept in mind that, the VDAC role of plant-necrotrophic pathogen interaction may be different than in plant-biotrophic pathogen relationship since HR like reactions are known to facilitate for some of the necrotrophic pathogen infection (Govrin and Levine, 2000).

EST-1749 (observed in uninfected samples C3 and u) and EST-384 (observed in mild isolate infected bulk B2 but not in B1) show high similarity to proteins related to vacuolar

ATPase subunit E and have vATP-synt_E (pfam01991) conserved domain. When aligned parts taken into consideration, they are 94% similar to each other. V-ATPases (V1V0-ATPases), which are primarily located in eukaryotic vacuole members, transport solutes (salts, metabolites, carbohydrates and amino acids) and lower pH of the vacuole lumen (Buchanan *et al.*, 2000, Kluge *et al.*, 2003). Subunit E may act as a stator and mediator of interactions with regulatory subunits (Jones *et al.*, 2005). Otte *et al.* (2001) reported the importance of vacuolar (H^+)-ATPase in chickpea defense response as stated in the Introduction. An H^+-transporting V-ATPase chain E was downregulated in Pti4-expressing *Arabidopsis* plants which express Pto interacting ERF and have resistance to fungal pathogen *Erysiphe orontii* and tolerance to the bacterial pathogen *Pst* strain DC3000 (Gu *et al.*, 2002; Chakravarthy *et al.*, 2002). Under the view of these reports and by considering the fact that variable aggressiveness of *A.rabiei* isolates may affect the expression level of VDAC negatively or positively, this EST should be considered for further work firstly to confirm its expression upon *A.rabiei* attack and secondly full-length cloning of the gene.

3.11.13 ESTs in MIPS 40: Metabolism

ESTs in Primary Metabolism: This group contains ESTs such as RGA-DD-RTPCR derived 2150, 2204; DDRT-PCR derived 1943-D5, 1943(P9), 1626, 1562, 1612, 1480; RGA-RT-PCR derived 116, 427, 818 and 1422a and GSP-RT-PCR derived FPIP. EST-FPIP was discussed in Section 3.7 and 3.8. EST-1943(P9) is highly similar to several plant unknown proteins probably located in the chloroplast thylakoid membrane, one of them has a SAM dependent methyltransferase (SAMMtase) region. SAMMtases were discussed for the EST-FPIP in Section 3.7. EST-1480 (in uninfected samples C2, C3) is similar to a plant unnamed protein which has a short chain dehydrogenase (SDR) region and it may have role in cell growth related processes (GO: 0008152).

The EST-1943-D5 (in infected samples H2 and H3) is highly similar to a chloroplast located unknown plant protein having RfaF region, i.e., ADP-heptose:LPS heptosyltransferase activity which may have role in cell envelope (membrane) biogenesis. *E.coli* RfaF catalyzes the transfer of heptose sugar onto the lipopolysaccharide inner core (Gronow *et al.*, 2000). No reports were found in plant-pathogen interaction. As a general interpretation, this EST may show the role of structural variations of plant membranes during pathogen infection. Due to its unknown role, further studies may be designed to investigate its role in plant-pathogen relationship.

EST-1612, (in infected samples H2 and H3) is highly similar to cellulose synthase-like proteins which transfer glycosyl groups. It also belongs to biogenesis of cellular components (MIPS 42). Cellulose synthase A (CesA) may be a glycosyltransferase having role in cellulose synthesis (Minorsky, 2002). Cellulose synthases (Ces) were shown to be involved in many plant-pathogen interactions. Ces and Ces-like genes were differentially regulated upon different treatments in Arabidopsis such as upon incompatible *A. brassicicola,* SA, MJ and E (Schenk *et al.*2000) and upon wounding (Cheong *et al.*, 2002). Moreover, mutation of *CEV1* (*CeSA3,* Ellis *et al.*, 2002a) results in constitutive activation of E and JA signaling; increased resistance to powdery mildew, *P. syringae* pv. *maculicola* and green peach aphid (*M. persicae*) in Arabidopsis (Ellis and Turner, 2001; Ellis *et al.*, 2002b). Mutations in subunits of *Ces* resulted in enhanced resistance of Arabidopsis to bacterium *Ralstonia solanacearum* and the necrotrophic fungus *Plectosphaerella cucumerina,* probably *via* changing secondary cell wall integrity by inhibiting cellulose synthesis (Hernandez-Blanco *et al.*, 2007). The Real-Time qRT-PCR data in PI and PII infected bulked FLIP84-92C(3) samples were 1.71 and 1.33 folds, respectively. Despite these measurements were obtained from bulked samples and needed to be confirmed in a time point basis; Real-Time qRT-PCR data showed that the induction profile was conserved for different chickpea genotype and isolates. This may be related to cell wall fortification of chickpea upon *A.rabiei* attack. As stated in Section 1.5.2.5, evidences of cell wall fortification as a disease response of chickpea are somewhat contradictory in previous works. Since above examples indicate that both active and inactive Ces genes may have effects in plant defense response, further analysis should be considered for EST-1612 in chickpea-*A.rabiei* system.

Another EST related to carbohydrate metabolism is EST-2204 (in uninfected samples C3 and u) which is very highly similar to galactose mutarotase like proteins (aldose 1-epimerase) having conserved domain aldose 1-epimerase (COG0676). *"Aldose 1-epimerase interconverts D-glucose and other aldoses in between their α and β forms"* (IPR008183). Yang *et al.* (2007) observed downregulation in a aldose-1-epimerase in canola (*Brassica napus* L.) upon necrotrophic fungal pathogen *S. sclerotiorum* at 48 hpi. De Paepe *et al.* (2004) described an aldose-1 epimerase family EST which was upregulated upon E treatment in wild type with respect to untreated wild type, but downregulated with respect to *ein2-1* mutant of Arabidopsis. Due to limited reports on this gene, further studies on EST-2204 may help to investigate its role in defense reactions of plants.

Two ESTs classified under amino acid metabolism are EST-1562 (in uninfected samples C1 and C2) and EST-116 (in bulk B1 but not in B2), both of which are similar to proteins related to aminotransferases having serine-pyruvate aminotransferase/archaeal aspartate aminotransferase domain (COG0075). These two sequences are nearly 75 % similar to each other. These enzymes are also products of genes called "enzymatic resistance (*eR*) genes" named by Taler *et al.* (2004) who found two constitutively expressed photorespiratory peroxisomal glyoxylate aminotransferases, *At1* and *At2* (similar to Ser and Ala glyoxylate aminotransferases of photorespiration, SGT and AGT1, respectively) which conferred HR associated resistance to *avr* expressing downy mildew oomycete *Pseudoperonospora cubensis* in melon. In peroxisomes, generation of H_2O_2 in the upstream of SGT/AGT is important for production of ROS/NO for defense responses (Taler *et al.*, 2004; Corpas *et al.*, 2001). Ditt *et al.* (2006) showed that an aminotransferase family protein was downregulated in *Arabidopsis* cell cultures upon *A. tumefaciens*. A class V aminotransferase and an aminotransferase 2 were shown to be differentially expressed upon virus infection in tolerant rice and in compatible interaction of citrus, respectively (Brizard *et al.*, 2006; Freitas-Astúa *et al.*, 2007). Under the view of the potential importance of *eR* genes for chickpea, the contradictory expression of these ESTs (from infected and uninfected samples; a similar case in ESTs 384 and 1749) should be resolved.

Another EST classified under the class of amino acid metabolism is EST-1626 (in uninfected samples C1, C2, C3) which is highly similar to fumarylacetoacetase having the conserved domain FAA_hydrolase (pfam01557). Fumarylacetoacetate is converted into fumarate and acetoacetate by fumarylacetoacetase (fumarylacetoacetate hydrolase, FAH) in the final step in phenylalanine and tyrosine degradation (Bateman *et al.*, 2001; IPR005959). Defense related FAH was differentially upregulated in resistant rice cultivar during bacterial leaf blight caused by *Xoo*; and it could be regulated by EREBP which is triggered by submergence or pathogen elicitors (Kottapalli *et al.*, 2007). FAH is also differentially expressed during raspberry ripening (Jones *et al.*, 2000) and upregulated in rice upon salt stress (Kim *et al.*, 2007). Fumarate is a member of citric acid cycle. Hence, probable downregulation of FAH may indicate downregulation of metabolism in chickpea upon *A.rabiei* attack. Although not many reports exist on FAH, further study may be performed to confirm its expression profile and its role in defense responses of chickpea.

Another EST related to amino acid metabolism is EST-1422a (observed in all samples B1, B2 and B3) which is highly similar to methionine synthase (MS) related proteins having the conserved domain called CIMS (Cobalamine-independent methonine synthase, cd03311). CIMS catalyzes the synthesis of methionine *via* transferring of a methyl group from methyltetrahydrofolate to homocysteine (Ferrer *et al.*, 2004). An MS was significantly upregulated upon A. *brassicicola* but not significantly affected by SA, E and MJ treatment (Schenk *et al.*, 2000). A CIMS was sharply upregulated upon *Blumeria graminis* f. sp. *tritici* (shortly *Bgt*) and upon wounding in diploid wheat (*Triticum monococcum*) (Bhuiyan *et al.*, 2007). A CIMS (CD051358) was upregulated in chickpea upon dehydration stress or ABA treatment (Boominathan *et al*., 2004). Since these reports show that expression of MS may be regulated upon stimuli, the expression of EST-1422a may be studied further to figure out the role of MS in chickpea defense responses.

EST-818 (in all samples B1, B2, B2) is very highly similar to a set of unnamed proteins as well as alanine racemase family protein and has a conserved domain cd00635 found in pyridoxal phosphate (PLP) dependent enzymes. PLP-dependent enzymes take role in the biosynthesis of amino acids, amino acid-derived metabolites and amino sugars (Mozzarelli and Bettati, 2006; IPR011078). Alanine racemase converts L-alanine to D-alanine (IPR001608). It is also highly similar to a putative proline synthetase associated protein. No examples were found related to alanine racemase in plant defense response. However, a well-known example is from a plant pathogenic fungi. An alanine racemase (*TOXG*) has role in the production of the virulence factor HC-toxin of *Cochliobolus carbonum*. (Brosch *et al.*, 1995; Cheng and Walton, 2000). Proline metabolism is environmentally regulated in plants. Transgenic tobacco plants over expressing exogenous proline synthetase are tolerant to osmotic stress (Kishor *et al.*, 1995). Due to its unknown role in plant defense response, EST-818 may be investigated for future work.

EST-2150 (in uninfected samples C3 and u) is highly similar to mitochondrial dihydroorotase (DHOase) having the conserved domain DHOase (cd01294). DHOase is the third enzyme of pyrimidine biosynthesis pathway and converts carbamoyl-aspartate into dihydroorotate (Buchanan *et al.*, 2000). No examples were found that report the involment of DHOase in plant-pathogen reaction in the literature survey for this study. In their patent, Kanhonou *et al.* (2007) described a stress responsive DHOase in sugar beet (*Beta vulgaris*) for the first time. Downregulation of EST-2150 might be due to allocation of resources for defense rather than growth-related nucleotide metabolism.

EST-427 (in all samples B1, B2, B3) is highly similar to inorganic pyrophosphatase (Ipp) which hydrolyzes pyrophosphate (PPi) produced from biosynthetic reactions using ATP (Cooperman *et al*., 1992; IPR008162). A rice Ipp responsive to both fungal blast *M. grisea* and bacterial blight *Xoo* was downregulated in both compatible and incompatible interactions in fungal blast; however it was upregulated in both compatible and incompatible interactions in bacterial blight (Li *et al.*, 2006). Microarray hybridization using cDNA from a cassava resistant variety MBra685 infected with bacterial blight causing *Xanthomonas axonopodis* pv. *manihotis* resulted in upregulation of an Ipp but downregulation of a vacuolar proton-inorganic pyrophosphatase at 7 dpi (Lopez *et al*., 2005). To understand the role of Ipp in chickpea response upon *A.rabiei*, the expression profile of EST-1422a should be investigated by taking time points into consideration.

ESTs in Secondary Metabolism: This group contains the following ESTs such as RGA-DDRT-PCR derived 2296(D19-4); DDRT-PCR derived 1538, 1633 and 1642; RGA-RT-PCR derived 241. The first EST classified under the secondary metabolism is EST-1538 (in infected samples H2 and H3) which is similar to a set of proteins related to uroporphyrinogen decarboxylase (UROD) and having URO-D (cd00717) domain. UROD is one of the enzymes of the biosynthetic pathway producing porphyrin which is the precursor of heme-containing molecules such as chlorophyll (von Wettstein *et al.*, 1995; Taylor, 1998). Since light activated uroporphyrinogen can donate electrons to molecular oxygen which gives rise to ROS production; Taylor (1998) suggested that manipulation of UROD levels could be used for developing HR like lesions. For example, deactivation of maize lesion mimic gene *Les22* encoding UROD produces necrotic lesions similar to HR triggered by plant pathogens (Hu *et al.*, 1998). UROD and coproporphyrinogen oxidase (CPO) antisense RNA expression in tobacco showed increased resistance to TMV, noticed by HR-like lesions which mean that decreased tetrapyrrole synthesis induced a set of defense responses similar to HR (Mock *et al.*, 1999). The literature from biotic examples reported the downregulation of this gene. On the contrary to many other ESTs whose down/up regulation may result in ROS production observed up to now, a possible increase in EST-1538 may indicate removing of uroporphyrinogen accumulation of which results in induction of ROS. Due to the fact that HR may facilitate the invasion of some necrotrophs (Govrin and Levine, 2000), upregulation of UROD may be necessary for chickpea defense to prevent enlargement of HR like lesions and consequently limit the spread of *A.rabiei*. However, this fact should be confirmed by further expression analysis of EST.

Two of the ESTs, EST-1642 (higher intensity in infected samples H1, H2, and H3) and EST-241 (observed in B1, B2, B3) are similar to two successive enzymes of the isoprenoid (terpenoid) biosynthesis pathway. EST-1642 is similar to 1-deoxy-D-xylulose-5-phosphate reductoisomerase, DXR, and EST-241 is similar to some proteins also involved in isoprenoid metabolism such as terpene cyclases. The plastid pathway of isoprenoinds-called as MEP (2C-methyl-D-erythritol 4-phosphate) pathway – condenses pyruvate and glyceraldehyde 3-phosphate by means of thiamine pyrophoshate *via* 1-deoxy-d-xylulose 5-phosphate synthase (DXS) and the resulting compound is further processed by NADPH dependent DXR into MEP (Buchanan *et al.*, 2000). The resulting isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate, are converted to several intermediates which are further used for biogenesis of monoterpenes, sesquiterpenes and diterpenes respectively, *via* terpenoid synthases (or terpene cyclases) (Bohlmann *et al.*, 1998). Isoprenoids are up most important compounds for plants as hormones (ABA, cytokinins, GBs and BRs), photosynthetic pigments, electron carriers, membrane components, phytoalexins, antimicrobial agents, etc. (Liu *et al.*, 2005). Examples for DXR and terpene cyclases are limited for microbial pathogen and plant host interaction. DXR was up-regulated genes in aphid *Manduca sexta*-infested *Nicotiana attenuata* and MJ-elicited *Solanum nigrum* (Schmidt *et al.*, 2005). Hans *et al.* (2004) characterized a DXR up-regulated in maize roots during mycorrhization. The patent application WO/2002/064764 (Aharoni *et al.,* 2002), claimed for a terpene synthase/cyclase conferring resistance in transgenic plants against insects and microorganisms. For the above mentioned importance of isoprenoids, possible upregulation of the EST-1642 (DXR) and expression profile of the EST-241 (terpene cyclase) should be investigated to find out whether or not terpenoid production is increased in chickpea upon *A.rabiei* attack.

The last two ESTs to be discussed are similar to two well-known enzymes of phenylpropanoid metabolism. The first one is EST-2296-D19 (in uninfected samples C3 and U) which is highly similar to an unknown protein and an Arabidopsis 4-coumarate-CoA ligase (4CL), both of which have the same regions and AMP-binding conserved domain pfam00501. The second one is EST-1633 (higher intensity in uninfected samples C1, C2, and C3) which is similar to a putative caffeic acid methyl transferase. Enzymes of phenylpropanoid pathway for lignin and flavonoid branches are 4CL and caffeic acid O-methyl- transferase (COMT). Being a key enzyme of phenylpropanoid metabolism, 4CL "*catalyzes the formation of CoA esters of p-coumaric acid, caffeic acid, ferulic acid, 5-hydroxyferulic acid, and sinapic acid*" (Raes *et al.* 2003); whereas COMT, which is

involved in lignin biosynthesis, methylates "*5-hydroxyconiferaldehyde and/or 5-hydroxyconiferyl alcohol to sinapaldehyde and/or sinapyl alcohol, respectively…"* (Raes *et al.* 2003). The importance of the products of phenylpropanoid pathway, especially pythoalexins medicarpin and maackiain, was stated in the Introduction. Literature stated induction of the enzymes of this pathway. Most of these data were derived from enzymatic assays but not from expression analysis. 4CL is also involved in the biosynthesis of chickpea medicarpin and maackiain (Barz and Mackenbrock, 1994). No special data was found on the expression change of 4CL in chickpea upon *A.rabiei* infection. However, upregulation of 4CL in Arabidopsis upon *A. brassicicola* (Uhlmann and Ebel, 1993; van Wees *et al.* 2003), wounding or *P. syringae* (Lee *et al.*, 1995) and regulation by E in Arabidopsis (De Paepe *et al.*, 2004) was reported. On the other hand, the situation for other lignin related transcripts were either constitutively expressed such as CAD or down regulated such as CCOM (Coram and Pang, 2005b). Some of the enzymes in this pathway were also downregulated (CCOM, GRP) upon salt stress as mentioned in the Introduction (Mantri *et al.*, 2007). CCOM is upregulated upon *Bgt* infection in *T. monococcum* highly and upon wounding, cold, drought and salinity stresses slightly (Bhuiyan *et al.*, 2007). The situation for EST-1633 is inline with the data for chickpea. Expression of EST-2296-D19 and EST-1642 should be confirmed by further experiments before concluding that *A.rabiei* repressed both of the branches of the phenylpropanoid pathway, i.e., flavonoid and lignin. Besides, the effect of using different isolates, chickpea genotypes and experimental conditions should not be underestimated. By considering the unforeseen reasons of contradictory results; the situation related to possible upregulation of EST-1612 (cellulose synthase-like) but down regulation of EST-1633 (COMT) should be resolved, since both of them are related to production of cell wall structural element.

3.11.14 ESTs with No Functional Annotation

RGA-DDRT-PCR-trial derived EST-R21 (unknown/zinc finger family protein) and EST-R44 (unnamed protein product); RGA-RT-PCR derived EST-372 (hypothetical/transducin family proteins, WD-40 repeat; chloroplast located), EST-505 (unknown protein), EST-489 (unnamed protein product of unknown function), EST-882 (COV1-like protein in integral membrane) and EST-1998 (unnamed/leucine-rich repeat family proteins having conserved domain LRR); DDRT-PCR derived EST-1609 (hypothetical protein located in cytoplasm), EST-1477 (P7) (unnamed protein product;), EST-1465a-2 (unnamed/unknown proteins having DUF246 unknown conserved domain) and EST-1597a (putative glycosyl

hydrolase of unknown function having DUF1680 domain), RGA-DDRT-PCR derived EST-2203 (unnamed proteins having DUF966 domain) and undetermined RGA EST-453 (unnamed protein product) were grouped under this topic and further details of ESTs are provided in Appendix D, E, and F. These ESTs may be as important as other genes with previously predicted functions. As a matter of fact, further elaborative analysis can be assigned for these genes in the future.

CHAPTER 4

CONCLUSION

After long period of traditional breeding efforts and classical genetics approaches, researchers working on chickpea have started to use molecular techniques to understand resistance mechanisms against the fungus *A.rabiei*. There are many unresolved questions related to chickpea- *A.rabiei* host-pathogen interaction system; so, investigation by use of new technical approaches is an open area for research. To design follow-up studies for investigating the chickpea / *A.rabiei* interaction, the proposals of Amelina-Torregrosa *et al.* (2006) related to the transcriptomic approaches for studying defense and resistance mechanisms of legumes may be useful: i) identifying diagnostic defense and signaling ESTs (such as identification of main defense mechanisms against compatible/incompatible pathogens/treatments or identification of resistance markers), ii) high throughput EST analysis (such as identification of novel genes-networks-mechanisms), iii) "Interactome" analysis to investigate plant and pathogen transcriptomes simultaneously to understand successive "attack and defense" steps (identification of components of plant resistance or elicitors/suppressors of pathogen) and iv) combining arraying and genotyping (such as identification of polymorphisms, understanding of QTLs).

The necrotrophic character of the fungus *A.rabiei* and the polygenic character of counter defense responses of chickpea make it difficult to understand this relationship. Moreover, use of various fungus isolates which are not well-defined and various chickpea genotypes in studies contribute this complexity. Increasing the knowledge on the chickpea genome will aid to solve this problem by enlarging population of chickpea genes and ESTs.

To serve this aim, in this study ESTs from a wide range of possible molecular mechanisms were produced by means of RGA-RT-PCR, DDRT-PCR, RGA-DDRT-PCR and GSP-RT-PCR. It was generally observed that the target directed PCRs, i.e., RGA and GSP, were not so effective to get the target gene sequences. Especially high degeneracy of some RGA primers resulted in isolation of ESTs from various origins. Among them, nearly 100 ESTs

were found to be significantly similar to the data in NCBI database when BLASTX hits are considered. Collectively, it was observed that; predicted functions of the products coded by these ESTs either from *A.rabiei* challenged samples or control samples could be collected under the MIPS groups from various molecular events such as, "Protein Fate", "Metabolism", "Cell Rescue, Defense and Virulence", "Transcription", "Transport", "Energy", and "Cell Fate" in a decreasing number of members, respectively. So, it could be proposed that, these ESTs may give clues for members of pathways involved during *A. rabiei* defense. These were placed on the figure developed by Coram *et al.* (2007): signaling, ROS production, detoxification, transport, translation, protein degradation/modification, photosynthesis, isoprenoid synthesis, recognition, transcription, nucleic acid processing, transposable elements, cell wall fortification and other structural elements, metabolism of compounds such as amino acids (Figure 3.22). Besides previously known genes related to defense responses of plants, unreported transcripts which may have potential roles were isolated such as FKBP-type peptidyl-prolyl cis-trans isomerases (PPIases), SHOOT1 protein, *S*-adenosyl-L-methionine-dependent methyltransferases (SAMMtases), Cleft Lip and Palate Transmembrane Protein 1 (CLPTM1), Early-Responsive to Dehydration 4 (ERD4), transposable elements and many unknown transcripts. Besides, some ESTs which were not submitted to NCBI database before by other authors for chickpea were isolated such as the ones which may code DELLA proteins, dicer-like proteins, N3-like proteins, terpene cyclase and uroporphyrinogen decarboxylase (UROD).

This scheme arises many questions related to defense responses of chickpea to *A. rabiei* to be investigated in the future: how do HR and ROS play role in the defense responses? Is photosynthesis down-regulated upon *A.rabiei* attack? What is the role of transposable elements in chickpea defense response? Are there any functional R and eR gene products for recognition and defense? What kind of transcription factors and signaling cascades are involved in defense responses of chickpea? Is there any RNAi dependent gene regulation in the chickpea- *A. rabiei* interaction system? Which enzymes are differentially regulated in cell wall fortification? Do isoprenoids confer resistance against *A. rabiei*?

To generate EST markers for aiding marker assisted selection (MAS) and find out position of putative defense related ESTs on the genetic map of chickpea, EST specific primers were generated by using some of the sequenced data. Screening with EST primers showed that, parents of CRIL-7 population (Tekeoglu *et al.*, 2000) segregating for Ascochyta

blight resistance is quite monomorphic with respect to this EST marker population and also CAPS may be limited. This observation was in line with the reports of many authors reporting that chickpea has a narrow genetic base. Screening of these markers resulted in 3 polymorphic markers (including 2 SNPs) in the CRIL-7 population of chickpea. None of them were found to be linked to reported major QTLs of Ascochyta blight resistance, but two of them (EST-R46 marker and EST-FPIP marker) were located on two different linkage groups of chickpea.

A small scale preliminary Real-Time qRT-PCR analysis was also performed with some of the above mentioned EST primers to confirm/understand their induction/repression and to compare them in different *C.arietinum- A.rabiei* genotypes. This analysis revealed that FDH, an EST similar to serine carboxypeptidase and an EST slightly similar to acyl-CoA synthetase were highly upregulated in both pathotype I and II infected resistant chickpea genotype FLIP84-92C(3) within 6 to 24 h, but this induction was delayed in ank6 isolate of *A.rabiei* infected tolerant ILC195 variety (36 h). Induction of two further ESTs, one similar to metal ion transport proteins and the other the polymorphic marker EST-FPIP similar to SAMMtases, was not so significantly except the induction of EST-FPIP in one time point (36 hpi) of ILC195. It was also observed that selection of reference gene for such analysis is also important and use of actin as internal control for Real-Time qRT-PCR analysis in blight infections may be questionable. By not underestimating environmental variations and small population of ESTs analyzed, resistance of chickpea for a particular isolate/pathotype may be based on rapid responses by higher inductions. One of these ESTs, FDH, may be the most promising one as a marker gene. Upon *A.rabiei* attack formate may be formed by the induction of catabolic pathways such as cell wall fortification or degradation. Hydrolysis of this formate by FDH may lead to alternative production of NADH to meet chickpea's energy requirement during defense responses partly so that energy related resources may be allocated for biosynthesis of defense related compounds. Due to potential importance of FDH, it was also screened in a BAC library of chickpea to find putative physical map position of this gene. However, no positive signal was obtained.

To conclude, with this study, a population of chickpea ESTs representing of various molecular pathways possibly affected by *A. rabiei* attack were isolated to be a basis for further studies including transcriptome and genetic mapping analysis.

REFERENCES

Aarts M.G., Hekkert, B.L., Holub, E.B., Beynon, J.L., Stiekema, W.J., Pereira, A. 1998. Identification of R-gene homologous DNA fragments genetically linked to disease resistance loci in *Arabidopsis thaliana*. Mol. Plant-Microbe. Interact. 11:251-258.

Abbo, S., Berger, J. and Turner, N. C. 2003. Evolution of cultivated chickpea: four bottlenecks limit diversity and constrain adaptation. Funct. Plant Biol 30:1081-1087.

Agarwal, P. K., Agarwal, P., Reddy, M. K., Sopory, S. K. 2006. Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell. Rep. 25: 1263-1274.

Agrios, G. N. 1988. Plant Pathology. (London: Academic Press). In: Hammond-Kosack, K. E. and Jones, J. D. G. 1996. Resistance gene-dependent plant defense responses. Plant Cell. 8:1773-1791.

Aharoni, A., Jongsma, M.A., Verhoeven, H.A. and Bouwmeester, H.J., Publication Date: 22.08.2002. Terpene synthase/cyclase and olefin synthase and uses thereof. WO/2002/064764. International Patent Application No.: PCT/NL2002/000089.

Alba, M. M. and Pages, M. 1998. Plant proteins containing the RNArecognition Motif. Trends Plant Sci. 3:15-21.

Alfano, G., Lewis Ivey, M. L., Cakir, C., Bos, J. I. B., Miller, S. A., Madden, L. V., Kamoun, S. And Hoitink, H. A. J. 2007. Systemic modulation of gene expression in tomato by *Trichoderma hamatum* 382. Am. Phytopathol. Soc. 97:4.

Alonso, J. M., Hirayama, T., Roman, G., Nourizadeh, S., Ecker, J. R. 1999. EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis. Science. 284(5423):2148-52.

Alvarez, M. E., Pennell, R. I., Meijer, P. J., Ishikawa, A., Dixon, R. A. and Lamb, C. 1998. Reactive oxygen intermediates mediate a systemic signal network in the establishment of plant immunity. Cell. 92: 773-784.

Amelina-Torregrosa, C., Dumas, B., Krajinski, F., Esquerre-Tugaye, M. T. and Jacquet, C. 2006. Transcriptomic approaches to unravel plant–pathogen interactions in legumes. Euphytica. 147: 25-36.

AmiGo. The Gene Ontology. The Gene Ontology Consortium. Gene Ontology: tool for the unification of biology. 2000. Nature Genet. 25: 25-29. http://amigo.geneontology.org/cgi-bin/amigo/go.cgi.

Anderson, J. P., Badruzsaufari, E., Schenk, P. M., Manners, J. M., Desmond, O. J., Ehlert, C., Maclean, D. J., Ebert, P. R., Kazan, K. 2004. Antagonistic interaction between abscisic acid and jasmonate-ethylene signaling pathways modulates defense gene expression and disease resistance in Arabidopsis. Plant Cell. 16: 3460-3479.

Angelini, R., Bragaloni, M., Federico, R., Infantino, A., Portapuglia, A. Involvement of polyamines, diamine oxidase and peroxidase in resistance of chickpea to *Ascochyta rabiei*.-J. Plant Physiol. 142: 704–709, 1993.

Ansari, M. A., Patel, B. A., Mhase, N. L., Patel, D. J., Douaik, A. and S. B. 2004. Tolerance of chickpea (*Cicer arietinum* L.) lines to root-knot nematode, *Meloidogyne javanica* (Treub) Chitwood. Genet. Resour.Crop Ev. 51: 449-453.

Arumuganathan, K. and Earle, E. D. 1991. Nuclear DNA content of some important plant species. Plant Mol. Biol. Rep. 9:208-218.

Asselbergh, B., Curvers, K., Francxa, S. C., Audenaert, K., Vuylsteke, M., Van Breusegem, F., Höfte, M. 2007. Resistance to Botrytis cinerea in sitiens, an Abscisic Acid-Deficient Tomato Mutant, Involves Timely Production of Hydrogen Peroxide and Cell Wall Modifications in the Epidermis. Plant Physiol. 144:1863-1877.

Atkinson, M. M. 1993. Molecular mechanisms of pathogen recognition by plants. Adv. Plant Pathol. 10:35-64.

Audenaert K., De Meyer G.B., Höfte, M.M. 2002. Abscisic acid determines basal susceptibility of tomato to *Botrytis cinerea* and suppresses salicylic acid-dependent signaling mechanisms. Plant Physiol. 128: 491–501

Austin, M. J., Muskett, P., Kahn, K., Feys, B. J, Jones, J. D, Parker, J. E. 2002. Regulatory role of SGT1 in early R gene-mediated plant defenses. Science. 295:2077-208.

Azevedo, C., Sadanandom, A., Kitagawa, K., Freialdenhoven, A., Shirasu, K. and Schulze Lefert, P. 2002. The RAR1 interactor SGT1, an essential component of R gene-triggered disease resistance. Science. 295:2073-2076.

Azevedo, H., Lino-Neto, T., Tavares, R. M. 2008. The necrotroph *Botrytis cinerea* induces a non-host type II resistance mechanism in *Pinus pinaster* suspension-cultured cells. Plant Cell Physiol. 49:386-395.

Bachmair, A., Novatchkova, M., Potuschak, T., Eisenhaber, F. 2001. Ubiquitylation in plants: a postgenomic look at a post-translational modification. Trends Plant Sci. 6:463-470.

Bantignies, B., Séguin, J., Muzac, I., Dédaldéchamp, F., Gulick, P., Ibrahim, R. 2000. Direct evidence for ribonucleolytic activity of a PR-10-like protein from white lupin roots. Plant Mol. Biol. 42: 871-881.

Barz, W. and Mackenbrock, U. 1994. Constitutive and elicitation induced metabolism of isoflavones and pterocarpans in chickpea *(Cicer arietinum)* cell suspension cultures. Plant Cell, llssue and Organ Culture. 38:199-211.

Bateman, R. L., Bhanumoorthy, P., Witte, J. F., McClard, R. W., Grompe, M., Timm, D. E. 2001. Mechanistic inferences from the crystal structure of fumarylacetoacetate hydrolase with a bound phosphorus-based inhibitor. J. Biol. Chem. 276(18):15284-15291.

Bayraktar, H. and Dolar, F. S. 2002. Induction of resistance in chickpea to Ascochyta Blight [*Ascochyta rabiei* (Pass.) Labr.] by salicylic acid. J. Turk. Phytopath. 31:49-61.

Bennett, M. D. and J. B. Smith. 1976. Nuclear DNA amounts in angiosperms. Phil. Trans. Royal Soc. Lon. 274 (Ser 13), 227–274.

Berrocal-Lobo, M., Molina, A., Solano, R. 2002a. Constitutive expression of ethylene-response-factor 1 in Arabidopsis confers resistance to several necrotrophic fungi. Plant J. 29:23–32.

Berrocal-Lobo, M., Segura, A., Moreno, M., Lopez, G., Garcia-Olmedo, F., Molina, A. 2002b. Snakin-2, an antimicrobial peptide from potato whose gene is locally induced by wounding and responds to pathogen infection. Plant Physiol. 128:951-961.

Bertolaet, B. L., Clarke, D. J., Wolff, M., Watson, M. H., Henze, M., Divita, G., Reed, S. I. 2001. UBA domains of DNA damage-inducible proteins interact with ubiquitin. Nat. Struct. Biol 8:5

Bhuiyan, N. H., Liu, W., Liu, G., Selvaraj, G., Wei, Y., King, J. 2007. Transcriptional regulation of genes involved in the pathways of biosynthesis and supply of methyl units in response to powdery mildew attack and abiotic stresses in wheat. Plant Mol. Biol. 64:305-318.

Bhushan, D., Pandey, A., Chattopadhyay, A., Choudhary, M. K., Chakraborty, S., Datta, A. and Chakraborty, N. 2006. Extracellular matrix proteome of chickpea (*Cicer arietinum*

L.) illustrates pathway abundance, novel protein functions and evolutionary perspect. J. Proteome Res. 5:1711-1720.

Blanco, F., Garreton, V., Frey, N., Dominguez, C., Perez-Acle, T., Van der Straeten, D., Jordana, X., Holuigue, L. 2005. Identification of NPR1-dependent and independent genes early induced by salicylic acid treatment in Arabidopsis. Plant Mol. Biol. 59:927-944.

Bian, X. Y., Ford, R., Han, T. R., Coram, T. E., Pang, E. C. K. and Taylor, P. W. J. 2007. Approaching chickpea quantitative trait loci conditioning resistance to *Ascochyta rabiei* via comparative genomics. Australas. Plant Pathol. 36:419-423.

Bio-Rad Laboratories, Inc. 2007. Normalization Methods for qPCR. BioRadiations 121. http://www.bmskorea.co.kr/bms_Product/MakerImage/notice/bio-rad/Normalization_Methods_for_qPCR.pdf

Bohlmann, J., Gauen-Meyer, G., Croteau, R. 1998. Plant terpenoid synthases: Molecular biology and phylogenetic analysis (terpene cyclase/isoprenoids/plant defense/genetic engineering/secondary metabolism. PNAS. 95:4126-4133.

Bolle, C. 2004. The role of GRAS proteins in plant signal transduction and development. Planta. 218: 683-692.

Bolwell, G. P., Butt, V. S., Davies, D. R., Zimmerlin, A. 1995. The origin of the oxidative burst in plants. Free Radic. Res. 23:517-532.

Bonnet, S., Prévot, G. and Bourgouin, C. 1998. Efficient reamplification of differential display products by transient ligation and thermal asymmetric PCR. Nucleic Acids Res. 26: 1130-1131.

Boominathan, P., Shukla, R., Kumar, A., Mana, D., Negi, D., Verma, P. K. and Chattopadhyay, D. 2004. Long term transcript accumulation during the development of dehydration adaptation in *Cicer arietinum*. Plant Physiol. 135:1608-1620.

Boquet, P. and Lemichez, E. 2003 Bacterial virulence factors targeting Rho GTPases: parasitism or symbiosis? Trends Cell Biol. 13 (5):238-246.

Boyko, A. and Kovalchuk, I. 2008. Epigenetic control of plant stress response. Environ. Mol. Mutagen. 49:61-72.

Bozkurt, O., Unver, T., Akkaya, M.S. 2007. Genes associated with resistance to wheat yellow rust disease identified by differential display analysis. Physiol. Mol. Plant Pathol. 71: 251– 259.

Brandwagt, B. F., Mesbah, L. A., Takken, F. L., Laurent, P. L., Kneppers, T. J., Hille, J. and Nijkamp, H. J., A. 2000. Longevity assurance gene homolog of tomato mediates resistance to *Alternaria alternata* f. sp. *lycopersici* toxins and fumonisin B1. Proc. Natl. Acad. Sci.USA. 97:4961-4966.

Brizard, J. P., Carapito, C., Delalande, F., Dorsselaer, A. and Brugidou, C. 2006. Proteome analysis of plant-virus interactome: comprehensive data for virus multiplication inside their hosts. Mol. Cell. Proteom. 5 (12):2279.

Brosch, G., Ransom, R. F., Lechner, T., Walton, J. D., Loidl, P. 1995. Inhibition of maize histone deacetylases by hc toxin, the host-selective toxin of *Cochliobolus carbonum*. Plant Cell. 7:1941-1950.

Btadley, D. J., Kjellbom, P., Lamb, C. J. 1992. Elicitor- and wound-induced oxidative cross-linking of a proline-rich plant cell wall protein: A novel, rapid defense response. Cell 70:21-30.

Buchanan, B. B., Gruissem, W. and Jones, R. L. 2000. Biochemistry and molecular biology of plants. Am. Soc. Plant Physiol. Rockville, Md. USA.

Buhariwalla, H. K., Jayashree, B., Eshwar, K., Crouch, J. H. 2005. Development of ESTs from chickpea roots and their use in diversity analysis of the *Cicer* genus. BMC Plant Biol. 5:16.

Bundock, P. and Hooykaas, P. 2005. An Arabidopsis hAT-like transposase is essential for plant development. Nature. 436 (7048):282-284.

Burd, C. G. and Dreyfuss, G. 1994. Conserved structures and diversity of functions of RNA-binding proteins. Science. 265:615-621.

Burkhard, P., Stetefeld, J., Strelkov, S. V. 2001. Coiled coils: a highly versatile protein folding motif. Trends Cell. Biol. 11:81-82.

Cao, H., Glazebrook, J., Clarke, J. D., Volko, S., Dong, X. 1997. The Arabidopsis NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell. 88:57-63.

Cato, S., Gardner, R., Kent, J., Richardson, T. 2001. A rapid PCR-based method for genetically mapping ESTs. Theor. Appl. Genet. 102:296–306.

Century, K. S., Holub, E. B., Staskawicz, B. J. 1995. NDR1, a locus of arabidopsis thaliana that is required for disease resistance to both a bacterial and a fungal pathogen. Proc. Natl. Acad. Sci. U S A. 92: 6597-6601.

Cervantes, E., De Diego, J. G., Gómez, M. D., Rivas, J. D. L., Igual, J. M., Velázquez, E., Grappin, P., Cercós, M., Carbonell, J. 2001. Expression of cysteine proteinase mRNA in chickpea (*Cicer arietinum* L.) is localized to provascular cells in the developing root. J. Plant Physiol. 158:1463-1469.

Chakravarthy, S., Tuori, R. P., D'Ascenzo, M. D., Fobert, P. R., Després, C., Martin, G. B. 2003. The tomato transcription factor Pti4 regulates defense-related gene expression via GCC box and non-GCC box cis elements. Plant Cell. 15:3033-3050.

Chen, W. and Muehlbauer, F.J. 2003. An improved technique for virulence assay of *Ascochyta rabiei* on chickpea. International Chickpea Nda Pigeonpea Newslett.10: 31-33.

Chen, W., Coyne, C. J., Peever, T. L., Muehlbauer, F. J. 2004. Characterization of chickpea differentials for pathogenicity assay of ascochyta blight and identification of chickpea accessions resistant to *Didymella rabiei*. Plant Pathol. 53:759-769.

Chen, X. M., Line, R. F. Leung, H. 1998. Genome scanning for resistance-gene analogs in rice, barley and wheat by high-resolution electrophoresis. Theor. Appl. Genet. 97:345-355.

Cheng, Y. Q. and Walton, J. D. 2000. A eukaryotic alanine racemase gene involved in cyclic peptide biosynthesis. J. Biol. Chem. 275(7):4906-4911.

Cheong, Y. H., Chang, H. S., Gupta, R., Wang, X., Zhu, T., Luan, S. 2002. Transcriptional profiling reveals novel ınteractions between wounding, pathogen, abiotic stress, and hormonal responses in Arabidopsis. Plant Physiol. 125:661-677.

Cheong, Y. H., Moon, B. C., Kim, J. K., Kim, C. Y., Kim, M. C., Kim, I. H., Park, C. Y, Kim, J. C., Park, B. O., Koo, S. C., Yoon, H. W., Chung, W. S, Lim, C. O., Lee, S. Y., Cho, M. J. 2003. BWMK1, a rice mitogen-activated protein kinase, locates in the nucleus and mediates pathogenesis-related gene expression by activation of a transcription factor. Plant Physiol. 132:1961-1972.

Chinnaiyan, A. M., Chaudhary, D., O'Rourke, K., Koonin, E. V., Dixit, V. M. 1997. Role of CED-4 in the activation of CED-3. Nature. 388:728-729.

Chitnis, P.R. and Nelson, N. 1992. Assembly of two subunits of the cyanobacterial photosystem I on the n-Side of thylakoid membranes. Plant Physiol. 99: 239-246.

Cho, S., Chen, W., Muehlbauer, F. J. 2005. Constitutive expression of the flavanone 3-hydroxylase gene related to pathotype-specific ascochyta blight resistance in *Cicer arietinum* L. Physiol. Mol. Plant Pathol. 67:100-107.

Cho, S., Chen, W., Muehlbauer, F.J. 2004. Pathotype-specific genetic factors in chickpea (*Cicer arietinum* L.) for quantitative resistance to Ascochyta blight. Theor Appl. Genet. 109:733-739.

Cho, S., Kumar, J., Shultz, J. L., Anupama, K., Tefera, F., Muehlbauer, F. J. 2002 Mapping genes for double podding and other morphological traits in chickpea. Euphytica. 128:285-292.

Cho, S. and Muehlbauer, F. J. 2004. Genetic effect of differentially regulated fungal response genes on resistance to necrotrophic fungal pathogens in chickpea (*Cicer arietinum* L.). Physiol. Mol. Plant Pathol. 64:57-66.

Chongo, G. and Gossen, B. 2001. Effect of plant age on resistance to *Ascochyta rabiei* in chickpea. Can. J. Plant Pathol. 23:358-363.

Choumane, W., Winter, P., Weigand, F., Kahl, G. 2000. Conservation and variability of sequence-tagged microsatellite sites (STMSs) from chickpea (*Cicer arietinum* L.) within the genus Cicer. Theor. Appl. Genet. 101:269-278.

Chu, K. T., Liu, K. H., Ng, T. B. 2003. Cicerarin, a novel antifungal peptide from the green chickpea. Peptides. 24:659-663.

Chu Z., Yuan, M., Yao, J., Ge, X.J., Yuan, B., Xu, C.G., Li, X.H., Fu B.Y., Li, Z.K., Bennetzen, J.L., Zhang, Q.F., Wang, S.P. 2006. Promoter mutations of an essential gene for pollen development result in disease resistance in rice. Gene Dev. 20:1250- 1255.

Chung, C. Niemela, S.L., Miller, R.H. 1989. One-step preparation of competent E. coli: Transformation and storage of bacterial cells in the same solution. Proc. Natl. Acad. Sci. U.S.A. 86:2172-2175.h. IN: David Scott Gilmour. Penn State Personal Web Server. http://www.personal.psu.edu/faculty/d/s/dsg11/labmanual/DNA_manipulations/Comp_bact_by_TSS.htm . Accesion date: December, 2005

Clarke, A., Desikan, R., Hurst, R. D., Hancock, J. T. ,Neill, S. J. 2000. NO way back: nitric oxide and programmed cell death in Arabidopsis thaliana suspension cultures. Plant J. 24: 667-677.

CLUSTALW. Kyoto University Bioinformatics Center, Institute for Chemical Research, Kyoto University. http://align.genome.jp/. Accession date: 26 June 2008

Collard, B. C. Y., Pang, E. C. K., Ades, P. K., Taylor, P. W. J. 2003. Preliminary investigation of QTLs associated with seedling resistance to ascochyta blight from *Cicer echinospermum*, a wild relative of chickpea. Theor. Appl. Genet. 107:719-729.

Colmenares, A. J., Aleu, J., Duran-Patron, R., Collado, I. G., Hernandez-Galan, R. 2002. The putative role of botrydial and related metabolites in the infection mechanism of *Botrytis cinerea*. J. Chem. Ecol. 28:997-1005.

Cooper, J. A. and Schafer, D. A. 2000 Control of actin assembly and disassembly at filament ends. Curr. Opin. Cell Biol. 12:97-103.

Cooperman, B. S. , Baykov, A. A., Lahti, R. 1992. Evolutionary conservation of the active site of soluble inorganic pyrophosphatase. Trends Biochem. Sci. 17:262-266.

Coppin, E., Debuchy, R., Arnaise, S., Picard, M. 1997. Mating types and sexual development in filamentous ascomycetes. Microbiol. Mol. Biol. Rev. 6:411-428.

Coram, T. E. and Pang, E. C. K. 2005a. Isolation and analysis of candidate ascochyta blight defence genes in chickpea. Part I. Generation and analysis of an expressed sequence tag (EST) library. Physiol. Mol. Plant Pathol. 66:192-200.

Coram, T. E. and Pang, E. C. K. 2005b. Isolation and analysis of candidate ascochyta blight defence genes in chickpea. Part II. Microarray expression analysis of putative defence-related ESTs. Physiol. Mol. Plant Pathol. 66:201-210.

Coram, T. E. and Pang, E. C. K. 2006. Expression profiling of chickpea genes differentially regulated during a resistance response to *Ascochyta rabiei*. Plant Biotech. J. 4:647-666.

Coram, T. E., and Pang, E. C. K. 2007. Transcriptional profiling of chickpea genes differentially regulated by salicylic acid, methyl jasmonate and aminocyclopropane carboxylic acid to reveal pathways of defence-related gene regulation. Funct. Plant Biol. 34:52-64.

Coram, T. E., Mantri, N. L., Ford, R., Pang, E. C. K. 2007. Functional genomics in chickpea: an emerging frontier for molecular-assisted breeding. Funct. Plant Biol. 34:861-873.

Cornels, H., Ichinose, Y., Barz, W. 2000. Characterization ofcDNAs encoding two glycine-rich proteins in chickpea (*Cicer arietinum* L.): Accumulation in response to fungal infection and other stress factors. Plant. Sci. 154: 83-88.

Corpas, F. J., Barroso, J. B., del Rio, L. A. 2001. Peroxisomes as a source of reactive oxygen species and nitric oxide signal molecules in plant cells. Trends Plant. Sci. 6:145-150.

Crino, B. P. 1990. Chickpea breeding for resistance to ascochyta. Options Méditerranéennes. Série Séminaires. no 9: 55-60.

Croft, K. P. C., Jüttner, F., Slusarenko, A. J. 1993. Volatile products of the lipoxygenase pathway evolved from *Phaseolus vulgaris* (L.) leaves inoculated with *Pseudomonas syringae* pv. *phaseolicola*. Plant Physiol. 101:13-24.

Croser, J. S., Clarke, H. J., Siddique, K. H. M., Khan, T. N. 2003. Low-temperature stress: Implications for chickpea (*Cicer arietinum* L.) improvement. Crit. Rev. Plant Sci. 22:185-219.

Cross, R. L. and Muller, V. 2004. The evolution of A-, F-, and V-type ATP synthases and ATPases: reversals in function and changes in the H+/ATP coupling ratio. FEBS Lett. 576:1-4.

Cvetkovska, M., Rampitsch, C., Bykova, N., Xing, T. 2005. Genomic analysis of MAP kinase cascades in *Arabidopsis* defense responses. Plant Mol. Biol. Reporter. 23: 331-343.

Dangl, J. L. and Jones, J. D. 2001. Plant pathogens and integrated defence responses to infection. Nature. 411:826-833.

Davidson, J. A. and Kimber, R. B. E. 2007. Integrated disease management of ascochyta blight in pulse crops. Eur. J. Plant Pathol. 119:99-110.

dCaps Finder 2.0. Reference: Neff M.M., Turk E., Kalishman M. 2002. Web-based primer design for single nucleotide polymorphism analysis. Trends Genet. 18 613-615. http://helix.wustl.edu/dcaps/dcaps.html Accession date: 05/21/2007.

De Paepe, A., Vuylsteke, M., Van Hummelen, P., Zabeau, M., Van Der Straeten, D. 2004. Transcriptional profiling by cDNA-AFLP and microarray analysis reveals novel insights into the early response to ethylene in Arabidopsis. Plant J. 39:537-559.

De Rosa Jr, V. E., Nogueira, F. T. S., Menossi, M., Ulian, E. C., Arruda, P. 2005. Identification of methyl jasmonate-responsive genes in sugarcane using cDNA arrays. Braz. J. Plant Physiol. 17(1):173-180.

Delta® Differential Display Kit User Manual PT1173-1 (PR19324) Published 05 September 2001 Catalog #: K1810-1.

Delledonne, M., Xia, Y., Dixon, R. A., Lamb, C. 1998. Nitric oxide functions as a signal in plant disease resistance, Nature. 394:585-588.

Desikan, R., Mackerness, S. A. H., Hancock, J. T., Neill, S. J. 2001. Regulation of the Arabidopsis transcriptome by oxidative stress. Plant Physiol. 127:159-172.

Devoto, A., Muskett, P. R., Shirasu, K. 2003. Role of ubiquitination in the regulation of plant defence against pathogens. Curr. Opin. Plant Biol. 6:307-311.

Devoto, A. and Turner, J. G. 2005. Jasmonate-regulated Arabidopsis stress signalling network. Physiol. Plantarum. 123:161-172.

Dey, S. K. and Singh, G. 1993. Resistance to ascochyta blight in chickpea-Genetic basis. Euphytica. 68:147-153.

Di Matteo, A., Giovane, A., Raiola, A., Camardella, L., Bonivento, D., De Lorenzo, G., Cervone, F., Bellincampi, D., Tsernoglou, D. 2005. Structural basis for the interaction between pectin methylesterase and a specific inhibitor protein. Plant Cell. 17:849-858.

Dievart, A. and Clark, S. E. 2004. LRR-containing receptors regulating plant development and defense. Development. 131:251-261.

Dita, M.A., Rispail, N., Prats, E., Rubiales, D., Singh, K.B., 2006. Biotechnology approaches to overcome biotic and abiotic stress constraints in legumes. Euphytica. 147: 1-24.

Ditt, R. F., Kerr, K. F., de Figueiredo, P., Delrow, J., Comai, L., Nester, F. W. 2006. The *Arabidopsis thaliana* Transcriptome in Response to *Agrobacterium tumefaciens.* MPMI. 19:665-681.

Dixon, R. A. 2001. Natural products and plant disease resistance. Nature. 411:843-847.

Dixon, R. A., Achnine, L., Kota, P., Liu, C. J., Reddy, M. S. S., Wang, L. 2002. The phenylpropanoid pathway and plant defence-a genomics perspective. Mol. Plant Pathol. 3:371-390.

Dolar, F.S. and Gürcan, A. 1992. Pathogenic variability and race appearance of *Ascochyta rabiei* (Pass.) Labr. In Türkiye. J. Turk. Phytopath. 21 (2-3): 61-65.

Domachowske, J. B. and Malech, H. L. 1997. Reamplification of differential display products: more is not better. www.bioscience.org/1997/v2/c/domachow/12-14.htm
Dreher, K. and Callis, J. 2007. Ubiquitin, hormones and biotic stress in plants. Annals of Botany. 99:787-822.

Du, L. and Chen, Z. 2000. Identification of genes encoding receptor-like protein kinases as possible targets of pathogen- and salicylic acid-induced WRKY DNA-binding proteins in *Arabidopsis*. Plant J. 24: 837-847.

Dugan, F. M., Lupien, S. L., Hernandez-Bello, M., Peever, T. L., Chen, W. 2005. Fungi resident in chickpea debris and their suppression of growth and reproduction of *Didymella rabiei* under laboratory conditions. J. Phytopathol. 153:431–439.

Dunoyer, P., Himber, C., Voinnet, O. 2006. Induction, suppression and requirement of RNA silencing pathways in virulent *Agrobacterium tumefaciens* infections. Nature Genet. 38:258-263.

Durner, J., Wendehenne, D., Klessig, D. F. 1998. Defence gene induction in. tobacco by nitric oxide, cyclic GMP, and cyclic ADP ribose, Proc. Natl. Acad. Sci. U.S.A. 95:10328-10333.

Dusunceli, F., Meyveci, K., Cetin, L., Avci, M., Surek, D., Albustan, S., Mert, Z., Akan, K., Karacam, M., Strange, R. N. 2007. Determination of agronomic practices for the management of blight of chickpea caused by *Ascochyta rabiei* in Turkey: 1. Appropriate sowing date. Eur. J. Plant Pathol. 119:449-456.

Ebel, J. and Mithöfer, A. 1998. Early events in the elicitation of plant defence. Planta. 206: 335-348.

Eckardt, N. A. 2001. A role for PsbZ in the core complex of photosystem II. Plant Cell. 13: 1245-1248.

Eisen, J. A., Sweder, K.S., Hanawalt, P. C. 1995. Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. Nucleic Acids Res. 23:2715-2723.

Ellis, C. and Turner, J. G. 2001. The Arabidopsis mutant *cev1* has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. Plant Cell. 13:1025-1033.

Ellis, C,, Karafyllidis, I., Wasternack, C.,Turner, J. G. 2002a. The Arabidopsis mutant *cev1* links cell wall signaling to jasmonate and ethylene responses. Plant Cell. 14:1557-1566.

Ellis, C., Karafyllidis, I., Turner, J. G. 2002b. Constitutive activation of jasmonate signaling in an Arabidopsis mutant correlates with enhanced resistance to *Erysiphecichoracearum*, *Pseudomonas syringae*, and *Myzus persicae*. Mol. Plant Microbe. Interact. 15:1025-1030.

Emi, T., Kinoshita, T., Sakamoto, K., Mineyuki, Y., Shimazaki, K. 2005. Isolation of a protein interacting with Vfphot1a in guard cells of *Vicia faba*. Plant Physiol. 138: 1615-1626

Eng, B. H., Guerinot, M. L., Eide, D., Saier, M. H. Jr. 1998. Sequence analyses and phylogenetic characterization of the ZIP family of metal ion transport proteins. J. Membrane Biol. 166:1-7.

fr33net. Pawlowski, N. Life Sciences Tools. Tools for DNA, RNA and protein sequences. http://www.fr33.net/translator.php

Falk, A., Feys, B. J., Frost, L. N., Jones, J. D., Daniels, M. J., Parker, J. E. 1999. EDS1, an essential component of R gene-mediated disease resistance in Arabidopsis has homology to eukaryotic lipases. Proc. Natl. Acad. Sci. U.S.A. 96:3292-3297.

Fan, W. and Dong, X. 2002. In vivo interaction between NPR1 and transcription factor TGA2 leads to salicylic acid-mediated gene activation in Arabidopsis. Plant Cell. 14:1377-1389.

Fanghänel, J. and Fischer, G. 2004. Insights into the catalytic mechanism of peptidyl prolyl *cis/trans* isomerases. Front. Biosci. 9:3453-3478.

Farmer, E. E. and Ryan, C. A. 1992. Octadecanoid precursors of jasmonic acid activate the synthesis of wound-inducible proteinase inhibitors. Plant Cell. 4:129-134.

Ferrari, S., Plotnikova, J. M., De Lorenzo, G., Ausubel, F. M.. 2003. Arabidopsis local resistance to *Botrytis cinerea* involves salicylic acid and camalexin and requires *EDS4* and *PAD2*, but not *SID2*, *EDS5* or *PAD4*. Plant J. 35:193-205.

Ferrer, J. L. , Ravanel, S., Robert, M., Dumas, R. 2004. Crystal structures of cobalamin-independent methionine synthase complexed with zinc, homocysteine, and methyltetrahydrofolate. J. Biol. Chem. 279:44235-44238.

Flandez-Galvez, H., Ford, R., Pang, E. C. K., Taylor, P. W. J. 2003a. An intraspecific linkage map of the chickpea (*Cicer arietinum* L.) genome based on sequence tagged microsatellite site and resistance gene analog markers. Theor. Appl. Genet. 106:1447-1456.

Flandez-Galvez, H., Ades, P. K., Ford, R., Pang, E. C. K., Taylor, P. W. J. 2003b. QTL analysis for ascochyta blight resistance in an intraspecific population of chickpea (*Cicer arietinum* L.). Theor. Appl. Genet. 107:1257-1265

Flor, H. H. 1971. Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. 9:275-296.

Freitas-Astúa, J., Bastianel, M., Locali-Fabris, E. C., Valdenice, M., Novelli, V. M., Silva-Pinhati, A. C., Basílio-Palmieri, A. C., Targon, M. L. P. N, Machado, M. A. 2007. Differentially expressed stress-related genes in the compatible citrus-citrus leprosis virus interaction. Genet. Mol. Biol. 30:980-990.

Frye, C. A, Tang, D., Innes, R.W. 2001. Negative regulation of defense responses in plants by a conserved MAPKK kinase. Proc. Natl. Acad. Sci. USA. 98:373-378.

Foyer C.H. and Noctor, G. 2003. Redox sensing and signaling associated with reactive oxygen species in chloroplasts, peroxisomes and mitochondria. Physiol. Plant 119: 355-364.

Fujita, M., Yasunari Fujita, Y., Noutoshi, Y., Takahashi, F., Narusaka, Y., Yamaguchi-Shinozaki, K., Shinozaki, K. 2006. Crosstalk between abiotic and biotic stres responses: a current view from the points of convergence in the stress signaling networks. Curr. Opin. Plant Biol. 9:436-442.

Gachomo, E. W., Shonukan, O. O., Kotchoni, S. O. 2003. The molecular initiation and subsequent acquisition of disease resistance in plants. Afr. J. Hüttel 2(2):26-32.

Gamas, P., Niebel, F.de C., Lescure, N. , Cullimore, J. 1996. Use of a subtractive hybridization approach to identify new *Medicago truncatula* genes induced during root nodule development. Mol. Plant Microbe Interact. 9:233-242.

Gau, A. E. , Thole, H. H. , Sokolenko, A., Altschmied, L., Hermann, R. G. , Pistorius, E. K. 1998. PsbY, a novel manganese-binding, low-molecular-mass protein associated with photosystem II. Mol. Gen. Genet. 260:56-68.

Germain, H., Rudd, S., Zotti, C., Caron, S., O'Brien, M., Chantha, S. C., Lagace, M., Major, F., Matton, D. P. 2005. A 6374 unigene set corresponding to low abundance transcripts expressed following fertilization in *Solanum chacoense* Bitt, and characterization of 30 receptor-like kinases. Plant Mol. Biol. 59:515–532.

Gibly, A., Bonshtien, A., Balaji, V., Debbie, P., Martin, G. B., Sessa, G. 2004. Identification and expression profiling of tomato genes differentially regulated during a resistance response to *Xanthomonas campestris* pv. *vesicatoria*. MPMI. 17(11):1212-1222.
Giri, A. P., Harsulkar, A. M., Patankar, A. G., Gupta, V. S., Sainani, M.N., Deshpande, V. V., Ranjekar, P. K.1998. Association of induction of protease and chitinase in chickpea roots with resistance to *Fusarium oxysporum* f.sp. *ciceri*. Plant Pathol. 47, 693–699.

Gitmans A., Thesis. 1998. Westfaelische Wilhelms-Universitaet Muenster, Institut für Biochemie und Biotechnologie der Pflanzen. Submitted to NCBI on 29-JUL-1998.

Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. 43:205-227.

Glickman, M. H. and Ciechanover, A. 2002. The ubiquitin-proteasome proteolytic pathway: destruction for the sake of construction. Physiol. Rev. 82:373-428.

Gomez, J., Sanchez-Martinez, D., Stiefel, V., Rigau, J., Puigdomenech, P., Pages, M. 1988. A gene induced by the plant hormone abscisic acid in response to water stress encodes a glycine rich protein. Nature. 334:262-264.

Gortner, G., Nenno, M., Weising, K., Zink, D., Nagl, W., Kahl, G. 1998. Chromosome localization and distribution of simple sequence repeats and the Arabidopsis-type telomere sequence in the genome of *Cicer arietinum* L. Chromosome Res. 6:97-104.

Govrin, E. M. and Levine, A. 2000. The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. Curr. Biol. 10:751-757.

Graham-Lorence, S., Amarneh, B., White, R. E., Peterson, J. A., Simpson, E. R. 1995. A three-dimensional model of aromatase cytochrome P450. Protein Sci. 4:1065-1080.

Gray, J.C., Sullivan, J.A., Wand, J.H., Jerome, C.A., MacLean, D.2003. Coordination of plastid and nuclear gene expression. Philos. Trans. R. Soc. Lond. B. Biol. Sci. 358: 135-145.

Grass, G., Wong, M. D., Rosen, B. P., Smith, R. L., Rensing, C. 2002. ZupT is a Zn(II) uptake system in *E. coli*. J. Bacteriol. 184: 864-866.

Greenberg, J. T. 1997. Programmed cell death in plant–pathogen interactions. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:525-545.

Greene, M. K., Maskos, K., Landry, S. J. 1998. Role of the J-domain in the cooperation of Hsp40 with Hsp70. Proc. Natl. Acad. Sci. U. S. A. 95:6108-6113.

Gronow, S., Brabetz, W., Brade, H. 2000. Comparative functional characterization in vitro of heptosyltransferase I (WaaC) and II (WaaF) from Escherichia coli. Eur. J. Biochem. 267(22):6602-6611.

Gu, Y. Q., Wildermuth, M. C., Chakravarthy, S., Loh, Y. T., Yang, C., He, X., Han, Y., Martin, G. B. 2002. Tomato transcription factors Pti4, Pti5, and Pti6 activate defense responses when expressed in Arabidopsis. Plant Cell. 14:817-831.

Yu, H. and Kumar, P. P. 2003. Post-transcriptional gene silencing in plants by RNA Plant Cell Rep. 22:167-174.

Hahn, M., Neef, U., Struck, C., Göttfert, M., Mendgen, K. 1997. A putative amino acid transporter is specifically expressed in haustoria of the rust fungus *Uromyces fabae*. Mol. Plant Microbe Interact. 10:438-445.

Hall, J. L. and Williams, L. E. 2003. Transition metal transporters in plants. J. Exp. Bot. 54:2601-2613.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids Symp. Ser.41:95-98.

Hammerschmidt, R. Editorial. 2004. Resistance to necrotrophs: is there a predictable defense pattern? Physiol. Mol. Plant Pathol. 64:55-56.

Hammond, S. M., Bernstein, E., Beach, D., Hannon, G. J. 2000. An RNA-directed nuclease mediates posttranscriptional gene silencing in *Drosophila* cells. Nature. 404:293-296.

Hammond-Kosack, K. E. and Jones, J. D. G. 1996. Resistance gene-dependent plant defense responses. Plant Cell. 8:1773-1791.

Hammond-Kosack, K. E. and Jones, D. G. 1997. Plant resistance genes. Annu.Rev.Plant Physiol. Plant Mol. Biol. 48:575-607.

Hammond-Kosack, K. E. and Parker, J. E. 2003. Deciphering plant–pathogen communication: fresh perspectives for molecular resistance breeding. Curr. Opin. Hüttel 14:177-193.

Han, W. and Christen, P. 2004. cis-Effect of DnaJ on DnaK in ternary complexes with chimeric DnaK/DnaJ-binding peptides. FEBS Lett. 563:146-150.

Hancock, , J. T., Desikan, R., Clarke, A., Hurst, R. D., Neill, S. J. 2002. Cell signalling following plant/pathogen interactions involves the generation of reactive oxygen and reactive nitrogen species. Plant Physiol. Biochem. 40:611-617.

Hans, J., Hause, B., Strack, D., Walter, M. H. 2004. Cloning, characterization, and immunolocalization of a mycorrhiza-inducible 1-Deoxy-D-Xylulose 5-Phosphate reductoisomerase in arbuscule-containing cells of maize. Plant Physiol. 134:614-624.

Hanselle, T. and Barz, W. 2001. Purification and characterisation of the extracellular PR-2b β-1,3-glucanase accumulation in different *Ascochyta rabiei*-infected chickpea (*Cicer arietinum* L.) cultivars. Plant Sci. 161:773-781.

Hanselle, T., Ichinose, Y., Barz, W. 2001. Biochemical and molecular biological studies on infection (*Ascochyta rabiei*)-induced thaumatin-like proteins from chickpea plants (*Cicer arietinum* L.). Z Naturforsch [C] 56:1095-1107.

Hao, L., Hsiang, T., Goodwin, P.H. 2006. Role of two cysteine proteinases in the susceptible response of *Nicotiana benthamiana* to *Colletotrichum destructivum* and the hypersensitive response to *Pseudomonas syringae* pv. tomato. Plant Sci. 170: 1001-1009

Hartl, F. U. and Hayer-Hartl, M. 2002. Molecular chaperones in the cytosol: from nascent chain to folded protein. Science. 295:1852-1858.

Haris, M. EBI; major revision 10-01-2006, Jane Lomax, EBI. Michael Ashburner, Cambridge; updated 20-21 Aug 2002, Midori Harris, ftp://ftpmips.gsf.de/catalogue/funcat-2.0_scheme. Version: Functional Classification Catalogue, Version 2.0, 18.03.2004. Reference: Nucleic Acids Res. 2004. 32(18):5539-45.

Havas, K., Whitehouse, I., Owen-Hughes, T. 2001. ATP-dependent chromatin remodeling activities. Cell. Mol. Life Sci. 58:673-682.

Heath, M. C. 1980. Reaction of nonsuscepts to fungal pathogens. Annu. Rev. Phytopathol. 18:211-236.

Heath, M. C. 2000. Hypersensitive response-related death. Plant Mol. Biol. 44:321-334.

Heaton, J. H., Dlakic, W. M., Dlakic, M., Gelehrter, T. D. 2001. Identification and cDNA cloning of a novel RNA-binding protein that interacts with the cyclic nucleotide-responsive sequence in the Type-1 plasminogen activator inhibitor mRNA. J. Biol. Chem. 276 3341-3347.

Heins, L., Mehrle, A., Hemmler, R., Wagner, R., Kuchler, M., Hormann, F., Sveshnikov, D., Soll, J. 2002. The preprotein conducting channel at the inner envelope membrane of plastids. EMBO J. 21: 2616-2625.

Hernandez-Blanco, C., Feng, D. X., Hu, J., Sanchez-Vallet, A., Deslandes, L., Llorente, F., Berrocal-Lobo, M., Keller, H., Barlet, X., Sanchez-Rodriguez, C. 2007. Impairment of cellulose synthases required for Arabidopsis secondary cell wall formation enhances disease resistance. Plant Cell. 19: 890-903.

Herr, A. J., Molnar, A., Jones, A., Baulcombe, D. C. 2006. Defective RNA processing enhances RNA silencing and influences flowering of Arabidopsis. PNAS. 103: 14994-15001.

Hofiusa, D., Tsitsigiannis, D. I., Jones, J. D. G., Mundya. J. 2007. Inducible cell death in plant immunity. Semin. Cancer Biol. 17:166–187.

Hohl, B., Arnemann, M., Schwenen, L., Stockl, D., Bringmann, G., Jansen, J., Barz, W., 1989. Degradation of the pterocarpan phytoalexin maackiain by *Ascochyta rabiei*. Z. Naturforsch. 44c:771–776.

Hohl, B., Pfautsch, M., Barz, W. 1990. Histology of disease development in resistant and susceptible cultivars of chickpea (*Cicer arietinum* L.) inoculated with spores of *Ascochyta rabiei*. J. Phytopathol. 129:31–45.

Höhl, B., Weidemann, C., Holh, U., Barz, W. (1991). Isolation of solanopyrones A, B and C from culture filtrates and spore germination fluids of *Ascochyta rabiei* and aspects of phytotoxin action. J. Phytopathol. 132:193–206.

Hourton-Cabassa, C., Ambard-Bretteville, F., Moreau, F., de Virville, J. D., Rémy, R. Francs-Small, C. 1998. Stress induction of mitochondrial formate dehydrogenase in potato leaves. Plant Physiol.116:627–635.

Hu, G., Yalpani, N., Briggs, S. P., Johal, G. S. 1998. A porphyrin pathway impairment ıs responsible for the phenotype of a dominant disease lesion mimic mutant of maize. Plant Cell. 10:1095-1105.

Hu, W., Wang, Y., Bowers, C., Ma, H. 2003. Isolation, sequence analysis, and expression studies of florally expressed cDNAs in *Arabidopsis.* Plant Mol. Biol. 53:545–563.

Huang, L., Grammatikos, N., Yoneda, M., Banerjee, S. D., Toole, B. P. 2000. Molecular characterization of a novel intracellular hyaluronan-binding protein. J. Biol. Chem. 275:29829-29839.

Hubert, D. A., Tornero, P., Belkhadir, Y., Krishna, P., Takahashi, A., Shirasu, K., Dang, J. L. 2003. Cytosolic HSP90 associates with and modulates the Arabidopsis RPM1 disease resistance protein. EMBO J. 22:5679-5689.

Hüttel, B., Winter, P., Weising, K., Choumane, W., Weigand, F., Kahl, G. 1999. Sequence-tagged microsatellite site markers for chickpea (*Cicer arietinum* L.). Genome 42:1–8.

Hüttel, B., Santra, D., Muehlbauer, F., Kahl, G.2002. Resistance gene analogues of chickpea (*Cicer arietinum* L.): isolation, genetic mapping and association with a Fusarium resistance gene cluster. Theor. Appl. Genet. 105: 479-490.

Huggett, J., Dheda, K., Zumla, A. 2005. Real-time RT-PCR normalisation; strategies and considerations. Genes Immun. 6:279–284.

Huitema, E., Vleeshouwers, V. G. A. A., Francis, D. M., Kamoun, S. 2003. Active defence responses associated with non-host resistance of *Arabidopsis thaliana* to the oomycete pathogen *Phytophthora infestans*. Mol. Plant Pathol. 4(6):487-500.

Ichinose, Y., Tiemann, K., Schwenger-Erger, C., Toyoda, K., Hein, F., Hanselle, T., Cornels, H., Barz, W. 2000. Genes expressed in *Ascochyta rabiei*inoculated chickpea plants and elicited cell cultures as detected by differential cDNA-hybridization. Zeitschrift für Naturforschung. C, J. Biosci. 55:44–54.

Ichinose,Y., Toyoda K., Barz, W. 1999. cDNAcloning and gene expression of three small GTP-binding proteins in defense response of chickpea. Biochem Biophys. Acta 1489: 462–466.

ICRISAT. 2008a. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Chickpea. http://www.icrisat.org/ChickPea/Chickpea.htm. Accession date: 30 May 2008.

ICRISAT. 2008b. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Chickpea Taxonomy and Biosystematics. http://www.icrisat.org/chickpea/taxonomy/frachc.htm. Accession date: 30 May 2008.

ICRISAT. 2008c. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT). Fungal Diseases of Chickpea Affecting Aerial Parts. http://vasatwiki.icrisat.org/index.php/Fungal_Diseases_of_chickpea_Affecting_Aerial_Parts. Last modified 09:23, 9 October 2007.

ICRISAT. 2008d. International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Fungal Diseases of Chickpea Affecting_Root and Stem http://vasatwiki.icrisat.org/index.php/Fungal_Diseases_of_chickpea_Affecting_Root_/_Stem. Last modified 09:23, 9 October 2007.

Interpro. InterPro - Integrated Resource of Protein Domains and Functional Sites. The European Bioinformatics Institute. Copyright (C) 2001 The InterPro Consortium. http://www.ebi.ac.uk/interpro/

Iruela, M., Rubio, J., Cubero, J. I., Gil, J. Millan, T. 2002. Phylogenetic analysis in the genus Cicer and cultivated chickpea using RAPD and ISSR markers. Theor. Appl. Genet. 104:643-651.

Iruela, M., Rubio, J., Barro, F., Cubero, J. I., Millán, T., Gil, J. 2006. Detection of two quantitative trait loci for resistance to ascochyta blight in an intra-specific cross of chickpea (*Cicer arietinum* L.): development of SCAR markers associated with resistance. Theor. Appl. Genet. 112:278–287.

Iruela, M., Castro, P., Rubio, J., Cubero, J. I., Jacinto, C., Millán, T., Gil, J. 2007. Validation of a QTL for resistance to ascochyta blight linked to resistance to fusarium wilt race 5 in chickpea (*Cicer arietinum* L.). Eur. J. Plant. Pathol. 119:29–37.

Jabs, T., Tschope, M., Colling, C., Hahlbrock, K., Scheel, D. 1997. Elicitor-stimulated ion fluxes and O_2 from the oxidative burst are essential components in triggering defense gene activation and phytoalexin synthesis in parsley. Proc. Natl. Acad. Sci. USA. 94:4800-4805.

Jaiswai, P., Singh, A., Kumar, V., Upadhyaya, K., Verma, P. 2003. Molecular analysis of chickpea genes involved in defense/resistance in response to Ascochyta infection. Poster no: S22-77. 7th International Congress of Plant Molecular Biology. . June 23-28, 2003. Barcelona, Spain. www.ispmb2003.com/abst/obtimpress.php?idAbst=4834

Jakoby, M., Weisshaar, B., Droge-Laser, W., Vicente-Carbajosa, J., Tiedemann, J., Kroj, T. 2002. bZIP transcription factors in Arabidopsis. Trends Plant Sci. 7:106–11.

Jayashree, B., Buhariwalla, H., Shinde, S., Crouch, J. 2005. A legume genomics resource: the chickpea root expressed sequence tag database. Electron. J. Biotechn. 8:128–133.

Jirage, D., Tootle, T. L., Reuber, T. L., Frost, L. N., Feys, B. J. 1999. *Arabidopsis thaliana PAD4* encodes a lipase-like gene that is important for salicylic acid signaling. Proc. Natl. Acad. Sci. USA. 96:13583–88.

Johnson, A and Booth, C. eds. Plant Pathologist's Pocketbook. Common-wealth Agricultural Bureaux. London, England, 1983. IN: Mmbaga, M.T. Pathogenic Variability of *Ascochyta rabiei* and Ascochyta Blight Resistance in Chickpea. IN: DNA Markers and Breeding for Resistance to Ascochyta Blight in Chickpea. Proceedings of the Symposium on 'Application of DNA Fingerprinting for Crop Improvement: Marker-asisted Selection of Chickpea for Sustainable Agriculture in the Dry Areas. 11-12 April 1994. edited by S.M. Udupa and F.Weigang. 1997. International Center for Agriculture Research in the Dry Areas. Aleppo, Syria.

Jones, C. S., Davies, H. V., Taylor, M. A. 2000. Profiling of changes in gene expression during raspberry (*Rubus idaeus*) fruit ripening by application of RNA fingerprinting techniques. Planta. 211: 708-714.

Jones, R. P., Durose, L. J., Findlay, J. B., Harrison, M. A. 2005. Defined sites of interaction between subunits E (Vma4p), C (Vma5p), and G (Vma10p) within the stator structure of the vacuolar H+-ATPase. Biochem. 44:3933-3941.

Jones, D. A. and Takemoto, D. 2004. Plant innate immunity-direct and indirect recognition of general and specific pathogen-associated molecules. Curr. Opin. Immunol. 16:48–62.

Jones, J. D. and Dangl, J. L. 2006. The plant immune system. Nature. 16; 444 (7117): 323-329.

Jones, P. G., Allaway, D., Gilmour, M. D., Haris, C., Rankin, D., Retzel, E. R., Jones, C. A. 2002. Gene discovery and microarray analysis of cacao (*Theobroma cacao* L.) varieties. Planta. 216: 255–264.

Joshi, C. P. and Chiang, V. L 1998. Conserved sequence motifs in plant S-adenosyl-L-methionine-dependent methyltransferases. Plant Mol. Biol. 37:663–674.

Kachroo, P., Shanklin, J., Shah, J., Whittle, E. J., Klessig, D. F. 2001. A fatty acid desaturase modulates the activation of defense signaling pathways in plants. Proc. Natl. Acad. Sci. USA. 98:9448–53.

Kahl, G., Molina, C., Udupa, S.M., Rotter, B., Horres, R., Jungmann, R., Belarmino, L.C., L'Taief, B., Drevon, J. J., Baum, M., Winter, P. SuperSAGE: Exploring The Stress Transcriptomes In Chickpea. W91. Plant and Animal Genomes XVI Conference. January 12-16, 2008. San Diego.

Kaiser, W.J. 1992. Epidemiology of *Ascochyta rabiei.* P:117-134. in Disease resistance Breeding in Chickpea (K.b. Singh and M.C. Saxene, eds.) ICARDA, Aleppo, Syria. IN: Kaiser, W.J. The teleomorph of *ascochyta rabiei* and its significance in breeding chickpea. IN: DNA Markers and Breeding for Resistance to Ascochyta Blight in Chickpea. Proceedings of the Symposium on 'Application of DNA Fingerprinting for Crop Improvement: Marker-asisted Selection of Chickpea for Sustainable Agriculture in the Dry Areas. 11-12 April 1994. edited by S.M. Udupa and F.Weigang. 1997. International Center for Agriculture Research in the Dry Areas. Aleppo, Syria.

Kaiser, W.J. The teleomorph of *Ascochyta rabiei* and its significance in breeding chickpea. IN: DNA Markers and Breeding for Resistance to Ascochyta Blight in Chickpea. Proceedings of the Symposium on 'Application of DNA Fingerprinting for Crop Improvement: Marker-asisted Selection of Chickpea for Sustainable Agriculture in the Dry Areas. 11-12 April 1994. edited by S.M. Udupa and F.Weigang. 1997. International Center for Agriculture Research in the Dry Areas. Aleppo, Syria.

Kaiser, W.J. 1997. Intra- and international spread of ascochyta pathogens of chickpea, faba bean, and lentil. Can. J. Plant Pathol. 19:215-224.

Kaiser, W. J. and Küsmenoglu, I. 1997. Distribution of mating types and the teleomorph of *Ascochyta rabiei* on chickpea in Turkey. Plant Disease. 81:1284-1287.

Kammerer, B., Karsten Fischer, K., Hilpert, B., Schubert, S., Gutensohn, M., Weber, A., Flügge, U. 1998. Molecular Characterization of a Carbon Transporter in Plastids from Heterotrophic Tissues: The Glucose 6-Phosphate/Phosphate Antiporter. Plant Cell. 10(1):105-117.

Kanhonou, A.R., Salom, R.S., Palau, R.R. Issued on June 5, 2007. Sugar beet genes involved in stress tolerance. US Patent 7227053.

Kanazin, V., Marek, L. F., Shoemaker, R. C. 1996. Resistance gene analogs are conserved and clustered in soybean. Proc. Natl. Acad. Sci. USA. 93:11746–11750.

Karen, R., Niclas, O., James, S., Fred, P., Steven, L. 2006. An optimized grapevine RNA isolation procedure and statistical determination of reference genes for real-time RT-PCR during berry development. BMC Plant Biol. 6:27.

Kariola, T. 2006. Pathogen-induced defense signaling and signal crosstalk in *Arabidopsis.* Doctoral dissertation. University of Helsinki, Faculty of Biosciences, Department of Biological and Environmental Sciences, Division of genetics. Helsinki.

Kato, M., Takashima, K., Kakutani, T. 2004. Epigenetic control of CACTA transposon mobility in *Arabidopsis thaliana*. Genetics. 168:961-969.

Kaur, S. 1995. Phytotoxicity of solanapyrones produced by the fungus *Ascochyta rabiei* and their possible role in blight of chickpea (*Cicer arientinum*). Plant Sci.109:23-29.

Kawakamia, K., Iwaib, M., Ikeuchic, M., Kamiyad, N., Shen, J. 2007. Location of PsbY in oxygen-evolving photosystem II revealed by mutagenesis and X-ray crystallography. FEBS Lett. 581: 4983-4987.

Kazan, K and Muehlbauer, F. J. 1991. Allozyme variation and phylogeny in annual species of *Cicer* (Leguminosae). Pl. Syst. Evol. 175: 11-21

Kempken, F., and Windhofer, F. 2001. The hAT family: a versatile transposon group common to plants, fungi, animals and man. Chromosoma. 110: 1-9.

Keppler, L.D., and Novacky, A. 1986. lnvolvement of membrane lipid peroxidation in the development of a bacterially induced hypersensitive reaction. Phytopathol. 76:104-108.

Khurana, S. M. P., Pandey, S. K., Sarkar, D., Chanemougasoundharam, A. 2005. Apoptosis in plant disease response: A close encounter of the pathogen kind. Curr. Sci. 88:740-752.

Kieber, J. J., Rothenberg, M., Roman, G., Feldmann, K.A., Ecker, J.R. 1993. *CTR1,* a negative regulator of the ethylene response pathway in *Arabidopsis*, encodes a member of Raf family of protein kinases. Cell. 72:427-551.

Kim, D. W., Shibato, J., Agrawal, G. K., Fujihara, S., Iwahashi, H., Kim, D. H., ShimI, E., Rakwal, R. 2007. Gene transcription in the leaves of rice undergoing salt-induced morphological changes (*Oryza sativa* L.). Mol. Cells. 24(1):45-59.

Kim, H. S. and Delaney, T. P. 2002. Arabidopsis SON1 is an F-box protein that regulates a novel induced defense response independent of both salicylic acid and systemic acquired resistance. Plant Cell. 14:1469-1482.

Kinkema, M., Fan, W. H., Dong, X. N. 2000. Nuclear localization of NPR1 is required for activation of PR gene expression. Plant Cell. 12:2339-2350.

Kishor, P. B. K., Hong, Z., Miao, C., Hu, C. A., Verma, D. P. S. 1995. Overexpression of A1-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol. (108):1387-1394.

Kiyosue, T., Yamaguchi-Shinozaki, K., Shinozaki, K. 1994 Cloning of cDNAs for genes that are early-responsive to dehydration stress (ERDs) in *Arabidopsis thaliana* L.: identification of three ERDs as HSP cognate genes. Plant Mol Biol. 25(5):791-798.

Klug, W.S. and Cummings, M.R. 2003. Concepts of Genetics. Seventh Edition. International Edition. Pearson Education. Inc. Upper Saddle River, New Jersey, 07458.

Kluge, C., Lahr, J., Hanitzsch, M., Bolte, S., Golldack, D., Dietz, K. 2003. New insight into the structure and regulation of the plant vacuolar H+-ATPase. J. Bioenerg. Biomembr. 35(4):377-388.

Knop, M., Finger, A., Braun, T., Hellmuth, K., Wolf, D. H. 1996. Der1, a novel protein specifically required for endoplasmic reticulum degradation in yeast. EMBO J. 15:753-763.

Kobayashi, I., Kobayashi, Y., Hardham, A. R. 1994 Dynamic reorganization of microtubules and microfilaments in flax cells during the resistance response to flax rust infection. Planta. 195: 237-247.

Koch, E. and Slusarenko, A. 1990. Arabidopsis is susceptible to infection by a downy mildew fungus. Plant Cell. 2:437–445.

Kohler, G., Linkert, C. and Barz, W. 1995. Infection studies of *Cicer arietinum* (L.) with GUS-*E. coli*-glucuronidase transformed *Ascochyta rabiei* strains. *J. Phytopathol*. 143:589-595.

Kokkinos, C. D. Assessment of interactions among viruses ınfecting sweet potato. 2006. A Dissertation Submitted to the Graduate Faculty of the Louisiana State University and Agricultural and Mechanical College in partial fulfillment of the requirements for the degree of Doctor of Philosophy in The Department of Plant Pathology and Crop Physiology. Louisiana.

Komatsu, M., Chiba, T., Tatsumi, K., Lemura, S., Tanida, I., Okazaki, N., Ueno, T., Kominami, E., Natsume, T., Tanaka, K. 2004. A novel protein-conjugating system for Ufm1, a ubiquitin-fold modifier. EMBO J.23, 1977–1986

Kombrink, E. and Somssich, I.E. 1995. Defense responses of plants to pathogens. In: Advances in Botanical Research Vol. 21. (Andrews, J.H. and Tommerup, I.C. eds.), Academic Press Limited. pp 1- 34, 1995.

Kottapalli, K. R, Sarla, N., Kikuchi, S. 2006. In silico insight into two rice chromosomal regions associated with submergence tolerance and resistance to bacterial leaf blight and gall midge. Hüttel Adv. 24:561-589.

Kottapalli, K. R., Satoh, K., Rakwal, R., Shibato, J., Doi, K., Nagata, T., Kikuchi, S. 2007. Combining in silico mapping and arraying: An approach to identifying common candidate genes for submergence tolerance and resistance to bacterial leaf blight in rice. Mol. Cells. 24(3):394-408.

Krüger,A., Pescaronkan-Berghöfer, T., Frettinger, P., Herrmann, S., Buscot, F., Oelmüller, R. 2004. Identification of premycorrhiza-related plant genes in the association between *Quercus robur* and *Piloderma croceum*. New Phytologist. 163 :149-157.

Lacomme, C. and Roby, D., 1999. Identification of new early markers of the hypersensitive response in *Arabidopsis thaliana*. FEBS Lett. 459:149-153.

Ladizinsky, G. and Adler, A., 1976. Genetic relationships among the annual species of Cicer L. Theor. Appl. Genet., 48: 197-204.

Lagudah, E.S., Moullet, O., Appels, R. 1997. Map-based cloning of a gene sequence encoding a nucleotide-binding domain and a leucine-rich region at the Cre3 nematode resistance locus of wheat. Genome. 40: 659-665.

Lamb, C. and. Dixon, R. A. 1997. The oxidative burst in plant disease resistance. Annu. Rev. Plant Physiol. Plant Mol. Biol. 48:251-75.

Latif, Z., Strange, R. N., Bilton, J., Riazuddin, S. 1993. Production of phytotoxins, solanapyrones A and C and cytochalasin D among nine isolates of *Ascochyta rabiei*. Plant Pathology. 42:172-180.

Lee, D., Ellard, M., Wanner, L. A., Davis, K. R, Douglas, C. J. 1995. The *Arabidopsis thaliana* 4-coumarate:CoA ligase (4CL) gene: stress and developmentally regulated expression and nucleotide sequence of its cDNA. Plant Mol. Biol. 28(5):871-884.

Leister, D., Ballvora, A., Salamini, F., Gebhardt, C. 1996. A PCR-based approach for isolating pathogen resistance genes from potato with potential for wide application in plants. Nat. Genet. 14:421-429.

León, J., Lawton, M. A., Raskin, I. 1995. Hydrogen peroxide stimulates salicylic acid biosynthesis in tobacco. Plant Physiol.108:1673-1678.

Levine, A., Tenhaken, R., Dixon R. A., Lamb, C. 1994. H_2O_2 from the oxidative burst orchestrates the plant hypersensitive response. Cell. 79:583-593.

Levine, A., Pennell, R. I., Alvarez, M. E., Palmer, R., Lamb, C. 1996. Calcium-mediated apoptosis in a plant hypersensitive disease resistance response. Curr. Biol. 6:427-437.

Levy-Strumpf, N., Deiss, L. P., Berissi, H., Kimchi, A. 1997. DAP-5, a novel homologue of the translation initiation factor 4γ, isolated as a positive modulator of interferon-γ-induced programmed cell death. Mol. Cell. Biol. 17:1615-1625.

Leung, H. in: Zhang, L.P., Khan, A., Nino-Liu, D., Foolad, M.R. 2002. A molecular linkage map of tomato displaying chromosomal locations of resistance gene analogs based on a *Lycopersicon esculentum* x *Lycopersion hirsutum* cross. Genome. Vol: 45. p:133-146.

Li, J. and Chory, J. 1997. A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell. 90:929-938.

Li, Z., Pandit, S., Deutscher, M. P. 1999. RNase G (CafA protein) and RNase E are both required for the 59 maturation of 16S ribosomal RNA. EMBO J. 18:2878–2885.

Li, J., Lease, K. A., Tax, F. E., Walker, J. C. 2001. BRS1, a serine carboxypeptidase, regulates BRI1 signaling in *Arabidopsis thaliana*. PNAS. 98:5916-5921.

Li, J., Brader, G., Palva, E. T. 2004. The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. Plant Cell.16:319-31.

Li, Q., Chen, F., Sun, L., Zhang, Z., Yang, Y., He, Z. 2006. Expression profiling of rice genes in early defense responses to blast and bacterial blight pathogens using cDNA microarray. Physiol. Mol. Plant Pathol. 68: 51-60.

Li, L., Cheng, H., Gai, J. D., Yu, D. 2007. Genome-wide identification and characterization of putative cytochrome P450 genes in the model legume *Medicago truncatula*. Planta. 226:109-123.

Liang, P. and Pardee, A. B. 1992. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Science. 257:967.

Lichtenzveig, J., Scheuring, C., Dodge, J., Abbo, S., Zhang, H. B. 2005. Construction of BAC and BIBAC libraries and their applications for generation of SSR markers for genome analysis of chickpea,*Cicer arietinum* L. Theor. Appl. Genet. 110: 492-510.

Ligterink, W., Kroj, T., Nieden, U., Hirt, H., Scheel, D. 1997. Receptor-mediated activation of a MAP kinase in pathogen defense of plants. Science. 276:2054-2057.

Linder, B., Cabot, R. A., Schwickert, T., Rupp, R. A. 2004. The SNF2 domain protein family in higher vertebrates displays dynamic expression patterns in *Xenopus laevis* embryos. Gene. 326:59-66.

Liu, Y., Schiff, M., Serino, G., Deng, X. W., Dinesh-Kumar, S. P. 2002. Role of SCF ubiquitin-ligase and the COP9 signalosome in the N gene mediated resistance response to Tobacco mosaic virus. Plant Cell. 14:1483-1496.

Liu, X. D. and Shen, Y. G. 2004. NaCl-induced phosphorylation of light harvesting chlorophyll a/b proteins in thylakoid membranes from the halotolerant green alga, *Dunaliella salina.* FEBS Lett. 569: 337-340.

Liu, Y., Wang, H., Ye, H., Li, G. 2005. Advances in the plant ısoprenoid biosynthesis pathway and its metabolic engineering. Acta Bot. Sin. 47 (7):769-782.

Liu, X., Huang, B., Lina, J., Feib, J., Chena, Z., Panga, Y., Suna, X, Tang, K. 2006. A novel pathogenesis-related protein (SsPR10) from *Solanum surattense* with ribonucleolytic and antimicrobial activity is stress- and pathogen-inducible. J. Plant Physiol. 163:546-556.

Liu, J., Liu, X., Dai, L., Wang, G. 2007. Recent progress in elucidating the structure, function and evolution of disease resistance genes in plants. J. Gen. Genom.34(9):765-776.

Livak, K. J. and Schmittgen, T. D. 2001. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the $2^{-\Delta\Delta C}_{T}$ Method. Methods. 25: 402-408.

Llorente, F., Alonso-Blanco, C., Sanchez-Rodriguez, C., Jorda, L., Molina, A. 2005. ERECTA receptor-like kinase and heterotrimeric G protein from Arabidopsis are required for resistance to the necrotrophic *fungus Plectosphaerella cucumerina.*Plant J. 43:165-180

Lopez, C., Soto, M., Restrepo, S., Piegu, B., Cooke, R., Delseny, M., Tohme, J., Verdier, V. 2005. Gene expression profile in response to *Xanthomonas axonopodis* pv. *manihotis* infection in cassava using a cDNA microarray. Plant Mol. Biol. 57:393-410.

Lorenzo, O., Piqueras, R., Sánchez-Serrano, J. J., Solano, R. 2003. Ethylene Response Factor1 integrates signals from ethylene and jasmonate pathways in plant defense. Plant Cell. 15:165-178.

Lorenzo, W. O., Chico, J. M., Sanchez-Serrano, J. J., Solano, R. 2004. JASMONATE-INSENSITIVE1 encodes a MYC transcription factor essential to discriminate between different jasmonate-regulated defense responses in Arabidopsis. Plant Cell. 16:1938-1950.

Lu, G., Jantasuriyarat, C., Zhou, B., Wang, G. L. 2004. Isolation and characterization of novel defense response genes involved in compatible and incompatible interactions between rice and *Magnaporthe grisea*. Theor. Appl. Genet.108:525-534.

Lu, M., Tang, X.Y., Zhou, J. M. 2001. Arabidopsis NHO1 is required for general resistance against *Pseudomonas bacteria*. Plant Cell. 13:437-447.

Lucy, M. C., Matthews, H. D., VanEtten, H. D. 1988. Metabolic detoxification of the phytoalexins maackiain and medicarpin by *Nectria haematococca* field isolates: relationship to virulence on chickpea. Physiol. Mol. Plant. Pathol. 33:187-199.

Ludwig, A. A., Saitoh, H., Felix, G., Freymark, G., Miersch, O., Wasternack, C., Boller, T., Jones, J. D., Romeis, T. 2005. Ethylene-mediated cross-talk between calcium dependent protein kinase and MAPK signaling controls stress responses in plants. Proc. Natl. Acad. Sci. USA. 102:10736-10741.

Mackenbrock, U., Gunia, W., Barz, W. 1993. Accumulation and metabolism of medicarpin and maackiain malonylglucosides in elicited chickpea (*Cicer arietinum* L.) cell-suspension cultures. J. Plant Physiol. 142:385-391.

Mackenbrock, U. and Barz, W. 1991. Elicitor-induced formation of pterocarpan phytoalexins in chickpea (*Cicer arietinum* L.) cellsuspension cultures from constitutive isoflavone conjugates upon inhibition of phenylalanine ammonia-lyase. Z Naturforsch C. 46:43-50.

Mackey, D., Holt, B. F., Wiig, A., Dangl, J. L. 2002. RIN4 interacts with Pseudomonas syringae type III effector molecules and is required for RPM1-mediated resistance in Arabidopsis. Cell. 108:743-754.

Macknight, R., Bancroft, I., Page, T., Lister, C., Schmidt, R., Love, K., Westphal, L., Murphy, G., Sherson, S., Cobbett, C. 1997. FCA, a gene controlling flowering time in Arabidopsis, encodes a protein containing RNA-binding domains. Cell. 89: 737-745.

Mago, R., Nair, S. and Mohan, M. 1999. Resistance gene analogues from rice: cloning, sequencing and mapping. Theor. Appl. Genet. 99: 50-57.

Mahon, P. and Bateman, A. 2000. The PA domain: A protease-associated domain. Protein Sci. 9:1930-1934.

Mano, J., Torii, Y., Hayashi, S. I., Takimoto, K., Matsui, K., Nakamura, K., Inze, D., Babiychuk, E., Kushnir, S., Asada, K. 2002. The NADPH: quinine oxidoreductase PI-f-crystallin in Arabidopsis catalyzes the a, b-hydrogenation of 2-alkenals: detoxification of the lipid peroxide-derived reactive aldehydes. Plant Cell Physiol. 43:1445-1455.

Mantri, N. L., Ford, R., Coram, T. E., Pang, E. C. K. 2007. Transcriptional profiling of chickpea genes differentially regulated in response to high-salinity, cold and drought. BMC Genomics. 8:303.

Martin, G. B., Brommonschenkel, S. H., Chunwongse, J., Fra Ganal, M. W., Spivey, R., Wu, T., Earle, E. D., Tanksley, S. D. 1993. Map-based cloning of a protein kinase gene conferring disease resistance in tomato. Science. 262:1432-1436.

Matsumura, H., Reich, S., Ito, A., Saitoh, H, Kamoun, S., Peter Winter, P., Kahl, G., Reuter, M., Krüger, D. H., Terauchi, R. 2003. Gene expression analysis of plant host-pathogen interactions by SuperSAGE. PNAS. 100:15718-15723.

Mayrose, M., Ekengren, S. K., Melech-Bonfil, S., Martin, G. B., Sessa, G. 2006. A novel link between tomato GRAS genes, plant disease resistance and mechanical stress response. Mol. Plant Pathol. 7:593-604.

Tadashi Maruyama 1, Rintaro Suzuki 2 and Masahiro Furutani. 2004. Archaeal peptidyl prolyl *cis-trans* isomerases (PPIases) UPDATE 2004. Frontiers in Biosci. 9:1680-1700.

Meister, G and Tuschl, T. 2004. Mechanisms of gene silencing by double-stranded RNA. Nature. 431(7006):343-349.

Michelmore, R. W. and Meyers, B. C. 1998. Clusters of resistance genes in plants evolve by divergent selection and a birth-and-death process. Genome Res. 8:1113-1130.

Mikkelsen, M.D., Petersen, B.L., Glawischnig, E., Jensen, A.B., Andreasson, E., Halkier, B.A. 2003. Modulation of CYP79 genes and glucosinolate profiles in Arabidopsis by defense signaling pathways. Plant Physiol. 131: 298-308.

Millan, T., Rubio, J., Iruela, M., Daly, K., Cubero, J. I., Gil, J. 2003. Markers associated with Ascochyta blight resistance in chickpea and their potential in marker-assisted selection. Field Crops Res. 84:373-384.

Millan, T., Clarke, H. J., Siddique, K. H. M., Buhariwalla, H. K., Gaur, P. M., Kumar, J., Gil, J., Kahl, G., Winter, P. 2006. Chickpea molecular breeding: New tools and concepts. Euphytica:147: 81-103.

Minorsky, P. V. 2002. The wall becomes surmountable. Plant Physiol. 128:345-353.

Mittler, R. 2002. Oxidative stress, antioxidants and stress tolerance. Trends Plant Sci. 7:405-410.

Mmbaga, M.T. pathogenic variability of *ascochyta rabiei* and ascochyta blight resistance in chickpea. IN: DNA Markers and Breeding for Resistance to Ascochyta Blight in Chickpea. Proceedings of the Symposium on 'Application of DNA Fingerprinting for Crop Improvement: Marker-asisted Selection of Chickpea for Sustainable Agriculture in the Dry Areas. 11-12 April 1994. edited by S.M. Udupa and F.Weigang. 1997. International Center for Agriculture Research in the Dry Areas. Aleppo, Syria.

Mock, H. P., Heler, W., Antonio Molinai, A., Neubohn, B., Sandermann, H., Bernhard Grimm, B. 1999. expression of uroporphyrinogen decarboxylase or coproporphyrinogen oxidase antisense RNA in tobacco induces pathogen defense responses conferring increased resistance to tobacco mosaic virus. J. Biol. Chem. 274(7):4231-4238.

Moller, I. M. and Kristensen, B. K. 2004. Protein oxidation in plant mitochondria as a stress indicator. Photochem.Photobiol.Sci. 3:730-735.

Moller, I. M. 2001. Plant mitochondria and oxidative stress: Electron transport, NADPH turnover, and metabolism of reactive oxygen species. Annu. Rev. Plant. Physiol. Plant. Mol. Biol. 52:561-591.

Morel, M., Jacob, C., Kohler, A., Johansson, T., Martin, F., Chalot, M., Brun, A. 2005. Ectomycorrhizal Roots during *Paxillus involutus-Betula pendula* Ectomycorrhizal Symbiosis. Appl. Environ. Microbiol. 71(1): 382-391.

Morita, T, Maki, K. and Aiba, H. 2005. RNase E-based ribonucleoprotein complexes: mechanical basis of mRNA destabilization mediated by bacterial noncoding RNAs. Genes and Development. 19:2176-2186.

Morley, S. J., Coldwell, M. J., Clemens, M. J. 2005. Initiation factor modifications in the preapoptotic Phase. Cell Death Differ. 12:571-584.

Mou, L., Miller, H., Li, J., Wang, E., Chalifour, L. 1994. Improvements to the differential display method for gene analysis. Biochem. Biophys. Res. Commun. 199:564-569.

Mourelatos, Z. et al. 2002. miRNPs: a novel class of ribonucleoproteins containing numerous microRNAs. Genes Dev. 16:720–728.

Mozzarelli, A. and Bettati, S. 2006. Exploring the pyridoxal 5'-phosphate-dependent enzymes. Chem Rec. 6:275-87.

Mueller, M. J., Brodschelm, W., Spannagl, E., Zenk, M. H. 1993. Signaling in the elicitation process is mediated through the octadecanoid pathway leading to jasmonic acid. Proc. Natl. Acad. Sci. USA.90:7490-7494.

Murray, D. C. and Walters, D. R. 1992. Increased photosynthesis and resistance to rust infection in upper, uninfected leaves of rusted broad bean (*Vicia faba* L.). New Phytol. 120:235-242.

Mysore, K.S. and Ryu, C.M. 2004. Nonhost resistance: how much do we know? Trends Plant Sci. 9: 97–104.

Naqvi, S. M. S., Park, K. S., Yi, S. Y., Lee, H. W., Bok, S. H., Choi, D. 1998. A glycine rich RNA binding protein gene is differentially expressed during acute hypersensitive response following Tobacco Mosaic Virus infection in tobacco. Plant Mol. Biol. 37:571-576.

Navarro, L., dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Etselle, M., Voinnet, O., Jones, J. D. G. 2006. A Plant miRNA Contributes to Antibacterial Resistance by Repressing Auxin Signaling. Science. 312:436-439.

Navas-Cortés, J. A., Perez-Artes, E., Jimenez-Diaz, R. M., Llobell, A., Bainbridge, B. W., Heale, J. B. 1998. Mating type, pathotype and RAPD analysis in *Didymella rabiei*, the agent of Ascochyta blight of chickpea. Phytoparasitica 26:199-212.

Nawrath, C., Heck, S., Parinthawong, N., Metraux, J. P. 2002. *EDS5*, anessential component of salicylic acid-dependent signaling for disease resistance in Arabidopsis, is a member of the MATE transporter family. Plant Cell 14:275-86.

NCBI. National Library of Medicine. National Center for Biotechnology Information. http://www.ncbi.nlm.nih.gov/ Revised: July 23, 2008.

Nehra, K.S., Chugh, L.K., Dhillon, S., Singh, R. 1994. Induction, purification and characterization of chitinases from chickpea (*Cicer arientinum* L.) leaves and pods infected with *Ascochyta rabiei*. J. Plant Physiol. 144:7-11.

Nelson, M. A. 1996. Mating systems in Ascomycetes: a romp in the sac. Trends Genet. 12:69-74.

Nene, Y. L., Reddy, M. V., 1987. Chickpea diseases and their control. In: Sexena MC, Singh KB, eds. The Chickpea. Wallingford, UK: CAB International, 233–370. IN: Udupa, S., Weigand, F., Saxena, M., Kahl, G. 1998. Genotyping with RAPD and microsatellite markers resolves pathotype diversity in the ascochyta blight pathogen of chickpea. Theor. Appl. Genet. 97:299-307.

Nene Y.L. and. Sheila, V. K. primary collators. Common Names of Plant Diseases. APSnet.The American Phytopathological Society. http://www.apsnet.org/online/common/names/chickpea.asp . Last updated 6/25/96.

Nersissian, A. M., Immoos, C., Hill, M. G., Hart, P. J., Williams, G., Herrmann, R. G., Valentine, J.S. 1998. Uclacyanins, stellacyanins, and plantacyanins are distinct subfamilies of phytocyanins: Plant-specific mononuclear blue copper proteins. Protein Sci. 71915-1929.

Nguyen, T. T., Taylor, P. W. J., Redden, R. J., Ford, R. 2004. Genetic diversity estimates in Cicer using AFLP analysis. Plant Breeding. 123:173-179.

Nicholson, D. W. and Thornberry, N. A 1997. Caspases: killer proteases. Trends Biochem. Sci. 22:299-306.

Nicot, N., Hausman, J.F., Hoffmann, L., Evers, D. 2005. Housekeeping gene selection for real-time RT-PCR normalization in potato during biotic and abiotic stress. J. Exp. Bot. 56(421): 2907-2914.

Nielsen, H. B., Zhu, S., Newman, M. A., Shokat, K. M., Rietz, S., Parker, J., Mundy, J. 2006. Arabidopsis MAP kinase 4 regulates salicylic acid- and jasmonic acid/ethylene-dependent responses via EDS1 and PAD. Plant J. 447:532-546.

Nimbalkara, A. B., Harsulkara, A. M., Giria, A. P., Sainania, M. N., Franceschib, V., Gupta, V. S. 2006. Differentially expressed gene transcripts in roots of resistant and susceptible chickpea plant (*Cicer arietinum* L.) upon *Fusarium oxysporum* infection. Physiol. Mol. Plant Pathol. 68:176-188.

Noctor, G. and Foyer, C.H. 1998. Ascorbate and glutathione: Keeping active oxygen under control. Ann. Rev. Plant Physiol. 49: 249-279.

Nuhse, T. S., Peck, S. C., Hirt, H., Boller, T. 2000. Microbial elicitors induce activation and dual phosphorylation of the *Arabidopsis thaliana* MAPK 6. J. Biol. Chem. 275:7521-7526.

Oliver, R. P. and Ipcho, S. V. S. 2004. Arabidopsis pathology breathes new life into the necrotrophs-vs.-biotrophs classification of fungal pathogens. Mol Plant. Pathol. 5:347-52.

Otani, H., Kohnobe, A., Kodama, M., Kohmoto, K. 1998. Production of a host specific toxin by germinating spores of *Alternaria brassicicola*. Physiol. Mol. Plant Pathol. 52:285-295.

Otte, O. and Barz, W. 1996. The elicitor-induced oxidative burst in cultured chickpea cells drives the rapid insolubilization of two cell wall structural proteins. Planta. 200: 238-256.

Otte, O. and Barz, W. 2000. Characterization and oxidative in vitro cross-linking of an extensin-like protein and a proline-rich protein purified from chickpea cell walls Phytochem. 53:1-5.

Otte, O., Pachten, A., Hein, F., Barz, W. 2001. Early elicitor-induced events in chickpea cells: Functional links between oxidative burst, sequential occurrence of extracellular alkalinisation and acidification, K+/H+ exchange and defense-related gene activation. Z. Naturforsch [C]. 56: 65-76.

Overkamp, S., Hein, F., Barz, W. 2000. Cloning and characterization of eight cDNAs from chickpea (*Cicer arietinum* L.) cell suspension cultures. Plant Sci. 155:101-108.

Palomino, C., Satovic, Z., Cubero, J. I., Torres, A. M. 2006. Identification and characterization of NBS-LRR class resistance gene analogs in faba bean (Vicia faba L.) and chickpea (*Cicer arietinum* L.). Genome. 49(10):1227-1237.

Panabières F., Joëlle Amselemb J., Galiana E., Berre J.L. 2005. Gene identifcation in the oomycete pathogen *Phytophthora parasitica* during in vitro vegetative growth through expressed sequence tags. Plant Cell. 10: 105-117.

Pandey, B., Singh, U., Chaube, H. 1987. Mode of infection of ascochyta blight of chickpea caused by *Ascochyta rabiei*. J. Phytopathol. 119:88-93.

Park, J. H., Oh, S. A., Kim, Y. H., Woo, H. R., Nam, H. G. 1998. Differential expression of senescence associated mRNAs during leaf senescence induced by different senescence inducing factors in *Arabidopsis*. Plant Mol. Biol. 37: 445-454.

Parker, J. E., Holub, E. B., Frost, L. N., Falk, A., Gunn, N. D., Daniels, M. J. 1996. Characterization of EDS1, a mutation in Arabidopsis suppressing resistance to Peronospora parasitica specified by several different RPP genes. Plant Cell. 8:2033-2046.

Parlevliet, J.E., 1979. Components of resistance that reduce the rate of epidemic development. Annu. Rev. Phytopathol. 17:203-222.

Peck, S. C. 2003. Early phosphorylation events in biotic stres. Curr. Opin. Plant. Biol. 6:334-338.

Park, J.H., Oh, S.A., Kim, Y.H., Woo, H.R., Nam, H.G. 1998. Differential expression of senescence associated mRNAs during leaf senescence induced by different senescence inducing factors in *Arabidopsis.* Plant Mol. Biol. 37: 445–454.

Pasquer, F. 2005. Effects of plant protection compounds on wheat gene expression. Dissertation zur Erlangung der naturwissenschaftlichen Doktorwürde (Dr. sc. nat.) vorgelegt der Mathematisch-naturwissenschaftlichen Fakultät der Universität Zürich.

Pedras, M. S. C. and Ahiahonu, P. W. K. 2005. Metabolism and detoxification of phytoalexins and analogs by phytopathogenic fungi. Phytochem. 66:391-411.

Pelloux, J., Rusterucci, C., Mellerowicz, E. J. 2007. New insights into pectin methylesterase structure and function. Trends Plant Sci. 12(6) 267-277.

Peng, M. and Kuc, J. 1992. Peroxidase-generated hydrogen peroxide as a source of antifungal activity in vitro and on tobacco leaf disks. Phytopathol. 82:696-699.

Pfaff, T. and Kahl, G. 2003. Mapping of gene-specific markers on the genetic map of chickpea (*Cicer arietinum* L.). Mol. Gen. Genom. 269:243-251.

Pfam. The Wellcome Trust. Sanger Institute. The Pfam protein families database*:* R.D. Finn, J. Tate, J. Mistry, P.C. Coggill, J.S. Sammut, H.R. Hotz, G. Ceric, K. Forslund, S.R. Eddy, E.L. Sonnhammer and A. Bateman Nucleic Acids Research (2008) Database Issue 36:D281-D288. Release 1.1 (18th September 2007) Release 1.2 (15th October 2007). http://pfam.sanger.ac.uk/

Pickart C.M. 2001. Ubiquitin enters the new millennium. Mol. Cell. 8:499-504.

Pieterse C.M.J., van Wees S.C.M., Ton J, van Pelt J.A., van Loon L.C. 2002. Signalling in Rhizobacteria-induced systemic resistance in *Arabidopsis thaliana*. Plant Biol. 4:535-544.

Pontig, C. P.2000. Novel eIF4G domain homologues linking mRNA translation with nonsensemediated mRNA decay. Trends Biochem. Sci. 25(9):423-426.

Porta-Puglia, A. Crino, P., Mosconi, C. 1996. Variability in virulence to chickpea an Italian population of *Ascochyta rabiei*. Plant Dis. 80: 39-41.

Potuschak, T., Lechner, E., Parmentier, Y., Yanagisawa, S., Grava, S., Koncz, C., Genschik, P. 2003. EIN3-dependent regulation of plant ethylene hormone signaling by two arabidopsis F box proteins: EBF1 and EBF2. Cell. 115:647-648.

Pouteau, S., Grandbastien, M. A., Boccara, M. 1994. Microbial elicitors of plant defense responses activate transcription of retrotransposon. Plant J. 5:535-542.

Prell., H. H. and Day, P. R. 2001. Plant-fungal pathogen interaction. A classical and molecular view. Springer-Verlag Berlin Heilderberg. Germany.

Raab, T., López-Ráez, J. A., Klein, D., Caballero, J. L., Moyano, E., Schwab, W., Muñoz-Blanco, J. 2006. FaQR, required for the biosynthesis of the strawberry flavor compound 4-hydroxy-2,5-dimethyl-3(2H)-furanone, encodes an enone oxidoreductase. Plant Cell. 18(4):1023-1037.

Raes, J., Rohde, A., Christensen, J. H., Van de Peer, Y., Boerjan, W. 2003. Genome-wide characterization of the lignification toolbox in Arabidopsis**.** Plant Physiol. 133(3):1051-1071.

Rajesh, P. N. 2001. Chickpea genomics: BAC library construction, resistance gene analog (RGA) mapping and tagging double-podded trait. A thesis submitted to the University of Pune for the Degree of Doctor of Philosophy in Biotechnology. Plant Molecular Biology Division of Biochemical Sciences, National Chemical Laboratory Pune 411 008, India.

Rajesh, P. N., Tekeoglu, M., Gupta, V. S., Ranjekar, P. K., Muehlbauer, F. J. 2002. Molecular mapping and characterization of an RGA locus RGAPtokin1-2171 in chickpea. Euphytica 128: 427–433.

Rajesh, P. N., Coyne, C., Meksem, K., Sharma, K. D. Gupta, V., Muehlbauer, F. J. 2004. Construction of a HindIII Bacterial Artificial Chromosome library and its use in identification of clones associated with disease resistance in chickpea. Theor. Appl. Genet. 108:663-669.

Rajesh, P., O'Bleness, M., Main, D., Roe, B., Muehlbauer, F. 2007. Analysis of genome organization and composition using 500 Kb BAC sequences in chickpea. Plant and Animal Genome XV Conference. San Diego. (Eds G Lazo, D Grant, V Blake) p. 47. San Diego.

Rajesh, P.N. Avcioglu, B., Nayak, S., Winter, P., Varshney, R., McPhee, K., Zhang, H., Muehlbauer, F., Chen, W. Integration of additional molecular markers and genetic analysis of ascochyta blight resistance in chickpea. Poster no: P386. Plant and Animal Genomes XVI Conference. January 12-16, 2008. San Diego.

Rajput M.K. and Upadhyaya K.C. Posted 24 Aug 2007. *CARE*1, a TY3-gypsy long terminal repeat retrotransposon in the food legume chickpea (*Cicer arietinum* L). Nature Precedings: hdl:10101/npre.2007.819.1.

Raskin, I. 1992. Salicylate, a new plant hormone. Plant Physiol. 99:799-803.

Rasmusson, A. G., Soole, K. L., Elthon, T. E. 2004. Alternative nad(p)h dehydrogenases of plant mitochondria. Annu. Rev. Plant Biol. .55:23-39.

Rea, G., Metoui, O., Infantino, A., Federico, R., Angelini, R. 2002. Copper amine oxidase expression in defense responses to wounding and *Ascochyta rabiei* Invasion. Plant Physiol. 128:1-11.

Reddy, M.V., Singh, K.B., Malhotra, R.S. 1992. Multilocation evaluation of chickpea germplasm and breeding lines for resistance to *Ascochyta* blight. Phytopathol. Medit. 31:59-66.

Reddy, M.V. and Kabbabeh, S. 1985. Pathogenic variability *Ascochyta rabiei* (Pass.) lab. In Syria and Lebanon. Phytopathol. Medit. 24: 265-266.

Robinson, R. C, Turbedsky, K., Kaiser, D. A, Marchand, J. B, Higgs, H. N, Choe, S., Pollard, T. D. 2001. Crystal structure of ArPII/3 complex. Science. 294(5547):1679-1684.

Rospert, S., Dubaquie, Y., Gautschi, M. 2002. Nascent-polypeptide-associated complex. Cell Mol. Life. Sci. 59:1632-1639.

RRC Core Genomics Facility.2003. Real Time PCR Handbook. University of Illinois at Chicago. http://www.uic.edu/depts/rrc/cgf/realtime/index.html

Rubiales, D., Perez-de-Luque, A., Joel, D.M., Alcantara, C., Sillero, J. C. 2003. Characterization of resistance in chickpea to crenate broomrape (*Orobanche crenata*). Weed Science. 51:702-707.

Rudenko, G.N. and Walbot, V. 2001. Expression and post-transcriptional regulation of maize transposable element MuDR and its derivatives. Plant Cell. 13:553-570.

Rushton, P.J., Torres J.T., Parniske, M., Wernet, P., Hahlbrock, K., Somssich, I.E. 1996. Interaction of elicitor induced DNA binding proteins with elicitor response elements in the promoters of parsley PR-1 genes. EMBO J 15: 5690-5700.

Salzman, R. A., Fujta, T., Zhu-Salzman, K., Hasegawa, P. M., Bressan, R. A. 1999. An improved RNA isolation method for plant tissues containing high levels of phenolic compounds or carbohydrates. Plant Mol. Biol. Reporter. 17:11-17.

Sambrook, J., Fritsch, E. F., Manniatis, T. 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.

Sanabria, N. M and Dubery, I. A. 2004. Enchanced cloning efficiency of differential display PCR amplicons. Plant Mol. Biol. Reporter 22:309.

Sangster, T. and Queitsch, C. 2005. The HSP90 chaperone complex, an emerging force in plant development and phenotypic plasticity. Curr. Opin. Plant Biol. 8:86-92.

Santra, D.K., Tekeoglu, M., Ratnaparkhe, M., Kaiser, W.J., Muehlbauer, F.J. 2000. Identification and mapping of QTLs conferring resistance to ascochyta blight in chickpea. Crop Sci. 40, 1606-1612.

Santra, D., Singh, G., Kaiser, W., Gupta, V., Ranjckar, P. Muehlbauer, F. 2001. Molecular analysis of *Ascochyta rabiei* (Pass.) Lar., the pathogen of ascochyta blight in chickpea. Theor. Appl. Genet. 102:676-682.

Sato, K., Kohzuma, T. and Dennison, C. 2003. Active-site structure and electron-transfer reactivity of plastocyanins J. Am. Chem. Soc. 125:2101-2112.

Sawada, K., Hasegawa, M., Tokuda, L., Kameyama, J., Kodama, O., Kohchi, T., Yoshida, K., Shinmyo, A. 2004. Enhanced resistance to blast fungus and bacterial blight in transgenic rice constitutively expressing OsSBP, a rice homologue of mammalian selenium-binding proteins. Biosci. Hüttel Biochem. 68:873-880.

Schafleitner, R. and Wilhelm, E. 2002. Isolation of wound-responsive genes from chestnut (*Castanea sativa*) microstems by mRNA display and their differential expression upon wounding and infection with the chestnut blight fungus (*Chryphonectria parasitica*). Physiol. Mol. Plant Pathol. 61:339-348.

Schenk, P. M., Kazan, K., Wilson, I., Anderson, J. P., Richmond, T., Somerville, S. C., Manners, J. M. 2000. Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. PNAS. 97:11655-11660.

Schenk, P. M., Kazan, K., Manners, J. M., Anderson, J. P., Simpson, R. S., Wilson, I. W., Somerville, S. C., Maclean, D. J. 2003. Systemic gene expression in Arabidopsis during an incompatible interaction with *Alternaria brassicicola*. Plant Physiol. 132:999-1010.

Schmelzer, E. 2002. Cell polarization, a crucial process in fungal defence. Trends Plant Sci. 7(9):411-415.

Schmidt, D. D., Claudia, Voelckel, C., Hart, M., Schmidt, S., Baldwin, I. T. 2005. Specificity in ecological interactions. attack from the same lepidopteran herbivore results in species-specific transcriptional responses in two solanaceous host plants. Plant Physiol. 138: 1763-1773.

Schöttler, M. A., Kirchhoff, H., Weis, E. 2004. The role of plastocyanin in the adjustment of the photosynthetic electron transport to the carbon metabolism in Tobacco Plant Physiol. 136: 4265-4274.

Schulz, B. C4.12–Integrated approaches for gene analysis identify new signal transduction elements. 2002. C4–CELL SIGNALLING organized by M.R. McAinsh and A.A.R. Webb for the Cell Signalling Group. Ca2q Signalling and Ion Channels. Abstracts / Comparative Biochemistry and Physiology Part A 132. S195–S199.

Seah, S., Sivasithamparam, K., Karakousis, A., Lagudah, E. 1998. Cloning and characterisation of a family of disease resistance gene analogs from wheat and barley. Theor. Appl. Genet. 97: 937–945.

Seki, M., Narusaka, M., Abe, H., Kasuga M., Yamaguchi-Shinozaki, K., Carninci, P., Hayashizaki, Y., Shinozaki, K. 2001. Monitoring the expression pattern of 1300 Arabidopsis genes under drought and cold stresses by using a full-length cDNA microarray. Plant Cell. 13: 61-72.

Shaw, P. E. 2002. Peptidyl-prolyl isomerases: a new twist to transcription. EMBO reports. 3:521-526.

Shibahara, K., Asano, M., Ishida, Y., Aoki, T., Koike, T., Honjo, T. 1995. Isolation of a novel Mouse gene *MA-3* that is induced upon programmed cell death. Gene. 166:297-301.

Shibuya, N. and Minami, E. 2001. Oligosaccharide signalling for defence responses in plant. Physiol. Mol. Plant Pathol. 59:223-233.

Shirasu, K., Lahaye, T., Tan, M. W., Zhou, F., Azevedo, C., Schulze-Lefert, P. 1999. A novel class of eukaryotic zinc-binding proteins is required for disease resistance signaling in barley and development in *C. elegans*. Cell. 99:355-366.

Showalter, A. M. 1993. Structure and function of plant cell wall proteins. Plant Cell. 5:9-23.

Siezen, R. J. and Leunissen, J. A. M. 1997. Subtilases: The superfamily of subtilisin-like serine proteases. Protein Sci. 6:501-523.

Singh, K. B., Hawtin, G.C., Nene, Y.L., Reddy, M.V. 1981. Resistance in chickpeas to *Ascochyta rabiei*. Plant Disease. 65:586-587.

Singh, K. B., Malhotra, R. S., Saxena, M. C. 1993.Registration of ILC195 chickpea. Crop. Sci. 33:1409-1410.

Singh, K. B. 1997. Chickpea (*Cicer arietinum* L.). Field Crops Research. 53:161-170.

Singh, K. B. and Reddy, M. V. 1989. Genetics of resistance to ascochyta blight in four chickpea lines. Crop Sci. 29:657-659.

SMART (a Simple Modular Architecture Research Tool). European Molecular Biology Laboratory. Bork Group. Schultz et al. (1998) *Proc. Natl. Acad. Sci. USA* 95, 5857-5864 Letunic et al. (2006) *Nucleic Acids Res* 34, D257-D260. http://smart.embl-heidelberg.de/

Smith, L. M., Pontes, O., Searle, I., Yelina, N., Yousafzai, F. K., Herr, A. J., Pikaard, C., Baulcombe, D. C. 2007. An SNF2 protein associated with nuclear RNA silencing and the spread of a silencing signal between cells in Arabidopsis. Plant Cell. 19:1507-1521.

Smithson J.B., Thompson J.A., Summerfield R.J.1985. Chickpea (*Cicer arietinum* L.). In: R.J. Summerfield and E.H. Roberts (eds.), Grain Legume Crops. Collins, London, UK. pp. 312-390. IN: Rajesh, P. N. 2001. Chickpea genomics: BAC library construction, resistance gene analog (RGA) mapping and tagging double-podded trait. A thesis submitted to the University of Pune for the Degree of Doctor of Philosophy in Biotechnology. Plant

Molecular Biology Division of Biochemical Sciences, National Chemical Laboratory Pune 411 008, India.

Smolen, G. and Bender, J. 2002. Arabidopsis cytochrome P450 Cyp83B1 mutations activate the tryptophan biosynthetic pathway. Genetics. 160: 323–332.

Soanes, D. M. and Talbot, N. J. 2005. A bioinformatic tool for analysis of EST transcript abundance during infection-related development by *Magnaporthe grisea*. Mol. Plant Pathol. 6:503-512.

Song, W. Y, Pi, LY., Wang, G. L, Gardner, J., Holsten, T., Ronald, P. C. 1997. Evolution of the rice *Xa21* disease resistance gene family. Plant Cell. 9:1279-1287.

Song, W. Y., Wang, G. L., Chen, L. L., Kim, H. S., Pi, LY, Ho Gardner, J., Wang., B., Zhai, WX., Zhu, L. H., Fauquet, C., Ronald, P. A. 1995. Receptor kinase-like protein encoded by the rice disease resistance gene *Xa21*. Science. 270:1804-1806.

Spassieva, S. D., Markham, J. E., Hille, J. 2002. The plant disease resistance gene *Asc-1* prevents disruption of sphingolipid metabolism during AAL-toxin-induced programmed cell death. Plant J. 32:561-562.

Speulman, E., Bouchez, D., Holub, E.B., Beynon, J.L. 1998. Disease resistance gene homologs correlate with disease resistance loci of *Arabidopsis thaliana*. Plant J. 14(4): 467–474

Spreter, T., Pech, M., Beatrix, B. 2005. The Crystal Structure of Archaeal Nascent Polypeptide-associated Complex (NAC) Reveals a Unique Fold and the resence of a Ubiquitin-associated Domain. J. Biol. Chem. 280:15849-15854.

Staginnus, C., Winter, P., Desel, C., Schmidt, T., Kahl, G., 1999. Molecular structure and chromosomal localization of major repetitive DNA families in the chickpea (*Cicer arietinum* L.) genome. Plant Mol. Biol. 39:1037-1050.

Staswick, P. E., Tiryaki, I., Rowe, M. L. 2002. Jasmonate response locus *JAR1* and several related Arabidopsis genes encode enzymes of the firefly luciferase superfamily that show activity on jasmonic, salicylic and indole-3-acetic acids in an assay for adenylation. Plant Cell. 14:1405-15.

Stepanova, A. N. and Ecker, J. R. 2000. Ethylene signaling: from mutants to molecules. Curr. Opin. Plant Biol. 3:353-360.

Stokes, T. L., Kunkel, B. N., Richards, E. J. 2002. Epigenetic variation in Arabidopsis disease resistance. Genes Dev. 16:171-182.

Stoller, G., Rücknagel, K. P., Nierhaus, K. H., Schmid, F. X., Fischer, G., Rahfeld, J. U. 1995. A ribosome-associated peptidyl-prolyl *cis*/*trans* isomerase identified as the trigger factor. EMBO J. 14:4939-4948.

Strange, R. N. 2006. Counteracting virulence mechanisms of grain legume pathogens Euphytica. 147:49-65.

Stratagene.2007. Introduction to Quantitative PCR. Methods and Applications Guide. www.stratagene.com. Accesion date: 01.03.2007.

Struck, C., Ernst, M., Hahn, M. 2002. Characterization of a developmentally regulated amino acid transporter (AAT1p) of the rust fungus *Uromyces fabae*. Mol. Plant Pathol. 3:23-30.

Struck, C., Mueller, E., Martin, H., Lohaus, G. 2004. The *Uromyces fabae* UfAAT3 gene encodes a general amino acid permease that prefers uptake of in planta scarce amino acids. Mol. Plant Pathol. 5 (3):183-189.

Sturm, A. 1992. A wound inducible glycine rich protein from *Daucus carota* with homology to single stranded nucleic aid binding proteins. Plant Physiol. 99:1689–1692.

Sturtevant, J. 2000. Applications of differential-display reverse transcription-PCR to molecular pathogenesis and medical mycology. Clin. Microbiol. Rev. 13 (3): 408–427

Subramanian, B., Bansal, V. K., Kav, N. N. V. 2005. Proteome-Level Investigation of *Brassica carinata*-Derived Resistance to *Leptosphaeria maculans*. J. Agric. Food Chem. 53:313-324.

Sung, Y. J. and Denmen R. B. 1997. Use of two reverse transcriptase eliminates false-positive results in differential display. Biotechniques. 23:462-464.

Supek, F., Supekova, L., Nelson, H., Nelson, N. 1997. Function of metal-ion homeostasis in the cell division cycle, mitochondrial protein processing, sensitivity to mycobacterial infection and brain function. J. Exp. Biol. 200:321-330.

Suzuki, G., Kai, N., Hirose, T., Fukui, K., Nishio,T., Takayama, S., Isogai, A., Watanabe, M. Hinata K. 1999. Genomic organization of the *S* locus: identification and

characterization of genes in *SLG/SRK* region of *S9* haplotype of *Brassica campestris* (syn. *rapa*). Genet. 153:391-400.

Tai, Y., Bragg, J., Meinhardt, S. 2007. Functional Characterization of ToxA and Molecular Identification of its Intracellular Targeting Protein in Wheat. Am. J. Plant Physiol. 2:76-89.

Takada, Y., Ito, A., Ninomiya, C., Kakizaki, T., Takahata, Y., Suzuki, G., Hatakeyama, K., Hinata, K., Shiba, H., Takayama, S., Isogai, A., Watanabe, M. 2001. Characterization of Expressed Genes in the SLL2 Region of Self-Compatible *Arabidopsis thaliana*. DNA Res. 8:215-219.

Takagi-Morishita, Y., Yamada, N., Sugihara, A., Iwasaki, T., Tsujimura, T., Terada, N. 2002. Mouse uterine epithelial apoptosis is associated with expression of mitochondrial voltage-dependent anion channels, release of cytochrome c from mitochondria, and the ratio of Bax to Bcl-2 or Bcl-X1. Biology of Reproduction. 68(4):1178-1184.

Takahashi, A., Casais, C., Ichimura, K., Shirasu, K. 2003. HSP90 interacts with RAR1 and SGT1 and is essential for RPS2-mediated disease resistance in Arabidopsis. Proc. Natl. Acad. Sci. U.S.A. 100:11777-11782.

Taler, D., Galperin, M., Benjamin, I., Cohen, Y., Kenigsbuch, D. 2004. Plant *eR* genes that encode photorespiratory enzymes confer resistance against disease. Plant Cell. 16: 172-184

Tameling, W. I. L., Elzinga, S. D. J., Darmin, P. S., Vossen, J. H., Takken, F. L. W., Haring, M. A., Cornelissen, B. J. C. 2002. The tomato R gene products I-2 and Mi-1 are functional ATP binding proteins with ATPase activity. Plant Cell. 14:2929-2939.

Tao, Y., Xie, Z., Chen, W., Glazebrook, J., Chang, H. S., Han, B., Zhu, T., Zou, G., Katagiri, F. 2003. Quantitative nature of *Arabidopsis* responses during compatible and incompatible interactions with the bacterial pathogen *Pseudomonas syringae*. Plant Cell 15:317-330.

Taylor, C. B. 1998. Vampire Plants? Plant Cell. 10:1071-1073.

Tayyar, R.I. and Waines, J.G., 1995. Genetic relationships among annual species of *Cicer (Fabaceae)* using isozyme variation. Theoret. Appl. Genet., 92: 245-254.

Tekeoglu, M., Rajesh, P. N., Muehlbauer, F. J. 2002. Integration of sequence tagged microsatellite sites to the chickpea genetic map. Theor. Appl. Genet. 105:847-854.

Tekeoglu, M., Santra, D. K., Kaiser, W. J., Muehlbauer, F. J. 2000. Ascochyta Blight Resistance Inheritance in Three Chickpea Recombinant Inbred Line Populations. Crop. Sci. 40:1251-1256.

Tenhaken, R., and Barz, W. 1991. Characterization of pectic enzymes from the chickpea pathogen *Ascochyta rabiei*. Verlag der Zeitschrift fur Naturforschung. 46c:51-57.

Thaler, J. S., Owen, B., Higgins, V. J. 2004. The role of the jasmonate response in plant susceptibility to diverse pathogens with a range of lifestyles. Plant Physiol. 135:530-538.

Thomma, B. P. H. J., Nelissen, I., Eggermont, K., Broekaert, W. F. 1999. Deficiency in phytoalexin production causes enhanced susceptibility of *Arabidopsis thaliana* to the fungus *Alternaria brassicola*. Plant J. 19: 163-171.

Thomma, B. P., Penninckx, I. A., Broekaert, W. F., Cammue, B. P. 2001. The complexity of disease signalling in Arabidopsis.Curr. Opin. Immunol. 13:63-68.

Tiemann, K., Inze, D., Van Montagu, M., Barz, W. 1991. Pterocarpan phytoalexin biosynthesis in elicitor-challenged chickpea (*Cicer arietinum* L.) cell cultures. Purification, characterization and cDNA cloning of NADPH: Isoflavone oxidoreductase. Eur. J. Biochem. 15:751-757.

Tivoli, B. and Banniza, S. 2007. Comparison of the epidemiology of ascochyta blights on grain legumes. Eur. J. Plant Pathol. 119:59-76 .

Tivoli, B., Baranger, A., Avila, C. M., Banniza, S., Barbetti, M., Chen, W., Davidson, J., Lindeck, K., Kharrat, M., Rubiales, D., Sadiki, M., Sillero, J. C., Sweetingham, M., Muehlbauer, F. J. 2006. Screening techniques and sources of resistance to foliar diseases caused by major necrotrophic fungi in grain legumes. Euphytica 147:223-253.

Truernit, E., Schmid, J., Epple, P., Illig, J., Sauerai, N. 1996. The sink-specific and stress-regulated Arabidopsis SPP4 gene: enhanced expression of a gene encoding a monosaccharide transporter by wounding, elicitors, and pathogen challenge.Plant Cell. 8:2169-2182.

Tulu, A., Muehlbauer, F. J., Simon, C. J., Mayer, M. S., Kumar, J., Kaiser, W. J, Kraft, J. M. 1998. Linkage of the genes for resistance to Fusarium wilt race 4 with molecular markers in chickpea. Euphytica. 102:227-232.

Todd M.J.L. 1990. Primer Detective. (Version 1.01).

Turgeon, B. G., Bohlman, H., Ciufetti, L. M., Christiansen, S., Yang, G., Schafer, W., Yoder, O. C. 1993. Cloning and analysis of the mating-type genes from *Cochloibolus heterostrophus*. Mol. Gen. Genet. 238:270-284.

Udupa, S., Weigand, F., Saxena, M., Kahl, G. 1998. Genotyping with RAPD and microsatellite markers resolves pathotype diversity in the ascochyta blight pathogen of chickpea. Theor. Appl. Genet. 97:299-307.

Udupa, S. M. and Baum, M. 2003. Genetic dissection of pathotypespecific resistance to ascochyta blight resistance in chickpea (*Cicer arietinum* L.) using microsatellite markers. Theor. Appl. Genet. 106:1196-1202.

Uhlmann, A. and Ebel, J., 1993. Molecular cloning and expression of 4-coumarate:coenzyme a ligase, an enzyme lnvolved in the resistance response of soybean (*Glycine max* L.) against pathogen attack. Plant Physiol. 102: 1147-1156

UniProt. The UniProt Consortium. The Universal Protein Resource (UniProt) Nucleic Acids Res. 36:D190-D195(2008). http://www.expasy.uniprot.org/. July 22, 2008.

Vail, S.L. 2005. Population studies of *Ascochyta rabiei* on chickpea in Saskatchewan. A Thesis Submitted to the College of Graduate Studies and Research in Partial Fulfillment of the Requirements for the Degree of Masters of Agriculture in the Department of Plant Sciences University of Saskatchewan. Saskatoon.

Van der Biezen, E. A.,Jones, J. D. 1998. The NB-ARC domain: a novel signalling motif shared by plant resistance gene products and regulators of cell death in animals. Curr. Biol. 8: R226-R227.

Van Etten, H., Mansfield, J. W., Bailey, J. A., Farmer, E. E. 1994. Two classes of plant antibiotics: phytoalexins versus phytoanticipins. Plant Cell. 6:1191–1192.

Van der Hoorn, R. A. L., Jones, J. D. G. 2004. The plant proteolytic machinery and its role in defence. Curr. Opin. Plant Biol. 7:400-407.

Van Rheenen, H. A., Haware, M. P. 1994. Mode of inheritance to ascochyta blight (*Ascochyta rabiei* (Pass.) labr.) in chickpea (*Cicer arientum* L.) and its consequences for resistance breeding. Int. J. Pest. Man. 42:166-169.

Van Wees, S. C. M., Chang, H.S., Zhu, T., Glazebrook, J. 2003. Characterization of the early response of *Arabidopsis* to *Alternaria brassicicola* infection using expression profiling. Plant Physiol. 132: 606-617.

Van Zhong, G. and Burns, J.K. 2003. Profiling ethylene-regulated gene expression in *Arabidopsis thaliana* by microarray analysis. Plant Mol. Biol. 53: 117–131, 2003.

Vandenabeele, S., Van Der Kelen, K., Dat, J., Gadjev, I., Boonefaes, T., Morsa, S, Rottiers, P., Slooten, L., Van Montagu, , M., Zabeau, M., Inzé, D., Van Breusegem, F. 2003. A comprehensive analysis of hydrogen peroxide-induced gene expression in tobacco. PNAS. 100:16113–16118.

Vandesompele, J., De Preter, K., Pattyn, F., Poppe, B., Van Roy, N., De Paepe, A., Speleman, F. 2002. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3: research0034.1-0034.11.

Venu, R. C., Jia, Y., Gowda, M., Jia, M. H., Jantasuriyarat, C., Stahlberg, E., Li, H., Rhineheart, A., Boddhireddy, P., Singh, P., Rutger, N., Kudrna, D., Wing, R., Nelson, J. C., Wang, G. L. 2007. RL-SAGE and microarray analysis of the rice transcriptome after *Rhizoctonia solani* infection . Mol. Genet. Genomics. 278:421-431.

Veronese, P., Nakagami, H., Bluhm, B., AbuQamara, S., Chen, X., Salmeron, J., Dietrich, R. A., Hirt, H., Mengiste, T. 2006. The membrane-anchored Botrytis-induced kinase1 plays distinct roles in Arabidopsis resistance to necrotrophic and biotrophic pathogens. Plant Cell. 18:257-273.

Viard, M. P., Martin, F., Pugin, A., Ricci, P., Blein, J. P. 1994. Protein phosphorylation ıs ınduced in Tobacco cells by the elicitor cryptogein. Plant Physiol. 104:1245-1249.

Vlacilova, K., Ohri, D., Vrana, J., Cihalikova, J., Kubalakova, M., Kahl, G., Dolezel, J. 2002. Development of flow cytogenetics and physical genome mapping in chickpea (*Cicer arietinum* L.). Chromosome Res. 10: 695–706.

Vogelsang, R. and Barz, W. 1993a. Purification, characterization and differential hormonal regulation of a β-1,3-glucanase and two chitinases from chickpea (*Cicer arietinum* L.) Planta. 189 (1): 60-69.

Vogelsang, R. and Barz, W. 1993b. Plant gene register. cloning of a class 111 acidic chitinase from chickpea. Plant Physiol. 103: 297-298.

Vogelsang, R., Berger, E., Hagedorn, T., Moi-Ilenbeck, U., Tenhaken, R., Barz, W. 1994. Characterization of metabolic changes involved in hypersensitive-like browning reactions of chickpea (*Cicer arietinum* L.) cell cultures following challenge *Ascochyta rabiei* culture filtrate. Physiol. Mol. Plant Pathol. 44, 141-155.

Voinnet, O. 2002. RNA silencing: small RNAs as ubiquitous regulators of gene expression. Curr. Opin. Plant. Biol. 5:444 - 451.

Von Dahl, C. C, Michael-Havecker, M., Robert-Schlögl, R., Baldwin, I. T. 2006. Caterpillar-elicited methanol emission: anew signal in plant-herbivore interactions? Plant J. 46: 948-960.

Von Wettstein, D., Gough, S., Kannangara, C. G. 1995. Chlorophyll biosynthesis. Plant Cell. 7:1039-1057.

Ward J.M., Pei, Z.M., Schroeder, J. 1995. Roles of ion channels in initiation of signal transduction in higher plants. Plant Cell 7: 833-844.

Walters, D. and Heil, M. 2007. Costs and trade-offs associated with induced resistance. Physiol. Mol. Plant Pathol. 71:3-17.

WatCut: An on-line tool for restriction analysis, silent mutation scanning, SNP-RFLP analysis. Maintained by Michael Palmer, University of Waterloo. http://watcut.uwaterloo.ca/watcut/watcut/template.php Accession date: May, 2007.

Weissman, A. M. 2001. Themes and variations on ubiquitylation. Nat. Rev. Mol. Cell. Biol. 2:169-178.

Werck-Reichhart D. 1995. Cytochromes P450 in phenylpropanoid metabolism. Drug Metab. Drug. Interact. 12:221–243

Whetten, R., and Sederoff, R. 1995. Lignin biosynthesis. Plant Cell. 7:1001-1013.

White, D. and Chen, W. 2007. Towards identifying pathogenic determinants of the chickpea pathogen *Ascochyta rabiei*. Eur. J. Plant Pathol. 119:3-12.

Wildermuth, M. C., Dewdney, J., Wu, G., Ausubel, F. M. 2001. Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature 414:562-65.

Wilson, A. and Kaiser, W. 1995. Cytology and genetics of sexual incompatibility in *Didymella rabiei*. Mycolgia. 87:795-804.

Winter, P., Pfaff, T., Udupa, S. M., Hüttel, B., Sharma, P. C., Sahim, S., Arreguin-Espinoza, R., Weigand, F., Muehlbauer, F. J., Kahl, G. 1999. Characterization and

mapping of sequence-tagged microsatellite sites in the chickpea (*Cicer arietinum* L.) genome. Mol. Gen. Genet. 262:90-101.

Winter, P., Benko-Iseppon, A. M., Huttel, B., Ratnaparkhe, M., Tulu, A., Sonnante, G., Pfaff, T., Tekeoglu, M., Santra, D., Sant, V. J., Rajesh, P. N., Kahl, G., Muehlbauer, F. J. 2000. A linkage map of the chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* X *C. reticulatum* cross: localization of resistance genes for *fusarium* wilt races 4 and 5. Theor. Appl. Genet. 101:1155-1163.

Wojtaszek, P. 1997. Oxidative burst: an early plant response to pathogen infection. Biochem J. 322:681-692.

Xie, D. X, Feys, B. F., James, S., Nieto-Rostro, M., Turner, J. G. 1998. COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. Science. 280:1091-1094.

Xu, L., Liu, F., Lecher, E., Genschik, P., Crosby, W.L., Ma, H., Wen Peng, W., Huang, D., Xie, D. 2002. The SCFCOI1 ubiquitin-ligase complexes are required for jasmonate response in Arabidopsis. Plant Cell. 14:1919-1935.

Yamamoto, K., Okamoto, A., Isonishi, S., Ochiai, K., Oh, Y. 2001. A novel gene, CRR9, which was up-regulated in CDDP-resistant ovarian tumor cell line, was associated with apoptosis. Biochem. Biophys. Res. Commun. 280:1148-1154.

Yan, S., Zhangcheng, T., Su, W., Sun, W. 2005. Proteomic analysis of salt stress-responsive proteins in rice root. Proteomics. 5:235-244.

Yang, Y., Shah, J., Klessig, D. F. 1997. Signal perception and transduction in plant defense responses. Genes and Development. 11:1621-1639.

Yang, B., Srivastava, S. , Deyholos, M. K., Kav, N.N.V. 2007. Transcriptional profiling of canola (*Brassica napus* L.) responses to the fungal pathogen *Sclerotinia sclerotiorum* Plant Sci. 173:156-171.

Ye, X.Y., Ng, T. B., Rao, P. F. 2002. Cicerin and arietin, novel chickpea peptides with different antifungal potencies. Peptides 23: 817-822.

Yi, H. and Richards, E. J. 2007. A cluster of disease resistance genes in Arabidopsis is coordinately regulated by transcriptional activation and RNA silencing. Plant Cell 19:2929-2939.

Yoshimura, S., Yamanouchi, U., Katayose, Y., Toki, S., Wang, Z.X, Kono, I., Kurata, N., Yano, M., Iwata, N., Sasaki, T. 1998. Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. Proc. Natl. Acad. Sci. USA. 95:1663–1668.

Yotov, W. V., Moreau, A., St-Arnaud, R. 1998. The alpha chain of the nascent polypeptide-associated complex functions as a transcriptional coactivator. Mol. Cell. Biol. 18:1303-1311.

Yu, Y. G., Buss, G. R., Maroof, M. A. 1996a. Isolation of a superfamily of candidate disease-resistance genes in soybean based on a conserved nucleotide-binding site. Proc. Natl. Acad. Sci. U.S.A. 93:11751-11756.

Yu, H. and Kumar, P.P. 2003. Post-transcriptional gene silencing in plants by RNA Plant Cell. Rep. 22:167 – 174.

Yu, K., Schafer, U., Glavin, T.L., Goring, D.R., Rothstein, S.J. 1996b. Molecular characterization of the *S* locus in two self incompatible *Brassica napus* lines. Plant Cell. 8: 2369-2380.

Zegzouti, H., Marty, C., Jones, B., Bouquin, T., Latché, A., Pech, J. C., Bouzayen, M. 1997. Improved screening of cDNAs generated by mRNA differential display enables the selection of true positives and the isolation of weakly expressed messages. Plant. Mol. Biol. Rep. 15:236-245.

Zhang, S. and Klessig, D. F. 2001. MAPK cascades in plant defense signaling. Trends Plant Sci. 6:520-527.

Zhang, Z., Collinge, D.B., Thordal-Christensen, H. 1995. Germin-like oxalate oxidase, a H_2O_2-producing enzyme, accumulates in barley attacked by the powdery mildew fungus. Plant J. 8:139-145.

Zhong, G. V. and Burns, J. K. 2003. Profiling ethylene-regulated gene expression in *Arabidopsis thaliana* by microarray analysis. Plant Mol. Biol. 53:117-131.

Zhou, N., Tootle, T. L., Glazebrook, J. 1999. Arabidopsis pad3, a gene required for camalexin biosynthesis,encodes a putative cytochrome P450 monooxygenase. Plant Cell. 11:2419-2428.

Zou, H., Henzel, W. J, Liu, X., Lutschg, A., Wang, X. 1997. Apaf-1, a human protein homologous to *C. elegans* CED-4, participates in cytochrome *c*-dependent activation of caspase-3. Cell. 90:405-413.

Zou, J., Rodriguez-Zas, S., Aldea, M., Li, M., Zhu, J., Gonzalez, D. O., Vodkin, L. O., DeLucia, E., Clough, S. J. 2005. Expression profiling soybean response to *Pseudomonas syringae* reveals new defense-related genes and rapid HR-specific downregulation of photosynthesis. MPMI. 18 (11): 1161-1174.

Zuo, K., Wang, J., Wu, W., Chai, Y., Sun, X., Tang, K. 2005. Identification and characterization of differentially expressed ESTs of *Gossypium barbadense* infected by *Verticillium dahliae* with suppression subtractive hybridization. Mol. Biol. 39:191–199.

APPENDIX A

CHICKPEA PRODUCTION OF TURKEY IN BETWEEN 1987-2006

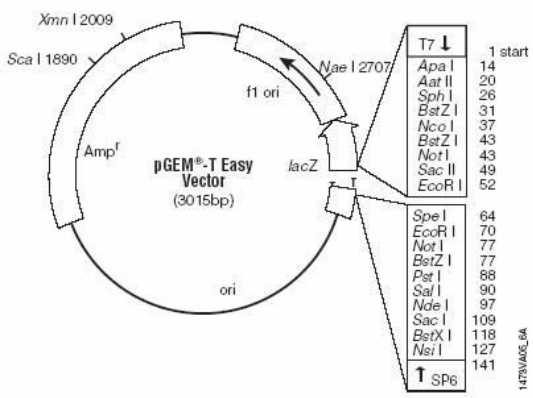
Table A.1 Data table of the Figure 1.1.

Years	Cultivated Area * (one-tenth of a hectare)	Production* (Tons)	Productivity (Kg / (one-tenth of a hectare)
1987	6 650 000	725 000	109
1988	7 780 000	777 500	100
1989	8 180 000	683 000	83
1990	8 900 000	860 000	97
1991	8 780 000	855 000	97
1992	8 560 000	770 000	90
1993	8 200 000	740 000	90
1994	7 600 000	650 000	86
1995	7 450 000	730 000	98
1996	7 800 000	732 000	94
1997	7 210 000	720 000	100
1998	6 650 000	625 000	94
1999	6 250 000	560 000	90
2000	6 360 000	548 000	86
2001	6 450 000	535 000	83
2002	6 600 000	650 000	98
2003	6 300 000	600 000	95
2004	6 060 000	620 000	102
2005	5 578 000	600 000	108
2006	5 243 672	551 746	105

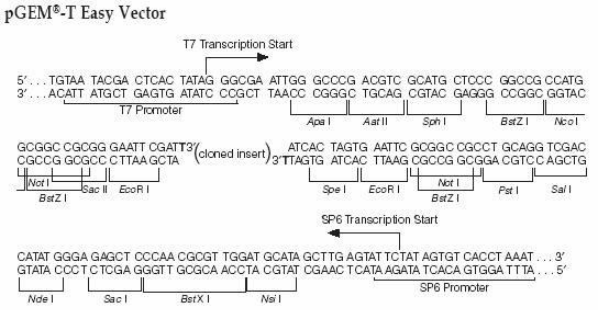
* Data taken from: Türkiye İstatistik Kurumu. Tarım/Bitkisel Üretim İstatistikleri. http://www.tuik.gov.tr/VeriBilgi.do?tb_id=45&ust_id=13. Accession date: 19 July 2008.

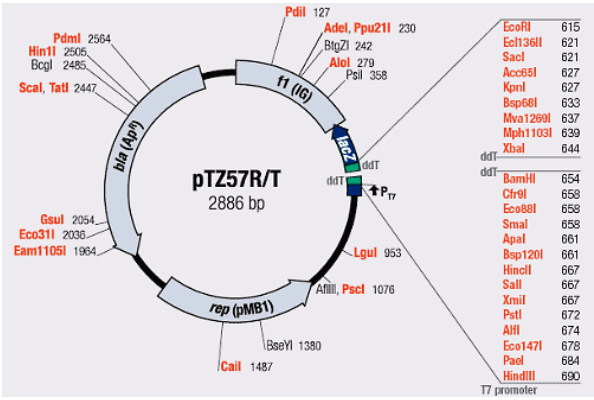
APPENDIX B

CLONING VECTORS



pGEM®-T Easy Vector

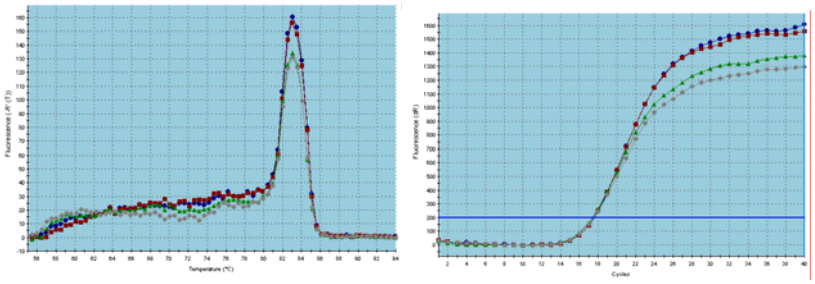




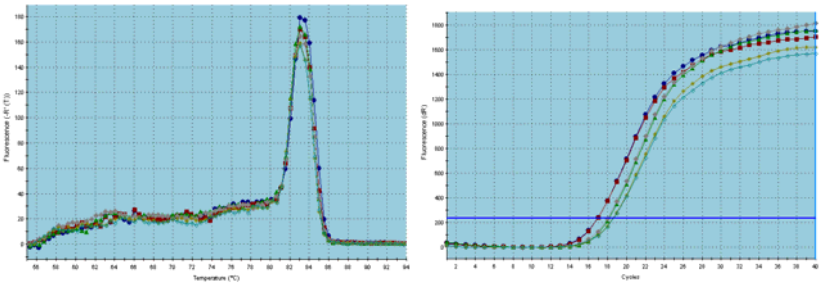
APPENDIX C

EXAMPLES FOR REAL-TIME qRT-PCR DISSOCIATION CURVES AND AMPLIFICATION PLOTS

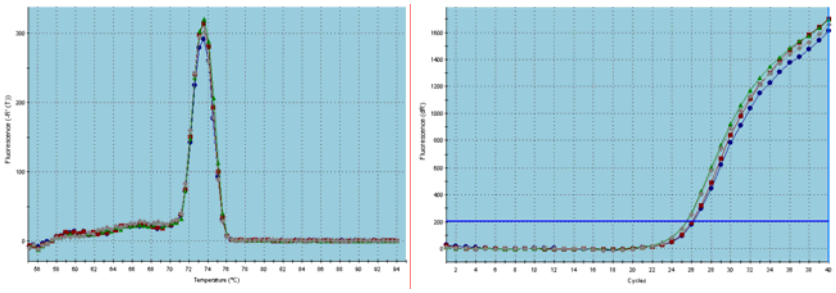
Stratagene MX4000



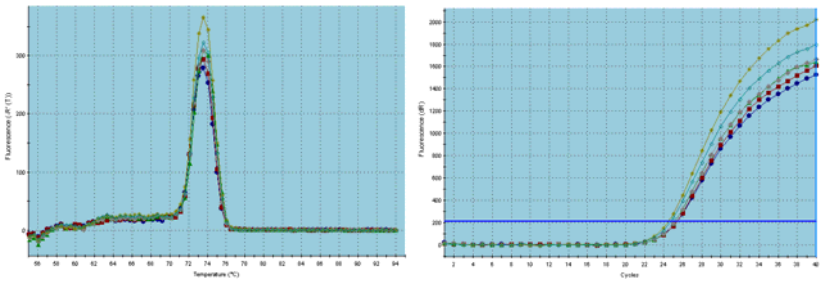
dissociation curve (left) and corresponding amplification plot of 18S in ILC195 infected and uninfected plants at 48 hpi



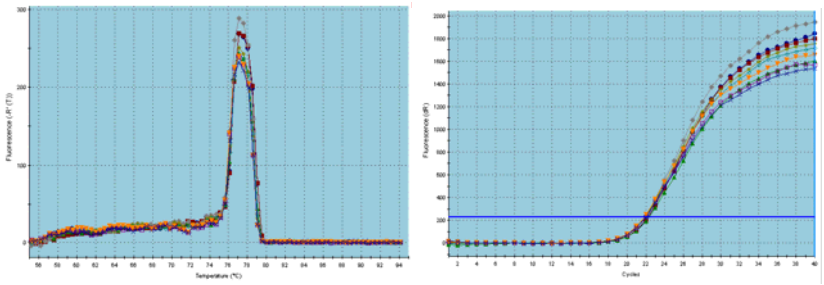
dissociation curve (left) and corresponding amplification plot of 18S in FLIP84-92(3) infected with PI and PII and uninfected plants at 24 hpi



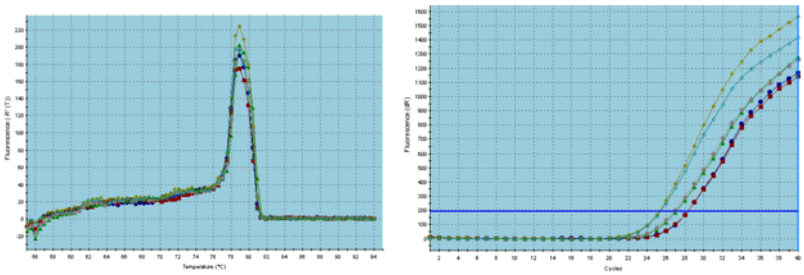
dissociation curve (left) and corresponding amplification plot of actin in ILC195 infected and uninfected plants at 48 hpi



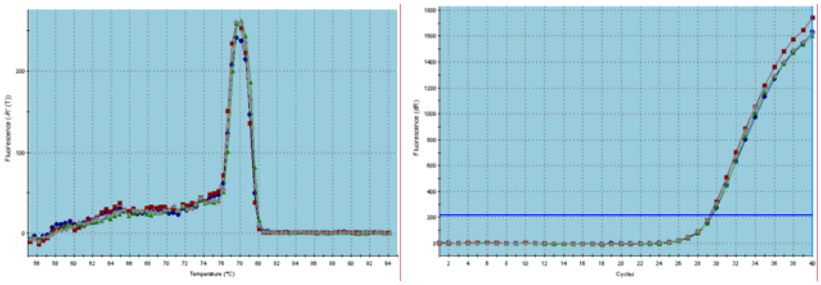
dissociation curve (left) and corresponding amplification plot of actin in FLIP84-92(3) infected with PI and PII and uninfected plants at 6 hpi



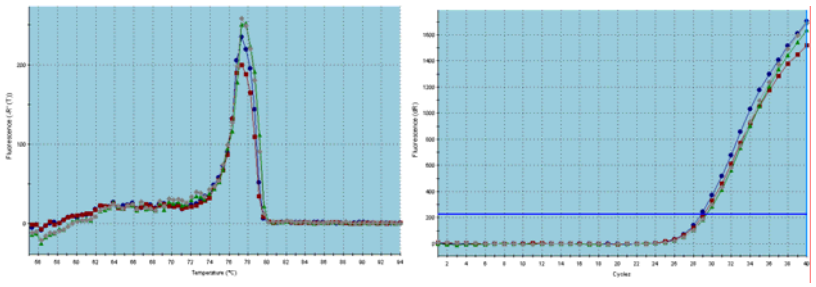
dissociation curve (left) and corresponding amplification plot of EST-FDH in FLIP84-92(3) infected with PI and PII and uninfected plants at 48 hpi



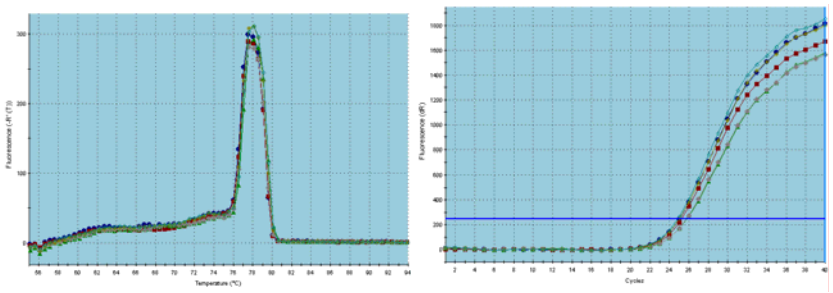
dissociation curve (left) and corresponding amplification plot of EST-1868 in FLIP84-92(3) infected with PI and PII and uninfected plants at 12 hpi



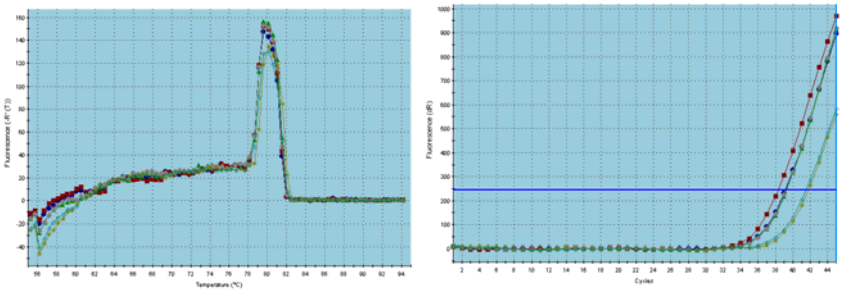
dissociation curve (left) and corresponding amplification plot of 1558 in ILC195 infected and uninfected plants at 24 hpi



dissociation curve (left) and corresponding amplification plot of 1468 in ILC195 infected and uninfected plants at 72 hpi



dissociation curve (left) and corresponding amplification plot of EST-FPIP in FLIP84-92(3) infected with PI and



dissociation curve (left) and corresponding amplification plot of EST-R46 in FLIP84-92(3) infected with PI and PII and uninfected plants at 12 hpi

APPENDIX D

SEQUENCES AND RELATED DATA

Table D.1 BLASTX done in September-November 2007 and EST sequence data. (↑) indicates up, (↓) indicates downregulation.

ESTs in RGA-DDRTPCR TRIALS AUTORADIOGRAPH FILM-1	
EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: R13 ↑ S2/T5, inf **DETAILS:** S2/T5 isst-1_ T7, **sequence size (bp):** 263 **BLASTX:** gb|AAY85658.1| plastid glucose-6-phosphate/phosphate transloc.. 80.1 4e-14	***CNTTATGCTGAGTGATATCTTTTTTTTTCC***ACTTCCGTTTATCTTTCGATTTTGA AAGCATCGCCGCTCCATATTATGACTAAAGTATGGCTGATAAATGTACACAA CAGGTGGGTAGCAGCTCAGAGTATATTCTATCATCTATACAATCAGGTGTCT TACATGTCCTTGGATGAGATCTCTCCCTTGACATTTAGCATTGGAAACACCAT GAAACGTATATCTGTCATAGTATCTTCAATT***GTCGTCTTCCCCACCCCCCC*** **Forward frame 1, 87 amino acids** **X**YAE*YLFFSTSVYLSILKASPLHIMTKVWLINVHNRWVAAQSIFYHLYNQVSY MSLDEISPLTFSIGNTMKRISVIVSSIVVFPTP
EST NO: R14 ↓ S2/T7, Unif **DETAILS:** S2/T7 iust_2_T7, **sequence size (bp):** 191 **BLASTX:** No significant similarity found **BlastN:** AF100336.1 Dendrobium grex Madame Thong-IN putative phenylalanine ammonia-lyase (ovg43) mRNA, partial cds 55.4 1e-05	***CATTATGCTGAGTGATATCTTTTTTTTTG***AANNTTACNTAGATAGGCGCTAAT TATGAAAAGGGAGTTCAAGTTTGCTACACGTACTTTTTCTATAAGCACACAT CATGACTGAGATCAATAAGAGTAATCCCTTTCTCATTTAATCATATGGTATA GATAGTGCCCTCTC***GYCGTCTTCCCCACCCCCCC***
EST NO: R15 ↓ S2/T7, Unif **DETAILS:** S2/T7 iust_3_T7, **sequence size (bp):** 173 **BLASTX:** No significant similarity found **BlastN:** AB049724.1 Pisum sativum ssa-15 mRNA for putative senescence-associated protein, complete cds 91.5 1e-16	***CATTATGCTGAGTGATATCTTTTTTTTTG***ATAATCCAAAAATGTTGATCTAAA ACAAAAGGTACAGCAACAAAATTTATAAGGAAACGAGTATGAAAATTCAAT CTAAAACATGAGTTTAGCATGAATTCAATTTTCAAATTCACTCATCATC***GTCG*** ***TCTTCCCCACCCCCCC***
EST NO: R17 ↑ S2/T7, inf **DETAILS:** S2/T7 iist4_T7, **sequence size (bp):** 233 **BLASTX:** No significant similarity found **BlastN:** AC144729.29 Medicago truncatula clone mth2-15j6, complete sequence 147 3e-33 AF100336.1 Dendrobium grex Madame Thong-IN putative phenylalanine ammonia-lyase (ovg43) mRNA, partial cds 59.0 1e-06	***GGGGGG****AT****GGGGAAGACGAC***GCGCATTTAAACAAGTGGGCTCTTAGGTCTCA GAAATTGGAGGTTTGATGGAGGGTGCATGCATTAGCATTATCATCATCATCA TCATCCATCCCTTTCTTCATTCTACCACCACACTTAACCCTTTGTGTTTGAGGT GACATTTATCTTCCTCTTTTGATGCTACCCCACTCCCACACCTAA***TCAAAAAA*** ***AAAGATATCACTCAGCATAATG***
EST NO: R18 ↑ S2/T7, inf **DETAILS:** S2/T7 iist_5_T7, **sequence size (bp):** 210 **BLASTX:** No significant similarity found **BLASTP** ref|XP_765437.1| hypothetical protein TP02_0869 [Theileria pa... 33.9 2.8	***CNTTATGCTGAGTGATATCTTTTTTTTTGA***GCAAAGAGGGTGGAAGGAGTTGG GATATTTTCTGGAGAATGTTCTGGAGGTGGTGGAGAACGTTCTGGTGTTGGT GGAGAATGTTCTGGTGTTGGGATTTTTTCTGTTGTTTCCTCAGGACCAGTAGG AGGTGATGAGAATGAAATAGGGTTTGTGGTAG***GTCGTCTTCCCCACCCCCCC*** **Reverse frame 3, 69 amino acids** GGGEDDLPQTLFHSHHLLLVLRKQQKKSQHQNILHQHQNVLHHLQNILQKISQL LPPSLL KKKDITQH**X**
EST NO: R21 ↑ S2/T2, inf **DETAILS:** S2/T2 iist_6_T2, **sequence size (bp):** 306 **BLASTX:** gb|ABK23952.1| unknown [Picea sitchensis] 58.2 2e-07 ref|NP_565854.1| zinc finger (C2H2 type) family protein [Arab.. 57.4 3e-07	***TTANGCTGAGTGATATCNTTTTTTTTAC***NAAAATATATANATTTGATTGANAT AATAACAAAATATTGCTTATCTCGNANTAATAAACTAACAAACATGCATAAT ATAACAACCAGTTTCTACTAAAATCGCCCAACCACCACAAAATAAAAAGTC ATATAGACNTAAGCTTTGGTCCATTATCANGCATTCCCATCCCTGCNGCTAA CTCGGCATCNNGNTGAGTGNGTGGTGCTTCACCCNTCNTTTGNTTCACACNT CTCCNATGTTTTTTGGNCTTAAAATG***GTCGTCTTCCCCACCCCCCC*** **Reverse frame 3, 101 amino acids** GGGEDDHFKXKKHXRXVXQXXGEAPXTXXDAELAAGMGMXDNGPKLXSI
EST NO: R22 ↓ S2/T3, Unif **DETAILS:** S2/T2, iist_7_T4, **sequence size (bp):** 138 **BLASTX:** No significant similarity found **BlastN:** AF100329.1 Dendrobium grex Madame Thong-IN ovg15 mRNA, partial sequence 59.0 5e-07	***CATTATGCTGAGTGATATCTTTTTTTTTAC***GCTNNCAATCAACCTCCTTATTGC TGCTTGTTTCCTCTCGCTTCTTTTCCCTGCGTAAGTCTCTATCCACATACCTGT CCCTACTCCTGTCGTCTTCCCCACCCCCCC **Forward frame 2, 45 amino acids** IMLSDIFFFTLXINLLIAACFLSLLFPA*

EST NO: R23 ↓ S2/T3, Unif **DETAILS:** S2/T3 iist8_T3, **sequence size (bp):** 303 **BLASTX:** No significant similarity found **BlastN:** DQ812977.1 Arachis hypogaea clone PTDI-1 mRNA sequence 55.4 2e-05 AF100330.1 Dendrobium grex Madame Thong-IN putative copper/zinc superoxide dismutase copper chaperone (ovg23) mRNA, partial cds 55.4 2e-05	***GGGGGGGTGGGGAAGACGAC***ATCAAAATATGTNNCTGGAAGGTTCCTTCGCT CTGCAGAATAAGAATTAAGAATTCAGTGATAATAGTGGAAATGACAAGCTG AGAAATGAAGAAAGGAGCACGCTACTGGGCTTAGGGTTATTAATGGGCTTTT AGCCCAAAACATTTTCAAACGGCCTAAACTCTCCCCGCCGACAATTGGCTAT TGTACCCCCCCATTTAAACCTATACCCCCAACATAAAAATTTTGTTGTAAAAT ACCTAAAATACCC***CTAAAAAAAAAGATATCACTCAGCATAATG***
ESTs in RGA-DDRTPCR TRIALS AUTORADIOGRAPH FILM-2	
EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: R46 ↓ R46-1-1, RLLRfwd/T5; Unif **DETAILS:** RLLRfwd/T5, U_HW-Plate2c06.b1, **sequence size (bp):** 261 **BLASTX:** emb|CAO62495.1| unnamed protein product [Vitis vinifera] 127 3e-28 emb|CAN66563.1| hypothetical protein [Vitis vinifera] 127 3e-28 dbj|BAB10839.1| receptor-like protein kinase [Arabidopsis thalia 120 4e-26 ref|NP_201077.2| leucine-rich repeat family protein / protein.. 120 4e-26 **CONSERVED DOMAIN:** cd00180, S_TKc, Serine/Threonine protein kinases, catalytic domain. Phosphotransferase.. 29142 Yes 5e-12	***CATTATGCTGAGTGATATCTTTTTTTTC***TCCAAAAAAAATACTGAACGACAACC CTTGAATTGGAGTGATCCCCTAAAGATAACCCTTGGTTCTGCTCGGGGTTTG GCATACCTGCACCATGAATGCAGCCCGAAAATTGTGCATCGCGACATAAAAT CTAGCAACATCCTTCTTGATGAAAACATGGAGCCTCACATCTCTGATTTTGGT CTTGCAAAGCTCTTGGTTGATGAAGATGCACAT***GTTACTCTAGTG****G****TTGCG*** **Forward frame 1, 87 amino acids** HYAE*YLFFSKKNTERQPLNWSDPLKITLGSARGLAYLHHECSPKIVHRDIKSSNI LLDENMEPHISDFGLAKLLVDEDAHVTLVVA
EST NO: R44 ↑ R44-a-a, RLLRfwd/T5; inf **DETAILS:** RLLRF/T5, U_HW-070116_Plate6b06 **sequence size (bp):** 473 **BLASTX:** emb|CAO40090.1| unnamed protein product [Vitis vinifera] 70.9 3e-11	***CATTATGCTGAGTGATATCTTTTTTTTC***TGGAAGTTTCAACCTTGTTGACTA TGACAACAGTCGCGACTATAAACAGACTCGCAACTATGGCAGGGCGGTACG CTCTGCTCTCTCGCGACCCCTACTCTAACGAGTAAGCAAGTAAACTACCTTTT GAAGCGCCAGTCGCGTAGGGCGACCGGGCCAGGCCGAGTCAAAAAGGCTTT GATGACTCAAGGTTAATCTTAGGGAAAGGAGAGTGAGGGGAAGAGGGGGC AGCCCTCGGCCCGATCATCCAATTCACTCCAACAGACAGGCATGGTTCTGTA GTCAAAGCAACTTCGTCACTTTCGTGTACCCATCGGACGGCAGCCCTTTCGG GGCTTCCTTAGGGACCGATTCACTCTGCGTAAATTGACTGAACGCAAAAAGC CTTCCACTGGCAGGCGATCGTGTTTTTCACAGGATTTATC***GTTACTCTAGTGG TTGCG*** **Forward frame 3, 157 amino acids** LC*VISFFFLEVSTLLTMTTVATINRLATMAGRYALLSRDPYSNE
EST NO: R48 ↓ R48-2-1, Ptokin-1/T4; uninf **DETAILS:** Ptokin-1/T4 U_HW-Plate2c08.b1, **sequence size (bp):** 394 **BLASTX:** dbj|BAA10929.1| cytochrome P450 like_TBP [Nicotiana tabacum] 121 1e-26 **BlastN:** Z11498.1 M.sativa 26S rRNA 562 8e-158	***CTTATGCTGAGTGATATCTTTTTTTTTCA***CTTTGACATTCAGAGCACTGGGCA GAAATCACATTGCGTCAACATCCGCAGGGACCATCGCAATGCTTTGTTTTAA TTAAACAGACGGATTCCCCTTGTCCGTACCAGTTCTGAGTTGACTGTTCGATG CCCGGGGAAAAGGCCCCAAAGGGCCCGTTCCCAATCCTTCCCCCGACCGGC ACGCGGCGACCCGCTCTCGCCGCGGGAGCAGCTCAAGCATTCCACCAACAG CCGACGGGTTCGGAACTGGGACCCCCGTGCCCAGCCCTCAAGCCAATCCTTT TCCCGAGGTTACGGATCCTTTTTGCCTACTTCTCTTGCCTATTTTGTTCCGACG ACCAGAGGCTG***TTCACCTTGTTCCATGG*** **Forward frame 3, 272 amino acids** AGLGVAGRAPILMLSDIFFFHFDIQSTGQKSHCVNIRRDHRNALF*LNRRIPLVRT SSELTVRCPGKRPQRARSQSFPRPARGDPLSPREQLKHSTNSRRVRNWDPRAQPS SQSFSRGYGSFLPTSLAYFVPTTRGCSPCSMENLYAFERISARILWPSFIHGGTGK TLRGHHSTRWMHILLFLWAK*
EST NO: R49 ↓ R49-2, Ptokin-1/T6; uninf **DETAILS:** Ptokin1/T5 U_HW-Plate2c10.b1 **sequence size (bp):** 209 **BLASTX:** emb|CAN64186.1| hypothetical protein [Vitis vinifera] 37.4 0.31 gb|EDL21732.1| pleckstrin homology-like domain, family A, mem.. 35.8 0.89	***CTTATGCTGAGTGATATCTTTTTTTTTCC***AACCAACCTCCTTTACCGCCCATGC CACCCCCACCAACACCCTCACCTGTAATATCACATGCTACCGATTCCATGCC CGGCCAGTCATCCTCATTCGCTAATTCTCCGGCAGGCTCACGGCGTCCAGTA GCATTCCAAGTTCAGTCGGATTATTCATCTCC*A****TTCACCTTGTTCCAATGC*** **Forward frame 1, 91 amino acids** WRASASRAGTDSYAE*YLFFSNQPPLPPMPPPPTPSPVISHATDSMPGQSSSFANS PAGSRRPVAFQVQSDYSSPFTLFQCNLDAFARYRA **Forward frame 2, 91 amino acids** GGPRHRGPGPILMLSDIFFFPTNLLYRPCHPHQHPHL*YHMLPIPCPASHPHSLILR QAHGVQ*HSKFSRIIHLHSPCSNAI*MHSRGTEL
ESTs in RGA-DDRTPCR TRIALS AUTORADIOGRAPH FILM-3	
EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: R50 ↑ R50-1; S1/T8; inf **DETAILS:** S2/T9 U_HW-Plate2c11.b1 **sequence size (bp):** 177 **BLASTX:** emb|CAC44142.1| putative polyprotein [Cicer arietinum] 89.0 9e-17 **BlastN:** AJ411810.1 Cicer arietinum Ty3-gypsy like Retrotransposon CaRep and partial pol gene for polyprotein including RNAse and integrase, clone DOM5x-9 215 5e-54	***CATAATGCTGAGTGATATCTTTTTTTTGG***AACCCGGATGAATACTCAGCTTAC TCTTATGCGCCTCTCCCAAAATCGTTTTCCGCATATCTGTAATGGCTGGTACA CAAATCCTACCATTACACCGCAAAACATTATCTGCCCTAATCTTGAACTCA***G*** ***TCGTCTTCCCCACCCCCCC*** **Reverse frame 3, 69 amino acids** SSVPRECI*IGGVGKTTEFKIRADNVLRCNGRICVPAITDMRKTILGEAHKSKLSIH PGSKKKISLSIM
ESTs in RGA-RT-PCR AUTORADIOGRAPH FILM-1	
EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**

EST NO: 2 WipK-1/ WipK-2; B1, B2, B3 **DETAILS:** WipK-1/ WipK-2 U_HW-Plate3a02.b1, **sequence size (bp):** 557 **BLASTX:** emb|CAA57721.1| protein kinase [Medicago sativa] 291 2e-77 gb|AAF73236.1|AF153061_1 MAP kinase 3 [Pisum sativum] 290 4e-77 gb|AAQ13491.1|AF104247_1 mitogen-activated protein kinase 1 [Gly 274 2e-72 emb|CAO21775.1| unnamed protein product [Vitis vinifera] 271 2e-71 gb|ABG54330.1| double HA-tagged mitogen activated protein kin.. 270 3e-71 dbj|BAA04866.1| MAP kinase [Arabidopsis thaliana] 270 3e-71 ref|NP_190150.1| ATMPK3 (MITOGEN-ACTIVATED PROTEIN KINASE 3);.. 270 3e-71 **CONSERVED DOMAIN:** cd00180, S_TKc, Serine/Threonine protein kinases, catalytic domain. Phosphotransferase.. 29142 Yes 1e-33	***CC*TTGA*G*GATGCACCGAC**TTTTTTTTTTCTATTGCATATGTTTAATCTGAGGAG TTTAACTCCAATTCGGGAGCCCTATCCCCTCTTGGGACTACATGTTCTGTCAT GAAGTCACTTTCCATAGTTGGTCGTGAAACACCAAAATCAATAATCTTCGAT TCGGATTTTGCATTCAACAACAGGTTGCTTGGTTTCAAATCTCTATGGATTAT GTTCGCAAAATGTATATACCTCAGACCACAAAGAATCTGATACAAAAAGTA CGGGCAGTGTTCATCTGACAGATTTTGATTGGAGCAAATGATTTGATGAAGA TCAGTATCCATGAGTTCGGTGGTAATGTAAACATCATTAAACTCTCGACCCG AGGGTGGAGGAAGAACATCTCGTAAACCAATTACATTTTCATGATCTAAATG CCTAAGAAGCTTACTCTCGCCCAGCATACGCTTAGCATCCATGTGATTATCA AAAGCATTCGCTATCTTCTTAACACCAACCAACTCGTTCTTCTCCGTGTGCAA TATCGAACAATCG***ATTCCATAC*GCACCACGACC** **Reverse frame 1, 185 amino acids** GRGAYGIDCSILHTEKNELVGVKKIANAFDNHMDAKRMLGESKLLRHLDHENV IGLRDVLPPPSGREFNDVYITTELMDTDLHQIICSNQNLSDEHCPYFLYQILCGLR YIHFANIIHRDLKPSNLLLNAKSESKIIDFGVSRPTMESDFMTEHVVPRGDRAPEL ELNSSD*TYAIEKKKSVHPQ
EST NO: 19 PtoFenS/PtoFenAS; B1, B2, B3 **DETAILS:** (PtoFenS/PtoFenAS) U_HW-070116_Plate6f07, **sequence size (bp):** 356 **BLASTX:** ref|NP_568958.1| unknown protein [Arabidopsis thaliana] >gb|A.. 183 3e-45	***TTGGCACAAATTCTCATCAAGC***ACGTCTTGAACCTCTGGAGGTTCCCCTAAAC TATCTCCAATTTCCTCAATCACTTCTGCTGCCAAGTTGTCATCAACCACATCT CGCCGCCGCTGAATGTGCCGAGTTTGGATTAATGTATGGAAATGTTGATCGA CAGCTTCTTCAAGAATGTTATCAAGCATGTTTTTGTCGAAGAAGTAGGGAGT ATACCATTTAATCCCATCTGTAGTTGCTATTGCAATATCCAAGTTCTGAGCAC TAAACACAGGCACCCCATCGACCTTTTTCCTGACACCATTGTGTGGGATTGT CTTCAGAAGTTTATTGGCT***GCCTTGAATACTTGCTTCCCAT*** **Reverse frame 3, 118 amino acids** GKQVFKAANKLLKTIPHNGVRKKVDGVPVFSAQNLDIAIATTDGIKWYTPYFFD KNMLDNILEEAVDQHFHTLIQTRHIQRRRDVVDDNLAAEVIEEIGDSLGEPPEVQ DVLDENLCQ
EST NO: 31 NLLRfwd/NLLRrev; B1, B2, B3 **DETAILS**: NLRRfwd/NLRRfwd**,** U_HW-070116_Plate6e11, **sequence size (bp):** 541 **BLASTX:** ref|NP_568011.1| RNA recognition motif (RRM)-containing prote.. 258 9e-68 **CONSERVED DOMAIN:** smart00360, RRM, RNA recognition motif; . 47687 No 1e-12	***TAGGGCCTCTTGCATCGTC***AACAGGTGTGGCCGAATCTATTGCAACCTCATG TCCAAGAATCTCATGAGGCCTTCGAGAGACACGATCTGCTACACCTTCTTCA GCAAAAGTAACTAATCCAAAACCCCTATGACCTGTTCGCTTAGGATCTCTGG GAATGTAAACATCTTCTATATGGCCAAATCTCCCAAAATATTGACGGAGATC GTCAGAATTTGCTTCTGGGGGTAAACGACCAACAAAAATCTTTTTACTAATT CTCCGACGAGGCTCTCCCCTTCCATAAATTGGGCCAGGATGGTCATACAAAG TAGGAGCTCCAAGGGCTGCATATCTAGTTGCTGTAGAAATATATGCATTGTA TGCACCATATCCTCCCTGTGAAATTCTGCCAGTTGGCTTAACGTCATCATCCT TAGGAGTAGCTCGATCAACCACCACAGCAGAACCTCCGAGTTCATGGGTTTC TGACATCAAATTCTCCACAGATTCTGCATTTGCAAAAGTGATAAAACCAATT CC***ACGATGCAAGAGGCCCTA*** **Reverse frame 1, 180 amino acids** *GLLHRGIGFITFANAESVENLMSETHELGGSAVVVDRATPKDDDVKPTGRISQG GYGAYNAYISTATRYAALGAPTLYDHPGPIYGRGEPRRRISKKIFVGRLPPEANS DDLRQYFGRFGHIEDVYIPRDPKRTGHRGFGLVTFAEEGVADRVSRRPHEILGHE VAIDSATPVDDARGP 259 ISKKI 1 MERKL
EST NO: 38 38-2, RLRRfwd/RLRRrev; B1, B2, B3 **DETAILS:** RLRRrev/RLRRrev, eU_HW-070116_Plate5b06 **sequence size (bp):** 737 **BLASTX:** emb|CAN77792.1| hypothetical protein [Vitis vinifera] 166 2e-39 emb|CAO70481.1| unnamed protein product [Vitis vinifera] 150 9e-35 ref|NP_001078319.1| EIF4G (EUKARYOTIC TRANSLATION INITIATION .. 138 3e-33 **CONSERVED DOMAIN:** pfam02847, MA3, MA3 domain. Domain in DAP-5, eIF4G, MA-3 and other proteins. Highly al.. 66525 No 2e-07	***ACACTGGTCCATGAGGTT***TTGCAAGGTCGATCAGCAGTTTGGCTAAAAGATC TCTCTCTGTGTCCTTTCTCTCGAACGAGTCTGTGACCCAGAGAGAAACCATG GAAGGATGAAAGCTTGGAGAGTTCAGATCTTTGATGCATAAAACAACTTCGT TCACATCTCTAGCACTATAGTATTCTTTAATTGCTGCCATGGACATATTCTGT AGTCGCTCCTCTGACCAACCCTTTTCTGAGGAGGTACTCTGGGATCCTACTGT CCCTTGTGCATGGACAAGAGGTGGATTTACAACAGGTCTATCAATAATCCTA TCAACATTTCTCATATCCTTGTTACCATAATTCATATTATGCTCTTCAATAATT GATTGATCGTAAGTAGTTGAACCAACAAATCTATCCTGAACGTATCTTGGGG TGAGGTCCTCCCTAGAGCTATATGAAGGGCGCTCTGGTAAATTACTGTAACC ATTAAGACCAATCGAACTTGATACTGCAGAAGGACCTCTAATAGACATTCCT CTAGCAAGACCACCCTGTGGTCCCAAGGTTATTGAGTCATCACCCAAAGGCC TTTGAGGCAAGGGAATACACAGTGTCCTGGCCCTCATAAGATTGCCTTTCAC CCCCACGAACATCCTGAGAACCATACCCACGAACTTGAGTAGGCACTCACGC AGTCCACTAATCTGGGCCATTAAGAGAGTTGACATAACG***AACCTCATGGACC AGTGT*** **Reverse frame 2, 245 amino acids** HWSMRFVMSTLLMAQISGLRECLLKFVGMVLRMFVGVKGNLMRARTLCIPLPQ RPLGDDSITLGPQGGLARGMSIRGPSAVSSSIGLNGYSNLPERPSYSSREDLTPRY VQDRFVGSTTYDQSIIEEHNMNYGNKDMRNVDRIIDRPVVNPPLVHAQGTVGS QSTSSEKGWSEERLQNMSMAAIKEYYSARDVNEVVLCIKDLNSPSFHPSMVSL WVTDSFERKDTERDLLAKLLIDLAKPHGPV
EST NO: 104 104-1-1, S2/NBS; B1, B2, B3 **DETAILS:** S2/NBS U_HW-Plate1a05.b1, **sequence size (bp):** 368 **BLASTX:** emb|CAC86495.1| RGA-F protein [Cicer arietinum] 194 1e-48 **CONSERVED DOMAIN:** pfam00931, NB-ARC, NB-ARC domain. 85132 Yes 9e-10	***GGGGGGGTGGGGAAGAAAC***AGAGGGTAAACTTCTTTACAATGATCCAGAAG TAAATGAAAAAATTGATTTGAAAGGGTGGGCATATATCTCGAAAGATTTTGA TATTGTTCGGGTCACTAAAACCCTTTTGGAATCTGCTACTTTAGAATCAATTG ATACTACCATACATACTGAGGTTGTCACCTCAAGAAGAACCGATACCAGTGA CCTGAATAATCTACAAGTGCAATTGCAGCAAAATTTAAATCATAAATCTTTT TTGCTATTACTGGATGACATGTGGGATGGAAGCTATGTGGATTGGGACAATC TCAAGGACATATTTAATGTTGGGGAAATGGGAA***GCTTAATCATAC*TCACAACT AGA*** **Forward frame 3, 130 amino acids** GGGEETEGKLLYNDPEVNEKIDLKGWAYISKDFDIVRVTKTLLESATLESIDTTI HTEVVTSRRTDTSDLNNLQVQLQQNLNHKSFLLLLDDMWDGSYVDWDNLKDI FNVGEMGSLIILTTRIPSEFAAA
EST NO: 116 116-2a, S2/NBSrev; B1, B3 **DETAILS:** S2/? or S2/P4? eU_HW-070116_Plate5e01 Related with 1562, **sequence size (bp):** 377 **BLASTX:** gb|AAZ94162.1| enzymatic resistance protein [Glycine max] 87.4 3e-16 gb|AAQ56193.1| aminotransferase 2 [Cucumis melo] 83.6 5e-15 **CONSERVED DOMAIN:** COG0075, COG0075, Serine-pyruvate aminotransferase/archaeal aspartate aminotransferase.. 30424 No 0.006	***GGGGGGGTGGGGAAGAC*T*CATTGAGGTGTAGTGGTATCTTTTTTTTTTTGT***TT TCCCGCCATCTGGAGTTTTGGAGAGAATTCTCCGACTTGCTCTTTGCTCTCCC GGAGATGGGACTGTATCGTTCTTGATTGGCCGTTCCTTTTGCTTTGGATTGTT CTTCAACATCCCCTTAAATTTAATGTTGATGTGTTGTGGCTGTGAATGGGGA AAATGTGCCTATCGTGACATTCTGGAATCAAAACTTGCTTCTGATTCTGCAC ACACTATTAAGGCTGTTTGCCTTGTGAATGATGAGACAATCACTGGTGGCCC GAATAAGTTGGCCAAAGTGAGACAGATTCTTGATGC***CTACCACCATTTTGTG AGGGTTAAT*** **Forward frame 1, 176 amino acids** WGGGEDFIEV*WYLFFFVFPPSGVLERILRLALCSPGDGTVSFLIGRSFCFGLFFNI PLNLMLMCCGCEWGKCAYRDILESKLASDSAHTIKAVCLVNDETITGGPNKLA KVRQILDAYHHFVRVNYRFLVAAMPIGTC*

Similar to 1562: Optimal Global aligment Alignment score: 430 Identities: 0,7545692	

ESTs in RGA-RT-PCR AUTORADIOGRAPH FILM-2

EST INFORMATION	**SEQUENCE**
EST NO: EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 154 Ptokin-1/T1; B2,B3 **DETAILS:** Ptokin-1/T9 eU_HW-070116_Plate6g06, **sequence size (bp):** 366 **BLASTX:** emb|CAN64195.1| hypothetical protein [Vitis vinifera] 107 3e-22 gb|AAK25760.1|AF334840_1 ribosomal protein L33 [Castanea sativa] 106 4e-22 ref|NP_174951.1| 60S ribosomal protein L35a (RPL35aB) [Arabid.. 101 1e-20 ref|NP_177567.1| 60S ribosomal protein L35a (RPL35aC) [Arabid.. 101 1e-20	***GCATTGGAACAAGGTGAA***GAAGGATGGTAGCCACTATCGTTGCATTTGGGGT AAGGTTACAGGGCCTCATGGTAACAGTGGCATAGTCCGCGCAAAGTTCAAG TCAAACCTTCCACCAAAACCGATGGGAGCCAGGGTTAGAGTCTTCATGTATC CAAGCAACATATAAGGTGATCCGAACCTTGGACTAGAAGACAAATGCACTA TGGAAGTTATATTTTTGATCATTTTTGTATCTCTAAACAGAATAGTTCCTTTT CCTGCATGGTGAAAGAACATGTATTAGTTATCCTCTGTTTTTTTCTGAATCCT GACTTCATTATGGATTTTTCTCCT***CCAAAAAAAAAGATATCACTCAGCATAATG*** **Forward frame 2, 121 amino acids** HWNKVKKDGSHYRCIWGKVTGPHGNSGIVRAKFKSNLPPKPMGARVRVFMYP SNI*GDPNLGLEDKCTMEVIFLIIFVSLNRIVPFPAW*
EST NO: 179 179-1, NLRRfwd/T2; B2, B3 **DETAILS:** NLRR frwd/T1 U_HW-Plate2a03.b1, **sequence size (bp):** 336 **BLASTX:** gb|ABU98947.1| dynein light chain [Lupinus albus] 74.3 2e-12 emb|CAN81003.1| hypothetical protein [Vitis vinifera] 74.3 2e-12 gb|AAL57365.1|AF404866_1 neuronal nitric oxide synthase prote.. 74.3 2e-12 ref|NP_193328.1| dynein light chain, putative [Arabidopsis th.. 74.3 2e-12 **CONSERVED DOMAIN:** pfam01221, Dynein_light, Dynein light chain type 1. 85315 No 2e-11	***TAGGGCCTCTTGCATCG***CCGGTCGCAATTTCGGTTCGTATGTGACTCATGAAA CAAACCACTTTGTTTATTTCTATTTGGATCAGAAAGCTGTTTTACTATTCAAG TCTGGCTAGCCTCATTCTGATGGAGACAAAAAAGGTCTACGAGGATTGTCTT GTGATTATGCTGCTATATTAACTCTTGTATGTATTAAATCCGCTGGCACAAAC TCTTTACAGGACATTCTTGTGCTTGCATATCTAATTTCATGCAAAATGGTTCT CAAGTTTGGATATGGATTACATTATTGATCTTTCTGCTTCTC***TTAAAAAAAAA*** ***GATATCACTCAGCATAATG*** **Forward frame 2, 251 amino acids** RASCIAGRNFGSYVTHETNHFVYFYLDQKAVLLFKSG*
EST NO: 253 253 (D10-1), PtoFenS/T1; B1 ,B2,B3 **DETAILS:** PtoFen-S/T1, U_HW-070116_Plate6b01, **sequence size (bp):** 571 **BLASTX:** sp|P14584|CB21_RAPSA Chlorophyll a-b binding of LHCII type 1 .. 149 1e-53 sp|P15773|CB2_MALDO Chlorophyll a-b binding protein AB10, chl.. 115 5e-51 gb|AAF20948.1|AF207690_1 chlorophyll a/b-binding protein [Daucus 134 3e-48 gb|ABD37900.1| light-harvesting chlorophyll-a/b binding prote.. 127 6e-44 emb|CAA43803.1| LHC II Type III chlorophyll a/b binding prote.. 104 7e-39 emb|CAA10284.1| chlorophyll a/b binding protein [Cicer arietinum 162 1e-38	***CATTATGCTGAGTGATATCTTTTTTTTTAA***AAAACAAGTGATTAACATTAAGT TGCATAGACATATGATAGATAATTCACAATCTAAGGGAAGTGCATTACAA GAACGTAAACTTCACCAAAAACACATAATGAAACCGAATTAAGGTCACTTTC CGGGAACAAAGTTGGTGGCATAGGCCCATGCATTGTTGTTGACGGGGTCAG AAAGATGGTCAGCAAGGTTCTCCAAAGGACCCTTTCCAGTGACAATAGCTTG AACAAAGAATCCAAACATAGAGAACATGGCTAATCTACCGTTCTTAAGTTCA TTGACCTTCAATTCTGCAAAAGCTTCAGGGTCGTCAGCTAAGCCCAATGGGT CAAAGCTACCACCAGGGTAGAGTGGGGTCAGTGGCCTCACCAAGAGGCCCA CCAGCAATACGGTAACCTTCAACAGCACCCATCAAAACAACTTGGGTAGCCC ATATAGCAAGGATACTTTGAGCATGGACCAAACTTGGGTTACCCAAGTAGTC AAGTCCACCCTCACTAAAGATTTGTGATCCA***GCCTTGAATACTTGCTTCCCAT*** **Reverse frame 1, 190 amino acids** MGSKYSRLDHKSLVRVDLTTWVTQVWSMLKVSLLYGLPKLF*WVLLKVTVLL VGLLVRPLTPLYPGGSFDPLGLADDPEAFAELKVNELKNGRLAMFSMFGFFVQA IVTGKGPLENLADHLSDPVNNNAWAYATNFVPGK*
EST NO: 241 CicerkinFwd /T1, B1, B2, B3 **DETAILS:** Primers found: ?/T1, U_HW-070116_Plate6f01, **sequence size (bp):** > 251 **BLASTX:** emb|CAO61326.1| unnamed protein product [Vitis vinifera] 81.3 2e-14 emb|CAN78410.1| hypothetical protein [Vitis vinifera] 81.3 2e-14 gb|AAK59445.2| unknown protein [Arabidopsis thaliana] 79.0 9e-14 ref|NP_195062.1| terpene cyclase/mutase-related [Arabidopsis .. 79.0 9e-14	***CATTATGCTGAGTGATATCTTTTTTTTTAA***AACAAGTTTCCTATTGTGTTATTG GTCTTGCGAAAAGTGATACACTATGAAGTAGTAGAACATGCCACTAATATCT TATTAATCCCAAGTTCTTCAACCAGAGTAGTACCTCCGCAAGCCCTTCTCTCA GGCTTCTGGGCTTATAGTCTAGCTCCCTTTTAGCTTTCTCGCAGGAATACCCC CACTGATGTCTTAAAACATTCACCGTCAGTGGACTAATG **Reverse frame 3** ISPLTVNVLRHQWGYSCEKAKRELDYKPRSLREGLAEVLLWLKNLGLIRY*
EST NO: 262 PtoFenS/T1; B1,B2,B3 **DETAILS:** PtoFen-S/PtoFen-S U_HW-070116_Plate6e12, **sequence size (bp):** 487 **BLASTX:** emb|CAO71692.1| unnamed protein product [Vitis vinifera] 211 1e-53 ref|NP_179336.1| leucine-rich repeat family protein [Arabidop.. 174 2e-42 ref|NP_195272.1| leucine-rich repeat family protein [Arabidop.. 173 4e-42 **CONSERVED DOMAIN:** COG4886, COG4886, Leucine-rich repeat (LRR) protein [Function unknown]. 34495 Yes 3e-06	***ATGGGAAGCAAGTATTCAAGGC***GTAGCAATCGGCTCAAAGAAGCAGGTAAG GTTGTTAAGTGGTTTCCCCTCAGATTTAGATAGACAAGACTTAGGAGATTTC CAACAGAATCAGGAATCTCTGTGATCCTATTGGAATGCAAGTCCAATTTGGT CAGTGAGGAAAGGCCACCAATTGTGGAAGGTAGAGCAACAATCCTATTCTC TGATAAATCAAGGGTGACCAAACTAGATAACTTTCCTATCGAATCAGGTAGC CAATCGACTCGGTCCACTAACTTGTTCTGGAGCTTGAGATCGCGAGTTCCTTT CTTTGCCGAAACCTCAATTATGCTAGCAAGTTTAATCAAACTCAACTTATCA CCATCTTGACCTGCAGCAGTAGTGGATTTTAATGATGAATCCAATATTTGGG GTTTGGATGTGATATTTGCTTCAATTGAATACCCATTAGGGTAGAATGTG***GC*** ***CTTGAATACTTGCTTCCCAT*** **Reverse frame 3, 161 amino acids** GKQVFKATFYPNGYSIEANITSKPQILDSSLKSTTAAGQDGDKLSLIKLASIIEVSA KKGTRDLKLQNKLVDRVDWLPDSIGKLSSLVTLDLSENRIVALPSTIGGLSSLTK LDLHSNRITEIPDSVGNLLSLVYLNLRGNHLTTLPASLSRLLRLEYLLP
EST NO: 283 PtoFenS/T1; B2,B3 **DETAILS:** PtoFenS/T1, U_HW-070501-Plate5g04.b1 **sequence size (bp):** 222 **BLASTX:** ref|NP_880000.1| hypothetical protein BP1228 [Bordetella pert.. 32.7 7.3	***CATTATGCTGAGTGATATCTTTTTTTTTAA***TGAATGAAAAACGAAAAAAGTGG GCAAAAAGAGGATAGCTATGATGATTTGCAGGTTGAGATCTCTGCTTTCTCT TGCCAAATGCAGTTCTCAACCCTTCCAATCACCGGCAGCAGCAGCAGCACGC ACTTTCCCTTCCTCCCATCACTTGCAAAATGTTGGATTCATTG***GCCTTGAATA*** ***CTTGCTTCCCAT*** **Reverse frame 1, 74 amino acids** MGSKYSRPMNPTFCK*WEEGKVRAAAAAGDWKG*ELHLARESRDLNLQIIIAIL FLPTFFVFHSLKKKISLSIM
EST NO: 304 304-2-1, Ptokin-1/Ptokin-2; B1, B3 **DETAILS:** Ptokin-1/ Ptokin-2 U_HW-Plate2a04.b1, **sequence size (bp):** 344 **BLASTX:** emb|CAN75214.1| hypothetical protein [Vitis vinifera] 36.6	***GCATTGGAACAAGGTGAAA***GAAGATTGGTTAATTTACCAAATGAGACGGAAA CAGTTAAACATATGAACTCTTTGGATAATTCGTTGGATATGCCCGCTACAAA TATGGACTGTTTCTCTCTTGCCACTCAAGTTAAATCTCCCAAACGGCAAAAG GTTTCTTTTTCTGATTCAAAGTTTATTGCTAGTAATGAATCTGACAGTTTCCA TTTGAGCTACTCAAAATTGGCTAGTCATGAAAAGAAAGGTCAAGTAGGAAG GGAAAACAGTAACAAAATCATCAGTGGGCCAGATAGGGCAGATGTCACAAG

0.51 ref|XP_001642980.1| hypothetical protein Kpol_1046p14 [Vander.. 35.4 1.1	CAGTAAATATGATGA***CTA****GG****GGGTGG****CCCCCCT*** **Forward frame 1, 114 amino acids** ALEQGERRLVNLPNETETVKHMNSLDNSLDMPATNMDCFSLATQVKSPKRQKV SFSDSKFIASNESDSFHLSYSKLASHEKKGQVGRENSNKIISGPDRADVTSSKYDD *

ESTs in RGA-RT-PCR AUTORADIOGRAPH FILM-3

EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 353 353-1-1, S2/T2; B2, B3 **DETAILS:** S2/T2 U_HW-Plate2d08.b1, **sequence size (bp):** 426 **BLASTX:** emb|CAO63363.1| unnamed protein product [Vitis vinifera] 59.7 6e-08 emb|CAN83758.1| hypothetical protein [Vitis vinifera] 59.7 6e-08 emb|CAO63363.1| unnamed protein product [Vitis vinifera] 59.7 6e-08 emb|CAN83758.1| hypothetical protein [Vitis vinifera] 59.7 6e-08	***GGGGGGGTGGGGAAGACGAC***AAAAGAGAAAATGGAAAACGCAGAAGAGGA ACAGCACCGAAAAGTCGAAGACCAGTATGCGCAGGTCCTTTCAGAGTCTCC AAGAGCAGCAGCAACAAGTGAACTGCACTCTTCCAAGCCTGCTCCTGCTCAG GGTTTGATCCTCTGATTCACTTCTTGTATATACCTTTTTTAAAACTCCTGCAA ATTGTTTCAATGTGAGCACACCTTTGACAGATTTTACTAATCACTTGGACAA GGTTGCAGCTATTTGATTTTCTTTTATAAGCAAAGAGAAATTGAATTTCCCAA CTTTGAAATTATATGCTTGTTATCTTTTTGCCCATTCACTACTGACTCCATGG AAAAAAAAAATAAGGGATTGGAAAGCTTTTCCA***GTAAAAAAAAAATATCACT CAACATAATG*** **Forward frame 1, 142 amino acids** GGVGKTTKEKMENAEEEQHRKVEDQYAQVLSESPRAAATSELHSSKPAPAQGL IL*
EST NO: 372 S1/T5; B1, B3 **DETAILS:** U_HW-070116_Plate6e08, **sequence size (bp):** 519 **BLASTX:** dbj|BAE71197.1| hypothetical protein [Trifolium pratense] 238 1e-61 dbj|BAF01924.1| hypothetical protein [Arabidopsis thaliana] 129 1e-28 ref|NP_563703.1| transducin family protein / WD-40 repeat fam.. 129 1e-28	***CATTATGCTGAGTGATATCTTTTTTTTTCC***AGAGAGCCAAGTGGATGGGAGTT ACCTTTTCTGCAAGGATGGTTGATGGGTCAAAGCCAAGTTGGTGCTCCCTCA ATGCTACCTCACATGGGTGTTAGTCGTGATACACAAGCTCAGCAGATTAGTT CTTCTGCAATGGCTAATAATCTTTCCACTTCTAATATTGATGTAGCAATGCCA CCTTCAGCAATGTCTGGCAGTATCAACATACCTGGATCTTCTATAAGATCAG GTTTACAGAATCATTTTTCACAATCGCGTACACCTGTATCTGAATCCGGAAA TCTTGCTGCTTCTATTACTACCCCACATGATGGATCTGACATCCAAACCATCA TGAGTCGAATCCAGTCAGAAATCGCTACATCTGTGGCTGCGGCTGCTGCTGC TGAACTGCCTTGTACCGTGAAGTTAAGAGTATGGTCTCATGACGTAAAAAAT CCCTCTACCCCACTAAATGCTGACAGATGTC***GTCTTCCCCACCCCCCC*** **Forward frame 1, 173 amino acids** HYAE*YLFFSREPSGWELPFLQGWLMGQSQVGAPSMLPHMGVSRDTQAQQISS SAMANNLSTSNIDVAMPPSAMSGSINIPGSSIRSGLQNHFSQSRTPVSESGNLAAS ITTPHDGSDIQTIMSRIQSEIATSVAAAAAAELPCTVKLRVWSHDVKNPSTPLNA DRCRLPHPP
EST NO: 384 384-2-2, S2/T7; B2, B3 **DETAILS:** S2/T7 U_HW-Plate3a08.b1 AND U_HW-070116_Plate6e07, **sequence size (bp):** 628 **BLASTX:** emb|CAO65845.1| unnamed protein product [Vitis vinifera] 125 2e-27 gb|AAO69667.1| vacuolar ATPase subunit E [Phaseolus acutifolius] 124 3e-27 ref|NP_176602.1| VHA-E3 (VACUOLAR H+-ATPASE SUBUNIT E ISOFORM.. 122 2e-26 sp|Q9MB46|VATE_CITUN Vacuolar ATP synthase subunit E (V-ATPas.. 118 2e-25 sp|Q9SWE7|VATE_CITLI Vacuolar ATP synthase subunit E (V-ATPas.. 118 2e-25 ref|NP_192853.1| TUF (VACUOLAR ATP SYNTHASE SUBUNIT E1) [Arab.. 118 3e-25 **CONSERVED DOMAIN:** PRK02292, PRK02292, V-type ATP synthase subunit E. 74167 No 2e-04 pfam01991, vATP-synt_E, ATP synthase (E/31 kDa) subunit. This family includes the vacu.. 79889 Yes 2e-14 **Similar to 1749** Optimal Global aligment Alignment score: 602 Identities: 0,6608280	***GGGGGGGTGGGGAAGACGAC***CTACACTTGGTAGAGGATGTGTTGGAATCAG CTGCTGAGGAGTATGCTGAGAAGGCAAAAGTTCATCTACCAGAGATTGTTGT TGATAAGGATGTCTATCTTCCACCTGCACCCAACCATAACAATCCCCATGAT CTTTATTGCTCTGGTGGCGTGGTATTGGCATCTCGAGATGGAAAGATTGTGTT TGAAAATACACTTGATGCACGTTTGGATGTAGTGTTCCGTAATAAACTCCCA CACATCCGCAAGGAGCTCTTTGGACAAGTTGCTGTTTGAAGAATTTGGTTGT ATTGTTTCGTTTCACCAAAAATATTTATAATTTACTGCAGAGATTGTCTGTGA GGGGAATATGTGTTGTGCCATTGCTCCTGTAAACTTACTATTATTGCCCTTTA GTAACTTCATTTTTTTTTTTGTGTCGTTCACTTTACTTTTTGTATTTGATGGGGC GTGCGATGCTCTAGAAGTTAGTGATAGCGATAAATGTTCTCAGCCAAAGAAT AAGTGCTTGCTAAAAGCATATCGCTGCTGTTAGCTTCTCTGTGATGAAGTAA AAAATCTATTGAACATTGCTGTC***AAAAAAAAAAATATCACTCAGCATAATG*** **Forward frame 3, 208 amino acids** GGGEDDLHLVEDVLESAAEEYAEKAKVHLPEIVVDKDVYLPPAPNHNNPHDLY CSGGVVLASRDGKIVFENTLDARLDVVFRNKLPHIRKELFGQVAV*
EST NO: 402 S1/T8; B1, B2, B3 **DETAILS:** S2/T8 e U_HW-070116_Plate6e06, **sequence size (bp):** 459 **BLASTX:** gb|ABO61516.1| GAI1 [Glycine max] 131 2e-29 dbj|BAF62636.1| DELLA protein [Phaseolus vulgaris] 121 1e-26 sp|Q5BN22|RGA2_BRACM DELLA protein RGA2 (RGA-like protein 2) .. 105 7e-22 emb|CAN67929.1| hypothetical protein [Vitis vinifera] 104 2e-21 sp|Q8S4W7|GAI1_VITVI DELLA protein GAI1 (Gibberellic acid-ins.. 104 2e-21 sp|Q7Y1B6|GAI_LYCES DELLA protein GAI (Gibberellic acid-insen.. 95.1 1e-18 emb|CAA72178.1| RGA2 protein [Arabidopsis thaliana] 95.1 1e-18 emb|CAA75492.1| GAI [Arabidopsis thaliana] 95.1 1e-18 ref|NP_172945.1| GAI (GA INSENSITIVE); transcription factor [.. 95.1 1e-18	***ATTATGCTGAGTGATATCTTTTTTTTTGC***GTTTCAACAACCGTAACAACGGAA CGAGTTGAGTCAGCTGAAGTTCGTTTGAGTCGTTTACTGGAAGGAAGAGATT CATCTGAGTCAGTAGTAGTTGTTGTAGTTGTAGTTGTGGTAGCATAGATCGC TTTACCTGGAATGGCGTTTAGGTCATTATCAGATGAATATGAAACCGAAGGG TTTTGAGGTTGAGGGTCAAAATTGGAAAGCATCGATTTAAGCCAGTTAGAAA TATCTGAAGGATTGTAATGAACGGTGTCGTTTGAGAGTTGTTGAGCGATAAT TGCTTCATCTTGGTCTTGGAAATTCCCCATGGCTTGTTCAAGTTGTTCAAGTT TTTGAGCAACTTCTCCCATGTCGGATGATTTCACCTTGTAACCAACCACCGCT AAAAGCTCATCCATACCACCGTC***GTCTTCCCCACCCCCCC*** **Reverse frame 3, 152 amino acids** GGGEDDGGMDELLAVVGYKVKSSDMGEVAQKLEQLEQAMGNFQDQDEAIIAQ QLSNDTVHYNPSDISNWLKSMLSNFDPQPQNPSVSYSSDNDLNAIPGKAIYATTT TTTTTTTDSDESLPSSKRLKRTSADSTRSVVTVVETQKKRYHSA*
EST NO: 405 Ptokin-1/T1; B1, B2, B3 **DETAILS:** Ptokin1?/ T1 eU_HW-070116_Plate5b04, **sequence size (bp):** >537 **BLASTX:** gb|ABK93583.1| unknown [Populus trichocarpa] >gb|ABK94271.1| .. 191 2e-47 gb|ABK93065.1| unknown [Populus trichocarpa] 190 4e-47	***CATTATGCTGAGTGATATCTTTTTTTTTAA***GAGGAGAAAAATCCATAATGAAG TCAGGATTCAGAACAAAACAGAGGATAACTAATACATGTTCTTTCACCATGC AGGAAAAGGAACTATTCTGTTTAGAGATACAAAAATGATCAAAAATATAAC TTCCATAGTGCATTTGTCTTCTAGTCCAAGGTTCGGATCACCTTATATGTTGC TTGGATACATGAATACTCTAACCCTGGCTCCCATTGATTTTGGTGGAAGGTTT GACTTGAACTTTGCGCGGACTATGCCACTGTTACCATGAGGCCTTGTAACCT TACCCCAAATGCAACGATAGTGGCTACCATCCTTCTTCACCTTGGCTTTGTAA ATGTCTGCCATGCGCTTCCCATCATACCAGGAAACCTCTTCTTTAGTGTTTAC TCCTTCAATCTGGACAAAAGAAGTGTTTGGGTACTGCTTCGACTTAGACCCC

emb|CAN64195.1| hypothetical protein [Vitis vinifera 190 4e-47 gb|AAK25760.1|AF334840_1 ribosomal protein L33 [Castanea sativa] 189 7e-47 ref|NP_177567.1| 60S ribosomal protein L35a (RPL35aC) [Arabid.. 188 2e-46 ref|NP_172188.1| 60S ribosomal protein L35a (RPL35aA) [Arabid.. 184 2e-45 **CONSERVED DOMAIN:** pfam01247, Ribosomal_L35Ae, Ribosomal protein L35Ae. 65072 No 2e-21 **Similar to 154:** Optimal Global aligment Alignment score: 610 Identities: 0,7924945 **Similar to 417:** Optimal Global aligment Alignment score: 643 Identities: 0,7420561	TTGCATCACCCATCTGTACCCCGAAAGTAAAGTCTAACACGCTCACCTCTGT CAAATGCAATCCAACTCCTGTTTGTCCATGCCTGTTTCGACTCTGTTCCAT **Forward frame 1, 177 amino acids** ALEQGERVRLYVRGTVLGYKRSKSNQYPNTSLVQIEGVNTKEEVSWYAGKRM AYIYKAKVKKDGSHYRCIWGKVTRPHGNSGIVRAKFKSNLPPKSMGARVRVFM YPSNI*
EST NO: 417 Ptokin-1/T3; B1, B2, B3 **DETAILS:** Ptokin-1/T3 e U_HW-070116_Plate6e05, **sequence size (bp):** 531 **BLASTX:** gb|AAK25760.1|AF334840_1 ribosomal protein L33 [Castanea sativa] 218 1e-55 emb|CAN64195.1| hypothetical protein [Vitis vinifera] 218 2e-55 ref|NP_177567.1| 60S ribosomal protein L35a (RPL35aC) [Arabid.. 211 2e-53 **CONSERVED DOMAIN:** pfam01247, Ribosomal_L35Ae, Ribosomal protein L35Ae. 65072 No 1e-28 **Similar to 405:** Optimal Global aligment Alignment score: 643 Identities: 0,7420561 **Similar to 154:** Optimal Global aligment Alignment score: 405 Identities: 0,5936330	***GCATTGGAACAAGGTGAA***CGTGTTAGGCTTTACGTCAGGGGTACAGTTCTTG GATACAAGAGGTCCAAGTCAAATCAATACCCAAACACCTCTCTTGTCCAGAT TGAGGGAGTAAACACTAAAGAAGAGGTTTCTTGGTATGCTGGGAAGCGCAT GGCATACATTTACAAGGCTAAAGTAAAGAAGGATGGTAGCCATTATCGTTGC ATTTGGGGTAAGGTTACAAGGCCTCATGGTAACAGCGGTATAGTCCGTGCAA AGTTCAAGTCAAATTTGCCACCAAAATCAATGGGAGCTAGGGTTAGAGTATT CATGTATCCAAGCAATATATAAGATTATCCTTACCTGTGTCTAGAAGGCAAA TGCATTATTGAAGTTAATTTTTGGTCTTTTTTTTTTTAATCTCTGAACAGAATA GTTCCCTTTTTAGGATGGTGAATGAACATGTATTACTCATCCTTTCTTTTGGT ATGAATCCTGATTTCCTAATAGATTTTCCCG***CTAAAAAAAAAGATATCACTCA GCATAATG*** **Forward frame 1, 177 amino acids** ALEQGERVRLYVRGTVLGYKRSKSNQYPNTSLVQIEGVNTKEEVSWYAGKRM AYIYKAKVKKDGSHYRCIWGKVTRPHGNSGIVRAKFKSNLPPKSMGARVRVFM YPSNI*
EST NO: 427 Ptokin-1/T5; B1, B2, B3 **DETAILS:** Ptokin-1/T5 U_HW-070116_Plate5b03, **sequence size (bp):** 585 **BLASTX:** ref|NP_190930.1| inorganic pyrophosphatase, putative (soluble.. 293 5e-78 **CONSERVED DOMAIN:** cd00412, pyrophosphatase, Inorganic pyrophosphatase. These enzymes hydrolyze inorganic.. 29533 No 3e-52	***GCATTGGAACAAGGTGAA***ATACGAGCTTGACAAAAAAACTGGACTTATTAAG GTTGACCGTGTACTTTACTCATCAGTTGTGTATCCTCACAACTATGGGTTTAT CCCCCGCACTATTTGTGAGGATGGCGACCCTATCGATGTCTTGGTTATTATGC AGGAGCCAGTTCTTCCTGGTTGCTTTCTTCGAGCCAAAGCTATTGGACTCATG CCTATGATTGATCAGGGCGAGAAAGATGACAAGATAACTGCTGTTTGTGCCG ATGATCCTGAGTACAGGCATTACAATGACATCAATGAGCTCCCTCCACATCG TTTAGCTGAAATTCGTCGTTTTTTTGAAGACTACAAAAAGAACGAGAACAAG GAAGTTGCGGTAGACGACTTTCTTCCTTCCTCAACTGCATATGAAGCAATTG AGCATTCCATGTCCTTGTATGCGGACTACGTTGTGGAGAGCTTGAGGCGGTA GTGTTGATGCAAATGGATTTTGTTTGGACGTGAAGGTATCTTTTGATGACTGC CATTTTATATCTTTATAATCATGATTACGAC***GGAAAAAAAAAGATATCACTCA GCATAATG*** **Forward frame 2, 194 amino acids** HWNKVKYELDKKTGLIKVDRVLYSSVVYPHNYGFIPRTICEDGDPIDVLVIMQE PVLPGCFLRAKAIGLMPMIDQGEKDDKITAVCADDPEYRHYNDINELPPHRLAEI RRFFEDYKKNENKEVAVDDFLPSSTAYEAIEHSMSLYADYVVESLRR*
EST NO: 447 447-1-2; B1, B2, B3 **DETAILS:** ?/T8, **sequence size (bp)**: >392 **BLASTX:** ref|NP_200901.2| chromosome-associated kinesin, putative [Arabid 47.4 3e-04 **BlastN:** CU104774.5 M.truncatula DNA sequence from clone MTH2-43L10 on chromosome 3, complete sequence 291 1e-75	***CTTTATGCTGAGTGATA****TC****TCTTTTTTTGC***AGAAAGTAATGACACCACACAAA TGGCTTCCCCCGAAATGACCAGTTCAAATGAAAAAAATGTTGAGAAGGAGA GAAGAGGTGGAGGGCTGCGCTGTTCTTGCAGTAAAAGGTCCTTGTGCAAGA CTACAAAGTGCAAATGCCGATCCATTGGTGGGAGCTGTGGACCATCTTGCGG CTGCACGCGCTTTAAGTGCACAAATAAGGAACTAAACACTCTAACAAAAAA CGAATCACTAAAATCTGATAATCCAAAATGCAGCGCTAACAAAGATGGGGG TGTGATTGCATCCAAATGTGCCAAACTATTAAAGAGTGCCCTTGTTCAAATC CTGCTAGTCGCATGACAACCTGCGGGTCCAT **Forward frame 3, 130 amino acids** FMLSDISFFAESNDTTQMASPEMTSSNEKNVEKERRGGGLRCSCSKRSLCKTTK CKCRSIGGSCGPSCGCTRFKCTNKELNTLTKNESLKSDNPKCSANKDGG*VIA*SKC AKLLKSALVQILLVA*
EST NO: 489 S1/T5; B1, B2, B3 **DETAILS:** U_HW-070116_Plate6e03, **sequence size (bp):** 374 **BLASTX:** emb|CAO64530.1| unnamed protein product [Vitis vinifera] 46.2 7e-04 emb|CAN77176.1| hypothetical protein [Vitis vinifera] 46.2 7e-04	***CATTATGCTGAGTGATATCTTTTTTTTTCC***CCATTAATTTATATTGCGACTTTA TATTCAAAGAAATAAAACGTGGTTTAACAGTACATTTGAAGCATAAATTTTT TGTCCTAGTGGTGTAGGTGTATATACATAATAATGAAGGATCACTTGCTTAA AGGATGGGATTAAGCATAGGCGTAAATTACTTAATGTAGAAAATCACTTGAC AAGTTGCTCCTTCTTTCCAGCTTCTTCAATTTCTCTCAACCCTCCAACTTTTTG TTCTATTTCCTCAATCAACTCCTCACGCCACAGAGCCTCCCTGGCCTCTGCTT CC*ATTATCTCTTGCTTCTCACAGATCCAGTTGCCA****GTGTCGTCTTCCCCACCCCC CC*** **Reverse frame 3, 124 amino acids** GGGEDDTGNWICEKQEIMEAEAREALWREELIEEIEQKVGGLREIEEAGKKEQL VK*FSTLSNLRLCLIPSFKQVILHYYVYTPTPLGQKIYASNVLLNHVLFL*
EST NO: 505 505-1-1, S2/T9; B1, B2, B3 **DETAILS:** S2/T9 U_HW-Plate3a10.b1, **sequence size (bp):** 317 **BLASTX:** ref|NP_564517.1| unknown protein [Arabidopsis thaliana] >gb|A.. 51.2 2e-05	***GGGGGGGTGGGGAAGACGAC***GATGACAATGACGATGATGATGAAGATGATG ATGATGACGATGATGATGGTGAAGACGATGATGACGAGGATGAGGAAGAGC ACGAGGAAGAGGAGGATTTGGGAACATATTACCTTGTTCGCCCTTTAGGCGC TGCTGAGGAGGAGGAAGCATCTGGTGATTTTGAACCTGTGGAAAACGGTGT GGAGGAAAACCAAGTGAAAAAAGATGATGGTGAAGAGGATGAAGGTCACC GTGATGATGATGTTGACAAGGCTGAGGTTCCA***CCAAAAAAAAAGATATCACT CACCCTACCG*** **Forward frame 3, 105 amino acids** GGGEDDDDNDDDDEDDDDDDDDGEDDDDEDEEEHEEEEDLGTYYLVRPLGAA EEEEASGDFEPVENGVEENQVKKDDGEEDEGHRDDDVDKAEVPPKKKISLTLP
EST NO: 542 Ptokin-1/T2; B1, B3 **DETAILS:** Ptokin1/T2 U_HW-070116_Plate6e02, **sequence size (bp):** 338 **BLASTX:** gb|EAY86395.1| hypothetical protein OsI_007628 [Oryza sativa .. 34.3 2.6 dbj|BAD33355.1| putative ABC transporter [Oryza sativa (japon.. 34.3 2.6	***GCATTGGAACAAGGTGAA***GAAACTGGACCAACAAGAGAGATTGATAACCGG CACTGCCTGAGTGGCTGAGTTCACCATCGACCTGTGAGAGCTGACCCAAAAG ATTTCAGTGTAATACATTGCGGTTATCATCCGCTTTTTCTTTTTTTTCTTTTTG CCTGTGCGCAATTTTACCTCAGAGGTGGTGCAGTGTAGCCATTGGCGTATGA GTATGAATCTCTGTACATGAGGTGTAATAGATGAAGAGATGTACATACTGTA CAATTGCAGGGTAAATATAAGCAGTATAGCACGGCTCGTGCAGCCTG***GTAAA AAAAAAGATATCACTCAGCATAATG*** **Forward frame 2, 112 amino acids** HWNKVKKLDQQERLITGTA*
EST NO: 617 S1/T4; B1	*T****GGGGGGGTGGGGAAGACGAC***CAACTGAGCGAATGTTCTATCCTGCTGTGAC TCAAAAAAGACCCGCGCCCCCTTCGGGATAGTGATACAACTCCTGCCTCATC

DETAILS: S2/? U_HW-070116_Plate6e01 **sequence size (bp):** ~299 **BLASTX:** ref|ZP_01897949.1| hypothetical molecular chaperone [Moritell.. 33.5 4.6	ATTTACCTAACAAAACCGCAACCACTAGGACATGTTATGTCAATATCGGGGGG AAATGTTGGCATCAACTATCTGGCCAATCTCCCTAAATATAGACGGACATTT TCTTATTTTGCTGGTGGGGGCAACGCACTTTTAACATCTTTTACTTATCTCC GATCACCCTCTCCCTTTCGAGAAATAGCGGCCGGAAGG **Forward frame 2, 99 amino acids** GGVGKTTN*ANVLSCCDSKKTRAPFGIVIQLLPHHLPNKTATTRTCYVNIGGNV GINYLANLPKYRRTFSYFAGGGNALLTSFYLSPITLSLSRNSGRK
EST NO: 619 619-1-1, S2/T4; B1, B3 **DETAILS:** S2/T4 U_HW-Plate2a07.b1, **sequence size (bp):** 257 **BLASTX:** No significant similarity found **BlastN:** AF100333.1 Dendrobium grex Madame Thong-IN putative DNA-binding protein (ovg30) mRNA, partial cds 57.2 4e-06	***CATTATGCTGAGTGATATCTTTTTTTTTCA***CCCCAGAAAATGCCACTACTTCAT TTTTTTTTGGTCCAACTCCAAATTAATAAGTTCCCAGATTCAAGAACTAGGA GAAACTCAACATAAAAAAGCCACCACTTTCTGCAATGTTCGACTTCTCACAG CTCAAGTTTGAATGTTGAATCTTCAACACATAACTTCAAGTCATAACAAAAA CTACCATCTTACACCATCTTCCCCACAG***TCGTCTTCCCCATCCCCCC*** **Forward frame 3, 85 amino acids** IMLSDIFFFHPRKCHYFIFFWSNSKLISSQIQELGETQHKKATTFCNVRLLTAQV* MLNLQHITSSHNKNYHLTPSSPQSSSPSP
EST NO: 645 Ptokin1/T4; B1, B2, B3 **DETAILS:** Ptokin1/T4, bU_HW-070116_Plate6d12; **sequence size (bp):** 222 ref|NP_001054720.1| Os05g0160200 [Oryza sativa (japonica cult... 142 7e-33 gb|ABK42077.1| ubiquitin extension protein [Capsicum annuum] 140 2e-32 pir||S42643 ubiquitin / ribosomal protein S27a - potato (fragmen 140 2e-32 **CONSERVED DOMAIN:** cd01803, Ubiquitin, Ubiquitin (includes Ubq/RPL40e and Ubq/RPS27a fusions as well as h... 29205 No: 2e-33 Similar to 2132 and 1934 Sequence 1: 645 Sequence 2: 2132 Optimal Global aligment Alignment score: 556 Identities: 0,9791667	***GCATTGGAACAAGGTGAA***GAGTTGATTCCTTCTGGATGTTGTAGTCGGCGAG AGTGCGGCCGTCTTCGAGTTGTTTTCCGGCGAAGATGAGTCGTTGTTGGTCC GGTGGAATCCCTTCCTTGTCTTGGATTTTGGCTTTGACGTTGTCGATTGTGTC AGAGGATTCAACCTCGAGGGTGATTGTTTTGCCCGTTAGGGTCTTCACGAAG ATCTGCATCTTCGGTCGATTAGGGTTTGCGCCGAGGGAAGGAAAATGAG***TGA*** ***AAAAAAAAGATATCACTCAGCATAATG*** **Reverse frame 3, 95 amino acids** LC*VISFFFTHFPSLGANPNRPKMQIFVKTLTGKTITLEVESSDTIDNVKAKIQDKE GIPPDQQRLIFAGKQLEDGRTLADYNIQKESTLHLVPM
EST NO: 737 737-1-1, Ptokin-1/T7, B1, B2, B3 **DETAILS:** Primers found: Ptokin-1/T7, U_HW-Plate2a08.b **sequence size (bp):** 229 **BLASTX:** sp|P13194|PSAE_HORVU Photosystem I reaction center subunit IV.. 38.9 0.11 sp|P12354|PSAE_SPIOL Photosystem I reaction center subunit IV.. 36.2 0.71 gb|AAT72503.1| AT4G28750 [Arabidopsis lyrata subsp. petraea] 35.0 1.6	***GCATTGGAACAAGGTGAA***AGAAGCGGGCGTGTCGACCAACAACTACGCCCTG GACGAGACCAAGGAGGTTGCTGCTTAAGCGAACGAGGCTAAGCTGCCGTGC GCTCCATCCAATGTTTGTATCAGTAGCTCGTCAAGTGACGATGTGAATGTTA GCCTCTCACAAACCTTATGTGTAATACCTCTGCGATTATATGTA***TCAAAAAAA*** ***AAGATATCCCTCAGCATAATG*** **Forward frame 2, 157 amino acids** HWNKVKEAGVSTNNYALDETKEVAA

ESTs in RGA-RT-PCR AUTORADIOGRAPH FILM-5

EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 818 RLLRfwd/T8; B1, B2, B3 **DETAILS:** RLLRfwd/T8 U_HW-070116_Plate6d10, **sequence size (bp):** 801 **BLASTX:** mb|CAO38916.1| unnamed protein product [Vitis vinifera] 382 1e-104 emb|CAN80917.1| hypothetical protein [Vitis vinifera] 382 1e-104 gb|AAK52989.1|AF375405_1 AT4g26860/F10M23_200 [Arabidopsis th.. 372 1e-101 emb|CAO69655.1| unnamed protein product [Vitis vinifera] 371 2e-101 ref|NP_567760.1| alanine racemase family protein [Arabidopsis th 369 9e-101 ref|NP_001054662.1| Os05g0150000 [Oryza sativa (japonica cult.. 358 3e-97 **CONSERVED DOMAIN:** cd00635, YBL036c_PLPDEIII, PLP dependent enzymes class III (PLPDE_III). The prototype .. 29733 No 7e-73	***CATTATGCTGAGTGATATCTTTTTTTTTGC***ATACTCTCTTGGTCCAAATATCGT TGATCCAACTCTCACATTAGTACTACCCGATTCAATCGCTAATTCAAAATCA CCAGACATGCCCATTGACAGTTCACATTGCTCCTCGTCCATTTCAAGTGCCTT GCAAACTTCAGTCCTGCAACTCGATAGGGTCTGAAAGTTCTGCGGAGTTGAG GTATAATCTAGCATTCCTACCGTCATTAAGCCAGAGAATTCTAAGTTTGGGC AGCTCAGCTTCACATGTTTTGCAAGATCAACACAATTGGAAGGATCAACACC AGATTTGGATGCTTCTCCACTGGTATTTACTTGCACCAAAACCCTCAGAGGA TTTCTTCCCAGGGTTGAAACCATACGGTCGAGATGATTTGCCACCTTCTGATT GTCCACCCCCTCAACCATGGCCAAGTTTGGAACTCCACCCAAAAGTGTTTTA ACTTTATTGCTTTGCAAATGTCCAATGAAGTGCCATTGAATGTCTTGTGGAA GCTGAGGAGCTTTATCGATGATTCCTTGGACGTAATTTTCGCCGAAGCAACG GTGACCTGCGTCATAGAGTTGGCGAATCATGGAAATAGGTTTGGTTTTGGAT ACTGCTACGACCCGTACTCGATCGGGTCTGGTACCGGATCTCTCTGCTGCTTG TTGGACACGGAGCATCACCGACCGGAGAGCTGCGACGGAGACAGTCTCGCA CAGCGAGAGTCATCAGGGACGTTGGATTATCGGACGAGCGAGACAGTGGA***G*** ***TTACTTCTAGTGGTTGCG*** **Reverse frame 1, 267 amino acids** RNH*K*LHCLARPIIQRP**LSLCETVSVAALRSVMLRVQQAAERSGTRPDRVRV VAVSKTKPISMIRQLYDAGHRCFGENYVQGIIDKAPQLPQDIQWHFIGHLQSNK VKTLLGGVPNLAMVEGVDNQKVANHLDRMVSTLGRNPLRVLVQVNTSGEASK SGVDPSNCVDLAKHVKLSCPNLEFSGLMTVGMLDYTSTPQNFQTLSSCRTEVCK ALEMDEEQCELSMGMSGDFELAIESGSTNVRVGSTIFGPREYAKKKISLSIM Query 729 ETVSVAALRS Sbjct 7 EGLAVTALRS
EST NO: 824 RLLRfwd/T9; B1, B2, B3 **DETAILS:**?/T9**,** eU_HW-070116_Plate5a08 **sequence size (bp):** > 498 **BLASTX:** gb|AAF64454.1|AF239932_1 DnaJ protein [Euphorbia esula] 35.4 3e-04 emb|CAC12824.1| putative DNAJ protein [Nicotiana tabacum] 33.1 5e-04 **Similar to 1901(or1772):** Optimal Global aligment Alignment score: 510 Identities: 0,6720143	***CAT****AA****ACGGAGTGA****ATCTTTTTTTTTGG***GGAAGGGTGGATTACTATGATTACG GCCCTCTTTCTTCTGTTTGAGCTTGTGATGTTCCTTTTGTTGTTCCGGAGGAA GTCCCCCCGCGGGGATTGTCTGGGGATTAGCGTGAGCATAAGCATCAAAATGT ACGCTCCTGTCAAGTGATGGTCCATTCTTTAGATTACTTCAATTACTTTCTTG CCTAGAGCGACCTTCTAAACCTTGCACTGCCCTCATCGATGTTTGTCTATGAG TGTGGAGCGGTGCCCTCACATCAATTGCGTGGATGCTGGATATGCTAATCAT AGTAGCACCCCGTGCGCATAGAAACCTTCATACCATTACATTGACAACCAGC AATGTCATCGAACCATCGTACTCAGAGCCTTTCCTTTTCACTTTGAGCACAAG ACCTTTCGAGAAGAGAAAGCTCCTTCGATGTTCCTAGTGTAAAGATCCTCCG TGAAACCTTTCATTGATGTCCCTC **Reverse frame 3, 165 amino acids** GTSMKGFTEDLYTRNIEGAFSSRKVLCSKCKRKGSEYDGSMTLLVVNVMV*
EST NO: 826 826 (D17-4) ; B1, B3; RLRR frwd/T7 **DETAILS:** Primers found: RLLRF/T7**,** U_HW-070116_Plate5h02 **sequence size (bp):** 458 **BLASTX:** emb|CAN78606.1| hypothetical protein [Vitis vinifera] 90.5 3e-17	***CATTATGCTGAGTGATATCTTTTTTTTTGA***TGAGACAATGAATAACCCACCGC CACAACACAAGTATTGTATTTAGCATTTTATTTACAAAATCGCAGTAGACGA TTTCTGATATTCTCCTTCATTGCTTCTCTTTCCACTCACCCAGGGTTTTACTTT TACCTTAAACCCCATGGCTTTGACGATGAGGGCTGCAGCGGCGGCGGCTCCC CGCGGTTTGCGCCGCCTTTTCTGCACTAACCCAACCTCTTCTTCTCCCTTTTTC CCTTTCACTTCAACACCGCCTTCAGGAGCTGCTCCCGCCCGACAGATGGCTG AGCCCAACACCAACCTCTTCGTCTCTGGGCTTAGCAAACGTACTACAACAGA GGGGCTGCGGGAAGAATTTCAAAAGTTTGGTGAAGTTGTTCATGCAAGGGT

ref|NP_200269.1| RNA recognition motif (RRM)-containing prote.. 90.5 3e-17 **CONSERVED DOMAIN:** smart00360, RRM, RNA recognition motif; . 47687 No 3e-05	GGTAACTGATCGAGTCTCAG***GTTACTCTAGTGGTTGCG*** **Forward frame 2, 152 amino acids** IMLSDIFFFDETMNNPPPQHKYCI*HFIYKIAVDDF*YSPSLLLFPLTQGFTFTLNP MALTMRAAAAAAPRGLRRLFCTNPTSSSPFFPFTSTPPSGAAPARQMAEPNTNLF VSGLSKRTTTEGLREEFQKFGEVVHARVVTDRVSGYSSGC
EST NO: 843 RLRRfwd/T8; **B1**, B3 **DETAILS:** 843 (RLRRfwd/T8; **B1**, B3) U_HW-070116_Plate6d07, **sequence size (bp):** 190 **BLASTX:** No significant similarity found **BlastN:** AY428546.1 Lycopersicon esculentum clone S21_200 RGA marker sequence 37.4 2.5	***CGCAACCACTAGAGTAAC***ATGCAGAACAATAGCATTGGTATTACTGTCGTGA GAGTAGCACATGGTCAGTAAATGTGCTATCAAATTTGTGCAGTTCCAATGGG TTGAAGCAATATTGGTGGTGTGAGATTTAAATGTAACTGCAAAAACACAATT CATG***GGCAAAAAAAAAGATATCACTCAGCATAATG*** **Reverse frame 2, 63 amino acids** IMLSDIFFFAMNCVFAVTFKSHTTNIASTHWNCTNLIAHLLTMCYSHDSNTNAIV LHVTLVVA
EST NO: 845 845-2a, RLLRfwd/T7; B1, B3 **DETAILS:** RLLRfwd/T7 U_HW-070501-Plate5h07.b1 **sequence size (bp):** 205 **BLASTX:** No significant similarity found **BlastN:** AM427426.2 Vitis vinifera contig VV78X251827.4, whole genome shotgun sequence 37.4 2.7	***CGCAACCACTAGAGTAAC***AGGTTATTTAAATAGTGCTAGTAGTATATTTTGTG AGTTGAAGGAGACCAAAATAGAGGAGAGAATTAAGAACCAGCTGCAGCA GGAACAAAATCTTATAAGACATGGTCGTACGGTTCATCCTAGTAAACCCTAT CCAAGTATCCATCAGTTAAG***TCAAAAAAAAAGATATCACTCAGCATAATG***
EST NO: 853 RLLRfwd/T5; B1, B2, B3 **DETAILS:** RLLRfwd/T5 U_HW-070501-Plate6e07.b1, **sequence size (bp):** 328 **BLASTX:** ref|NP_180560.1| NDA2 (ALTERNATIVE NAD(P)H DEHYDROGENASE 2); .. 147 2e-34 emb|CAB52796.1| putative internal rotenone-insensitive NADH d.. 147 3e-34 gb|AAM61225.1| putative NADH dehydrogenase (ubiquinone oxidor.. 146 3e-34 ref|NP_563783.1| NDA1 (ALTERNATIVE NAD(P)H DEHYDROGENASE 1); .. 146 3e-34 **CONSERVED DOMAIN:** COG1252, Ndh, NADH dehydrogenase, FAD-containing subunit [Energy production and conver.. 31444 Yes 2e-12	***CATTATGCTGAGTGATATCTTTTTTTTTCC***TTGCTAATTGTACCAGTATCAATG CTCATAAACATGAGGTGCAATGTGAGACTGTAACTGAAGGAACGCAAACAA TAGATCCTTGGAAGTTTACAATCTCCTATGACAAACTAGTAATTGCATTAGG AGCACAACCTACCACTTTTGGAATTCATGGAGTTCATGAACATGCATTTTTTC TTCGTGAAGTTTACCATGCACAGGAAATTCGTCGCAAGTTGCTCCTGAATTT GATGATGTCTGATGTTCCAGGGATTACGGAAAAGGAAAAGAAAAGGCT***GTT ACTCTAGTGGTTGCG*** **Forward frame 3, 108 amino acids** LC*VISFFFLANCTSINAHKHEVQCETVTEGTQTIDPWKFTISYDKL*VIA*LGAQPTT FGIHGVHEHAFFLREVYHAQEIRRKLLLNLMMSDVPGITEKEKKRLLL*
EST NO: 860 RLLRfwd/T4; B1, B2, B3 **DETAILS:** RLLRfwd/T4 U_HW-070116_Plate6d05 **sequence size (bp):** 564 **BLASTX:** emb|CAA08906.1| cysteine proteinase[Cicer arietinum] 188 1e-46 gb|AAO11786.1| pre-pro cysteine proteinase[Vicia faba] 169 1e-40 gb|AAB67878.1| pre-pro-cysteine proteinase[Vicia faba] 169 1e-40 sp|P25804|CYSP_PEA Cysteine proteinase 15A precursor 167 3e-40 emb|CAA82995.1| cysteine proteinase [Vicia sativa] 166 7e-40 emb|CAB53397.1| cysteine protease [Medicago sativa] 165 1e-39 ref|NP_567489.1| cysteine proteinase, putative [Arabidopsis t.. 145 1e-33 **CONSERVED DOMAIN:** cd02248, Peptidase_C1A, Peptidase C1A subfamily (MEROPS database nomenclature); compos.. 30292 No 2e-20	***CGCAACCACTAGAGTAAC***AAGCATACATAATTACCAACTACATAAATAACAT GATTGTGGTAGTTTTCTGATCCTATATGGAGATAGTTTTGCCAGAACCAGAA GCTAAATTCACAACTTCATGATTATATATTTACACAAATTGAACTAGAGTCG TGTTACATAGGATGAGACCACCAGAACCAGCATCTCCGTATTATTAATTGTT GGATGCATGAACTGCAGCTGCAGTTGAGACCATTGAATCCACTCCACATATA TTTCGACCTCTGCAGATCTTGTAATATCCTTCCTCACCCCAACTCTGTCCCCA TGAATTCTTTATGATCCAGTAAGGCTTTTCCTTCAATCGGATGGGAGCAAAA CCATTTCCAAAACCAACTAGAAGAACCCCATGATCCAAACGCGATTTTGCAC AGATGTATGGGCATGAGACACCACTCATATATGTTTGCATCCAGGCTGCGTT AATAGCAACTGCAAGAAGCAAAGCTTAAGTCAGTATTTGTAAAATTTGCCAT TCATTCTGTATCT***TGAAAAAAAAAGATATCACTCAGCATAATG*** **Reverse frame 3, 187 amino acids** LC*VISFFFKIQNEWQILQILT*ALLLAVAINAAWMQTYMSGVSCPYICAKSRLD HGVLLVGFGNGFAPIRLKEKPYWIIKNSWGQSWGEEGYYKICRGRNICGVDSM VSTAAAVHASNN**
EST NO: 863 (842+863) (RLRRfwd/T8; B1, B3) + (NLLRfwd/T2; B1, B3)] **DETAILS:** ?/T5 U_HW-Plate3b02.b1 Similar to U_HW-Plate3a02.b1, **sequence size (bp):** >520 **BLASTX:** emb|CAA57721.1| protein kinase [Medicago sativa] 68.6 1e-15 gb|ABF82263.1| MAP kinase [Cicer arietinum] 48.9 2e-15 **CONSERVED DOMAIN:** cd00180, S_TKc, Serine/Threonine protein kinases, catalytic domain. Phosphotransferase.. 29142 Yes 4e-07 **Similar to 2:** Optimal Global aligment Alignment score: 620 Identities: 0,7242583	CCTTAGGCAGACTGCGTCTTTTTTTTTTCCATGAATTGTGTTTTTTCCGAGGAT TTAAATCCAACTCCGGAGCCCTTGCTCCTCTTGGGACTACCTGTTCTGTTATG AAAGCACTTTTACTGATCGTGTGTGAAACACCAAACACAAAAACCTTCGCTA TTGTTTTTCATGTCAACAAAGGGTTGGTTGATTACAAGTCTCTTCGGATTATG TCCAAATAATGTTTATACCTCAGCCCTCAAAGAATCTTATACAAATAGTTTG GGTATTGTTCATCTGACATATTTGGATTGGACCAATCGAGGGGATAACTGTT TCCTGTGTGAGTTCTGTGCCGCTGTCAACTCCACTAAACTCTCGACCCAGGG GTGAAGGATGAACATCCCGGGATGCATTTAGAGAGAGCTAATCTAAATGAC TTGCGTTGCACTCTCTGCCCGCTTACGCTTAGCATCCTTGTGATTGTCAAATG CATTAGTGAATCTTCTTAACACGCGCGGAGAG***GTT***GTTTGCTTAGTGC… **Reverse frame 3, 270 amino acids** KRGNEARLYLCRVGLSSLHPGRGTL*FWLDAGGN*ETTATSPLYEL*RE*PIDLR VFLEYPPIHSKT*ELSSIVDMPVAAAFTTERT*SVWNEALDTALSKQPLRAC*EDS LMHLTITRMLSVSGQRVQRKSFRLALSKCIPGCSSFTPGSRV*WS*QRHRTHTGN SYPLDWSNPNMSDEQYPNYLYKIL*GLRYKHYLDIIRRDL*STNPLLT*KTIAKVF VFGVSHTISKSAFITEQVVPRGARAPELDLNPRKKHNSWKKKRRSLPK
EST NO: 879 RLLRfwd/T2; B1, B2, B3 **DETAILS:** RLLRfwd/? U_HW-070501-Plate5h09.b, **sequence size (bp):** > 419 **BLASTX:** ref|XP_421891.2| PREDICTED: similar to KIAA0572 protein [Gallus 35.8 0.86 ref|XP_001613744.1| hypothetical protein, conserved [Plasmodi.. 33.9 3.3 **first part similar to 892:** Optimal Global aligment Alignment score: 226 Identities: 0,5111773	***CGCAACCACTAAAGTACC***TACAAATTTCAAAGCCACCACCATTCAAAATAAC AAAAGGGTCTATTCTCTTCAATTGAAAACACCATCGTGGTTTCACCTTCGATT GCAATCTCGGGAGCATTATTTTCCCCACTTGCAACCTGGGATTCATCAACCG CACCTTAAAAGATAACCGATCTTGCGGCTTTCATCAAATAAAATAAGGTTGG ATGCCGCAATAATATTTTCCATGCTGGGGGGGGGGAGTGAGGCTAAATCCCT TTCCCCCCCCCCAATGTGCCTGATGCATTTTGTAGGGGGGTATAATCTAGTA AGGGAGTCGAGGGCCCCCAGCACGAAGAAATATGGGAAAAGCTTCCAACCC CCTGAAAGGAAAACTGATGATAATTCGAAAAAACCCCCGCAATCTGGATAG GGCC **Reverse frame 2, 139 amino acids** ALSRLRGFFRIIISFPFRGLEAFPIFLRAGGPRLPY*IIPPYKMHQAHWGGGKGFSL TPPPQHGKYYCGIQPYFI**
EST NO: 882 RLLRfwd/T2; B1, B3 **DETAILS:** U_HW-070116_Plate6d04, **sequence size (bp):** 373 **BLASTX:** gb|ABE11607.1| COV1-like protein [Solanum chacoense] 207 2e-52	***CATTATGCTGAGTGATATCTTTTTTTTTAC***ATGGTGGTTTGTTCAGTTCGTTGA TGGTTTCTTTAGTCCACTATATTCCAGTTTTGGCATTGAAATATTTGGGCTGG GTTTTATTACATCGTTGGCTTTTGTATTTGTTATTGGTGTTTTTGTTTCGTCAT GGATGGGTGCCACTGTCTTCTGGATTGGAGAATGGCTAATAAAGCAAATGCC CCTTGTTAGACATATATGCTCTGCATCCAAGCAGATTAGTGCCGCAATTTCTC CAGATCAAAATACCACTGCCTTTAAAGAGGTAGCAATTATCTGTCATCCCCG TGTTGGTGAATATGCTTTTGGCATCATTACATCAACT***GTTACTCTAGTGGTTG CG*** **Forward frame 2, 124 amino acids** IMLSDIFFFTWWFVQFVDGFFSPLYSSFGIEIFGLGFITSLAFVFVIGVFVSSWMGA

	TVFWIGEWLIKQMPLVRHICSASKQISAAISPDQNTTAFKEVAIICHPRVGEYAFG IITSTVTLVVA
EST NO: 892 892-2a, RLLRfwd/T1; B1, B2, B3 **DETAILS:** RLLRfwd/T1 U_HW-070501-Plate5h10.b1 **sequence size (bp):** 669 **BLASTX:** gb|AAM65279.1| unknown [Arabidopsis thaliana] 130 8e-29 ref|NP_176940.1| PSBY (photosystem II BY) [Arabidopsis thalia.. 130 8e-29 **BlastN:** AC123572.15 Medicago truncatula clone mth2-2b2, complete sequence 600 4e-169 ……. NM_105442.2 Arabidopsis thaliana PSBY (photosystem II BY) (PSBY) mRNA, complete cds 109 2e-21	***CGCAACCACTAGAGTAAC***CACAAATTTCAAAGCCACCACCATTCAAAATCTA CCAAAGGGTCTATTCTCTTCAATTGAAAACACCAACGTGGTTTCACCTTCCAT TGCAATCGCGGGCGCACTATTCTCGTCCCTTGCAACCTGTGACGCAGCTTTC GCAGCTCAACAAATAGCTGAAATTGCGGAAGGTGATAACAGCGGATTATCT TTGTTGTTGCCATTGGTTCCAGCCATAGGATGGGTTTTATTCAACATTCTCCA GCCAACTTTGAACCAAATCAACCGTATGCGTAACACAAGAGGGGTGATTATT GGGCTTGGGCTTGGACTTGGTGGGCTTGCAGCTTCGGGTATGATGTCAGCAT CTGCTAGTGAAATGGGTCTGATTGCTGATGCTGCTGCAGCTGGAAGTGACAA CAGGGGACAGCTTCTGTTGTTTGTGGTTTCACCATCTATTCTTTGGGTGCTTT ACAACATTCTGCAGCCAGCTCTCAACCAGATCAATAGGATGAGATCTGATTA AACTATGGGATTTTTGTGAAATGCTTTCTTGTAAAACTTGCTTGTAACCTGTA TCTAATGCTCTCCCATTCACTATATTATGTGTTGTATATCAAAAACATGTCTT TCTACTTACCC***TTAAAAAAAAAAGATATCACTCAGCATAATG*** **Forward frame 2, 222 amino acids** ATTRVTTNFKATTIQNLPKGLFSSIENTNVVSPSIAIAGALFSSLATCDAAFAAQQI AEIAEGDNSGLSLLLPLVPAIGWVLFNILQPTLNQINRMRNTRGVIIGLGLGLGGL AASGMMSASASEMGLIADAAAAGSDNRGQLLLFVVSPSILWVLYNILQPALNQI NRMRSD*
EST NO: 896 RLLRfwd/T1; B1, B3 **DETAILS:** RLLRfwd/T1 U_HW-070501-Plate6e10.b1, **sequence size (bp):** 219 **BLASTX:** No significant similarity found **BlastN:** AC126786.22 Medicago truncatula clone mth2-8c2, complete sequence 75.2 1e-11	***CATTATGCTGAGTGATATCTTTTTTTTTAA***TAGGCAAATGTTAGTATCTTAGTT GCTGTTATGTTAGTATTTTCTTTCTTCACAGGGTTTGAACCGGTCTTTTAACTC GTCAAACTCTTTTAAATCTTAGCTCAAACCAGTTGAAGCAACCACCCCCTCC TTTAGTGGCTCATATGTATGTAAATTAAGTGTTGTCAAATA***GTTACTCTAGTG GTTGCA*A**
EST NO: 928 NLLRfwd/T2; B1, B2, B3 **DETAILS:** ?/T2 U_HW-070501-Plate6e12.b1, **sequence size (bp):** 219 **BLASTX:** No significant similarity found **BlastN:** AC126786.22 Medicago truncatula clone mth2-8c2, complete sequence 75.2 1e-11	***CATTATGCTGAGTGATATCTTTTTTTTTAA***TAGGCAAATGTTAGTATCTTAGTT GCTGTTATGTTAGTATTTTCTTTCTTCACAGGGTTTGAACCGGTCTTTTAACTC GTCAAACTCTTTTAAATCTTAGCTCAAACCAGTTGAAGCAACCACCCCCTCC TTTAGTGGCTCATATGTATGTAAATTAAGTGTTGTCAAATAGTTACTCTAGGG GTTGCG
EST NO: 937 NLLRfwd/T2; B2, B3 **DETAILS:** NLLRfwd/T2 U_HW-070116_Plate6c11, **sequence size (bp):** 224 **BLASTX:** mb|CAN77440.1| hypothetical protein [Vitis vinifera] >emb|CA.. 53.9 3e-06 gb|AAN65067.1| Similar to CGI-126 protein [Arabidopsis thaliana] 53.9 3e-06 ref|NP_564289.1| unknown protein [Arabidopsis thaliana] >gb|A.. 53.9 3e-06 **CONSERVED DOMAIN:** pfam08694, UFC1, Ubiquitin-fold modifier-conjugating enzyme 1. Ubiquitin-like (UBL) po.. 87601 No 1e-04	***CATTATGCTGAGTGATATCTTTTTTTTTAC***ACAATGAAACAGCTCATTTTATTT CCCTTTGACAAGTTTGAAAACTATTAAGCCTGAGTTCTACTTTTACAGGTTTA AACAGACATGTGATAACTAAGATTCAGTCGATGTGGTTGCATCGTCTTTGTG CTTGATCATACCAGAATCCACAAGAATGGGAACCTCTGCTGCAAGCC***ACGAT GCAAGAGGCCCTA*** **Reverse frame 3, 73 amino acids** GPLASWLAAEVPILVDSGMIKHKDDATTSTES*
ESTs in RGA-RT-PCR AUTORADIOGRAPH FILM-6	
EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 997 RLLRfwd/AS7; B1, B3 **DETAILS:** RLLRfwd/AS7 U_HW-070116_Plate6c08, **sequence size (bp):** 376 **BLASTX:** gb|ABD32881.1| Nascent polypeptide-associated complex NAC; UB.. 219 4e-56 **CONSERVED DOMAIN:** pfam01849, NAC, NAC domain. 65629 No 1e-11 **BlastN:** AC149050.2 Medicago truncatula chromosome 7 BAC clone mth2-6j14, complete sequence 338 9e-90 NM_112074.3 Arabidopsis thaliana nascent polypeptide associated complex alpha chain protein, putative / alpha-NAC, putative (AT3G12390) mRNA, complete cds 223 5e-55	***CGCAACCACTAGAGTAAC***CACCAACTCAATATCCTTTGGATCTACACCAGTCT CGTCTACATCCTCATCTTCTGGAGCAACACCGGAGGATTCTGGTTTCGCTCCA ACGTTGCTCAAATTAGGGGCCTTGAACTGCTCTGCTGCCTGAGTTTGTAGCT GTGAGCCCAAGTCTTCAATCTTAGCTTCCCCGAATATAATGTAGGTGTCGGC AGTTGGGCTCTTGAAAACGTCAGGTTTGGAGATGACAAACAAGATATTCTTG CTCTTCTTGACTGTCACACGACTGACACCAGTAACTGGTTTCATTCCAAGTTT CAGCATTGCCTTACGACTCTTCTTTTCGCTTCGGGTCTGTTTG***GGCCTCCCCTT CGCCTTC*** **Reverse frame 2, 125 amino acids** KAKGRPKQTRSEKKSRKAMLKLGMKPVTGVSRVTVKKSKNILFVISKPDVFKSP TADTYIIFGEAKIEDLGSQLQTQAAEQFKAPNLSNVGAKPESSGVAPEDEDVDET GVDPKDIELVVTLVVA
EST NO: 955 RLLRfwd/AS8; B1, B2, B3 **DETAILS:** RLLRfwd/AS8 U_HW-070116_Plate6c10, **sequence size (bp):** 353 **BLASTX:** emb|CAD27943.1| PsbY-like protein precursor [Oryza sativa] 72.8 7e-12 sp|P80470|PSBY_SPIOL Photosystem II core complex proteins psb.. 72.4 9e-12 ref|NP_001060874.1| Os08g0119800 [Oryza sativa (japonica cult.. 71.6 1e-11 gb|AAM65279.1| unknown [Arabidopsis thaliana] 71.6 1e-11 dbj|BAD09053.1| putative photosystem II core complex proteins.. 71.6 1e-11 ref|NP_176940.1| PSBY (photosystem II BY) [Arabidopsis thalia.. 71.6 1e-11	***GAAGGCGAAGGGGAAGCC***CACCAAGTCCAAGCCCAAGCCCAATAATCACCC CTCTTGTGCTACGCATACGGTTGATTTGGTTCAAAGTTGGCTGGAGAATGTT GAATAAAACCCATCCTATGGCTGGAACCAATGGCAACAACAAAGCTAATCC TCTGTTATCACCTTCCGCAATTTCAGCTATTTGTTGAGCTGCGAAAGCTGCGT CACAGGTTGCAAGGGACGAGAATAGTGCGCCCGCGATTGCAATGGAAGGTG AAACCACGTTGGTGTTCTCAATTGAAGAGAATAGACCCTTTGGTAGATTCTG AATGGTGGTGGCTTTGAAATTTGTG***GTTACTCTAGTGGTTGCG*** **Reverse frame 2, 117 amino acids** ATTRVTTNFKATTIQNLPKGLFSSIENTNVVSPSIAIAGALFSSLATCDAAFAAQQI AEIAEGDNRGLALLLPLVPAIGWVLFNILQPTLNQINRMRSTRGVIIGLGLGLGGL PLRL
ESTs in RGA-RT-PCR AUTORADIOGRAPH FILM-7	
EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 1034	***TACTTGTGCTTGATGATGTGG***GTGGTGCAGTATTTGTTGGAGGTGGTGCTGCT

CicerKin/T3; B1, B3 **DETAILS:** U_HW-070501-Plate5d06.b1, **sequence size (bp):** 320 **BLASTX:** No significant similarity found **BlastN:** AY538265.1 Triticum aestivum cultivar H4564 cytosolic malate dehydrogenase mRNA, complete cds 42.8 0.098	GCTGCAGCTGCCCCTGCAGGAGGTGCCGCCCCTGCTGCCGATGCCGCCCCTG CCGCCGCAAAAAAAAAAAAAAAGGTTGAGGAAAAGGAGGAGGATGATGAT ATGGGATTCTCACTCTTCGATTAGAGTTTTCTAGTTTTTGTTCAATTTTGATTT AGGGGTTTTAGTCTTCAATCCTATGAATTTTGTGTATTGGCTTGCGCTTATTA ACGTACAATCTACCAAATAGAGTTTATG***CTAAAAAAAAAGATATCACTCAGCA TAATG***
EST NO: 1072 1072-1-1, S2/AS5; B1, B2, B3 **DETAILS:** S2?/AS5? U_HW-Plate1f01.b1 **sequence size (bp):** 504 **BLASTX:** gb|ABB85180.1| NBS-LRR type disease resistance protein [Vicia fa 269 4e-72 gb|ABB85195.1| NBS-LRR type disease resistance protein [Cicer.. 266 2e-71 emb|CAC86496.1| RGA-G protein [Cicer arietinum] 266 2e-71 **CONSERVED DOMAIN:** pfam00931, NB-ARC, NB-ARC domain. 85132 Yes 1e-06	***GAAGGTGAGGGGGAGG***AAGCGCTTACCTTAGTATGTCAAACGCAACCTTATC ATAAAAACGCAAAAATGATCAATTTTGAAAGCTTTTCAACAAAAGAATTGA AGCGAGTCAGACTTATTCAAGAGTGGAACTTTGTACACAACATCCACTCCAT ACTCTTTCAAGATATGCTCATCTCTAGAAATTATAACGACTCTACTCCCTGCA CCTAACCATTCACGCCTCACATCTAATTTCTCGAGTTGTTCAATTTGATCAAC ATTGTCAATAATTATAATGGCCTTTTCACGACATAGCCTATTTTGTATCAAGT TAGATGCGTTGAGAAGATTGCATGTTGGAAGGTATTCCTCGCCTAAATTTTG AAGTAGAATTTGCTTTTGTGCGCCGATTGGACCATCATGCCTATAAATTTTGC TCAGATCAGCAACACGGCATTACGCAGGAAATTGGTGAGAGATTTTGTTGTA TAAAAACAGAGGAAGG***GTCGTCTTCCCCCCCC*** **Reverse frame 2, 181 amino acids** *TSRRGRIKKKKKRGGKTTLPLFLYNKISHQFPA*CRVADLSKIYRHDGPIGAQK QILLQNLGEEYLPTCNLLNASNLIQNRLCREKAIIIIDNVDQIEQLEKLDVRREWL GAGSRVVIISRDEHILKEYGVDVVYKVPLLNKSDSLQFFC*KAFKIDHFCVFMIR LRLTY* Query 501 GGKTTL Sbjct 3 GGKTTL
EST NO: 1082 1082-1a, S2xAS5; B2, B3 **DETAILS:** S2/AS5 U_HW-070501-Plate5d07.b1 **sequence size (bp):** 303 **BLASTX:** ref|YP_001185853.1| binding-protein-dependent transport syste.. 131 1e-29 ref|NP_795033.1| putrescine ABC transporter, permease protein.. 101 1e-20 **CONSERVED DOMAIN** PRK10683, PRK10683, putrescine transporter subunit: membrane component of ABC superfamily. 78001 No 2e-11 **Similar to 1089:** Optimal Global aligment Alignment score: 594 Identities: 0,9867987	***GGGGGGGTGGGGAAGACGAC***AGCGGCGTGGTACTGCAATCATGAAACCGAG CAAACTGGCGCGCTATCTGCCCACTGGGCGGCATGCCGTTATCGGCATACCC TTCCTCTGGCTGTTCATGTTCTTCCTGCTGCCCTTCACCATCGTGCTGAAGAT CAGCTTCGCCGAAGCCGACGTGGCGATTCCGCCCTATACGGAAATCTACCAG TGGGCGGACAACAAGCTGACGCTGCTGTTGAATTTCGGCAACTACATCTTCC TCAGCGAAGATGCCCTGTACCTGTC***GGCCTCCCCCTCACCTTC*** **Forward frame 2, 100 amino acids** GGWGRRQRRGTAIMKPSKLARYLPTGRHAVIGIPFLWLFMFFLLPFTIVLKISFA EADVAIPPYTEIYQWADNKLTLLLNFGNYIFLSEDALYLSASPSP
EST NO: 1089 S2/AS5; B2, B3 **DETAILS: S2/AS3** U_HW-070116_Plate6h10, **sequence size (bp):** 303 **BLASTX:** ref|YP_001185853.1| binding-protein-dependent transport syste.. 131 1e-29 ref|NP_795033.1| putrescine ABC transporter.... 102 1e-20 **CONSERVED DOMAIN:** PRK10683, PRK10683, putrescine transporter subunit: membrane component of ABC superfamily. 83405 No 8e-12 **BlastN:** CP000680.1 Pseudomonas mendocina ymp …. 435 4e-119	***GAGGGTGAAGGGGAGGCC***GACAGGTACAGGGCATCTTCGCTGAGGAAGATG TAGTTGCCGAAATTCAACAGCAGCGTCAGCTTGTTGTCCGCCCACTGGTAGA TTTCCGTATAGGGCGGAATCGCCACGTCAGCTTCGGCGAAGCTGATCTTCAG CACGATGGTGAGGGGCAGCAGGAAGAACATGAACAGCCAGAGGAAGGGTA TGCCGATAACGGCATGCCGCCCAGTGGGCAGATAGCGCGCCAGTTTGCTCGG TTTCATGATTGCAGTACCACGCCGCTGTC***GTCTTCCCCACCCCCCC*** **Reverse frame 2, 100 amino acids** GGWGRRQRRGTAIMKPSKLARYLPTGRHAVIGIPFLWLFMFFLLPLTIVLKISFA EADVAIPPYTEIYQWADNKLTLLLNFGNYIFLSEDALYLSASPSP Query 263 MKPSK Sbjct 1 MKPSK
ESTs in RGA-RT-PCR AUTORADIOGRAPH FILM-8	
EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 1107 S2/AS1, B2, B3 **DETAILS:** Primers found S2/? U_HW-070116_Plate6h09, **sequence size (bp):** >319 **BLASTX:** ref|XP_001203258.1| PREDICTED: similar to toll-like receptor .. 34.3 2.7	***GGGGGGGTGGGGAAGAACAC***GTGTTAGGGTCTTGTTTCTTGAGAATATTTTT GATCAGATATTCAACCTCCCCAACTATGCGTCATCTGACGTGGGAATACCTT TCAGGCAGAAGAATCTGCCGTACCTTCGGCCTGATGTTCCACCCACAGAAGA ATACAATGTACCCACTGAATTCAAAGTTGATCGCAAACATAACCTTTTCTGG CTATCCCTGCACATGCCAAGATGCTCCTATAGTGATGCCTATACTAAGTTTTT TACTTCCCTGATCACTACTGCGCTTTACGATTGATCTACTTTTATTTGCCCGA GTAAT **Forward frame 3, 105 amino acids** GGGEEHVLGSCFLRIFLIRYSTSPTMRHLTWEYLSGRRICRTFGLMFHPQKNTMY PLNSKLIANITFSGYPCTCQDAPIVMPILSFLLP*
EST NO: 1130 1130-2-1, NLRRfwd/AS6; B1, B2, B3 **DETAILS:** NLRRfwd/? U_HW-Plate1g06.b1 **sequence size (bp):** > 220 **BLASTX:** emb|CAO17976.1| unnamed protein product [Vitis vinifera] 40.0 3e-07 gb|AAW38936.1| 3-phosphoinositide-dependent protein kinase-1 .. 40.0 3e-07 ref|NP_568138.1| PDK1 (3-PHOSPHOINOSITIDE-DEPENDENT PROTEIN K.. 40.0 3e-07	***TAGGGCCTCTTGCT***AGAAGGAGGGAGATCTGGAATCGAATTTCTCTTGCTAT AAAAAAAGAAAAGGAAACCATTTCGGGCATCTTTAAAGGGGGAAGTTCCAA AAAGCATTTGGTATAATGTGCAGCTCTCTTGCCCAGAGGTCATTTCCAAAAG TTGCTGGAGAGGAATTAAAACCCTCTGGAGAAAATAGTTCCTTCCCCACAAA CCTGGAGGCTTTG **Reverse frame 2, 73 amino acids** KASRFVGKELFSPEGFNSSPATFGNDLWARELHIIPNAFWNFPL*RCPKWFPFLFL *
EST NO: 1151 1151-1-2, CicerKinF/NBS; B1, B2, B3 **DETAILS:** CicerKin/NBS/? U_HW-Plate4a10.b1 **sequence size (bp):** > 303 **BLASTX:** No significant similarity found **BlastN:** EU024510.1 Corchorus olitorius acidic ribosomal protein P3 mRNA, complete cds 64.4 3e-08	***TACTTGTGCTTGATG****AA****GA***GGGGCGTGCAGTATTTGTGGAGGTGGTGCTGCTG AGAAACGGCACCTGCAGGAGGTGCCGCCGCTGCTGCCGATGCCGCCCCTGC CGCCGCAAAGAAAGAAGAAAAGGTTTTCTTAAGAGGAGGAGGATGATGATA TGGGATTCTCACTCTTCGATTAGAGTTTTCTAGTTTTTGTTCAATTTTGATTTA GGGGTTTTATTCTTCAATCCTATGAATTTTGTGTATTGGCTTGCGCTTATTAA CTTACATTCTACTTAATAGTGTTTTGCATAAAAAAAAGATT
EST NO: 1158 RLLRfwd/NBS; B1, B2, B3 **DETAILS:** RLLRfwd/NBS U_HW-070116_Plate6h06 **sequence size (bp):** 382 **BLASTX:** emb|CAA08906.1| cysteine proteinase [Cicer arietinum] 121 2e-26 **CONSERVED DOMAIN:**	***TCTAGTTGTGATGATTACTTTGC***CCCCATCCGATTGAAGGAAAAGCCTTACTG GATCATAAAGAATTCATGGGGACAGAATTGGGGTGAGGAAGGATATTACAA GATCTGCAGAGGTCGAAATATATGTGGAGTGGATTCAATGGTCTCAACTGTA GCTGCAGTTCATGCATCCAACAATTAATAATACGGAGATGCTGGTTCTGGTG GTCTCATCCTATGTGACGCGACTCTAGATCAATTTGTGTAAATATATAATCAT GAAGTTGTGAATTTAGCTTCTGGTTCTGGCAAAACTATCTCCATATAGGATC AGAAAACTACCACAATCATGTTATTTATGTAGTTGGTAATTATGTATGCTT***GT TACTCTAGTGGTTGCG***

cd02248, Peptidase_C1A, Peptidase C1A subfamily (MEROPS database nomenclature); compos.. 30292 No 3e-11 **BlastN:** AJ009878.1 Cicer arietinum mRNA for cysteine proteinase 628 5e-178 **similar to 860:** Optimal Global aligment Alignment score: 550 Identities: 0.6666667	**Forward frame 1, 127 amino acids** SSCDDYFAPIRLKEKPYWIIKNSWGQNWGEEGYYKICRGRNICGVDSMVSTVAA VHASNN**
EST NO: 1209 CicerkinF/T8; B1, B2, B3 **DETAILS:** CicerkinF/T8 U_HW-070116_Plate6h05 **sequence size (bp):** 298 **BLASTX:** gb|EAZ41154.1| hypothetical protein OsJ_024637 [Oryza sativa .. 39.3 0.081 gb|EAZ05205.1| hypothetical protein OsI_026437 [Oryza sativa .. 39.3 0.081 ref|NP_001060699.1| Os07g0688100 [Oryza sativa (japonica cult.. 39.3 0.081 **BlastN:** AC149547.26 Medicago truncatula chromosome 8 clone mth2-71b14, complete sequence 246 5e-63	***CATTATGCTGAGTGATATCTTTTTTTTTGC***AGACAAGATTTTTCACTAAATGAT AAAATAAAATATTGAAGCTCATGTTTGGCTTCGATGCTCTATTTCCATGATAC TTTACTCAACACAAGCCACATACCCCAAGTTACTTGTTACAATGTATTAATTC ACAATCTTTGTTAAATTGGATATACGCGCTGGTGGGTCACCAGAGCTCAATG TCTTGCTAAGGATACAAGTACAACACTACATACAGAGGGTAACAACAGATT ATTTAACCAGACAG***CCACATCATCAAGCACAAACA*** **Forward frame 2, 99 amino acids** HYAE**YLFFCRQDFSLNDKIKY**SSCLASMLYFHDTLLNTSHIPQVTCYNVLIHN LC*IGYTRWWVTRAQCLAKDTSTTLHTEGNNRLFNQTATSSSTN
ESTs in RGA-RT-PCR AUTORADIOGRAPH FILM-10	
EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 1422a PtoFenS/T4; B1, B2, B3 **DETAILS:** PtoFenS/PtoFenS U_HW-070116_Plate5d11, **sequence size (bp):** 501 **BLASTX:** gb|AAK64167.1| putative methionine synthase [Arabidopsis thalian 262 5e-69 ref|NP_187028.1| AtMS2 (Arabidopsis thaliana methionine synth.. 262 5e-69 **CONSERVED DOMAIN:** cd03311, CIMS_C_terminal_like, CIMS - Cobalamine-independent methonine synthase, or Me.. 48146 No 1e-43	***ATGGGAAGCAAGTATTCAAGGC***AGCCTCATCAATTTGGATAACACCAATACC ACCCTTCTCAAGGTCTTCTACTTCATCCTTGATAGCCAAAGCAATTTGATAGC AGGTTTCGGATCTAGGCTGGTCAACTCTAACGAAGGACCAGTTGAGAATGGT GACAGGGCCAGTAAGCAATCCCTTCATTGGACGCTTGGTAAAGCTCTGAGCA AGAGATGACCAGAAGACAGTCGTTGGCTTTGGGCGGCTCACATCACCGTAG ATGATTGGGGGCTTGGCGCAACGAGATCCATAGGATTGCACCCACCCATTAG CAGTAAAGGCAAATCCTGACAACTGCTCGCCAAAGTACTCAACCATGTCGTT CCTCTCGGGCTCTCCGAGTACTAGGACATCAATATCAAGCTCTTCCTGAAGC TCAACAACTTTGCGGATTTCCTCCTTAATTGCCTTAATGTACTCTTCCTCAGA GATCTTCTTA***GCCTTGAATACTTGCTTCCCAT*** **Reverse frame 1, 167 amino acids** YGKQVFKAKKISEEEYIKAIKEEIRKVVELQEELDIDVLVLGEPERNDMVEYFGE QLSGFAFTANGWVQSYGSRCAKPPIIYGDVSRPKPTTVFWSSLAQSFTKRPMKG LLTGPVTILNWSFVRVDQPRSETCYQIALAIKDEVEDLEKGGIGVIQIDEAALNTC FP
EST NO: 1450 PtoFenS/T2; B1, B3 **DETAILS:** PtoFenS/T2 U_HW-070116_Plate5d10, **sequence size (bp):** 334 **BLASTX:** ref|XP_951920.1| hypothetical protein TA15165 [Theileria annu.. 38.5 0.13 ref|XP_001547527.1| hypothetical protein BC1G_13975 [Botryoti.. 34.7 1.9 ref|XP_975514.1| PREDICTED: similar to CG8930-PA, isoform A [.. 34.7 1.9	***CATTATGCTGAGTGATATCTTTTTTTTTAC***GCTGATGAGAGTTCTCGATAGAG CTTGTACATTCATTGGCAATTTTCTGGCATTCTTCTTTATTTTTCGAAGGAAG AACTAAAGAATTGTCAGCATCCATATCCTGAGTTTCCTTGACAGAACCAAAC TGTGTTATTGTACAGCAACCGTTGTTCTCTTTCTGACTAAGAGCTTTAATAGT ATCAGTATCACTGTATGGCTTGCTATCAAGACCAGTACGCATGCTCTCACTA CTTATATACGATTCTGCCACTTCGTGATTTGGGAATTGTTTTTCTGCCG***GCCT TGAATACTTGCTTCCCAT*** **Reverse frame 1, 111 amino acids** MGSKYSRPAEKQFPNHEVAESYISSESMRTGLDSKPYSDTDTIKALSQKENNGC CTITQFGSVKETQDMDADNSLVLPSKNKEECQKIANECTSSIENSHQRKKKDITQ HN
ESTs in DDRT-PCR AUTORADIOGRAPH FILM-1	
EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 1465a ↑ 1465a-2-2, **DETAILS:** ?/T?; more in H3, less in H2, H1, C3, C2, C1. U_HW-Plate3b06.b1 **sequence size (bp):** > 427 **BLASTX:** emb|CAO62021.1| unnamed protein product [Vitis vinifera] 57.4 3e-07 ref|NP_565129.1| unknown protein [Arabidopsis thaliana] >gb|A.. 57.4 3e-07 **CONSERVED DOMAIN:** pfam03138, DUF246, Plant protein family. The function of this family of plant proteins.. 66790 No 2e-08	*GGGGGGGTGGGG****AACATCTTTTTTTTTTTAA***TGAGGGAGGTTAGAACGATGG GGGAAAATTAAGACGTGATAAGTAAATTACTAGATATGCTCAGCGCTTTTCC AGGAACTTCAATTGCGCCTGCCTTTTCACCATGCGGGAGTGCTCCCAACACC TAGGAGGTATTTGTTGATTGCACCTAGGGGATTCTAAAAACCGGGAGAACTG GGACCCCAGATGTTGTTGTTGTGGAAAAAATTTCGACTCCCACTCTGGCTGT ACCGAACTGCCACCAACGCTCTAACTGAAAAGCTTGGGGTGCCTTCTCACAG ATCTTTGACGTTGGTTGGTTCTCCTGCTATCTGCTCAATGATGTCCAATTAAT CAACCCCCTCCCTCTTCACAAAGGAATAAATCCCAGAAGACAAACAGCCATT TATTGGGCGT **Forward frame 1, 142 amino acids** GGVGNIFFFLMREVRTMGEN*DVISKLLDMLSAFPGTSIAPAFSPCGSAPNT*EVF VDCT*GILKTGRTGTPDVVVVEKISTPTLAVPNCHQRSN*KAWGAFSQIFDVGW FSCYLLNDVQLINPLPLHKGINPRRQTAIYWA
EST NO: 1468 ↑ 1468-1-2; P7/T2; more H3, H2, H1, C3; less in C1, C2, **DETAILS:** P7/T2; U_HW-Plate3b08.b1 **Sequence size (bp):** 483 **BLASTX:** ref|NP_001046190.1| Os02g0196000 [Oryza sativa (japonica cult.. 132 5e-30 emb|CAO21935.1| unnamed protein product [Vitis vinifera] 131 1e-29 gb|EAZ22087.1| hypothetical protein OsJ_005570 [Oryza sativa .. 129 6e-29 gb|AAZ20768.1| branchy [Setaria italica] 129 6e-29 ref|NP_566669.1| metal transporter family protein [Arabidopsi..	***CTTTATGCTGAGTGATATCTTTTTTTTTAC***ACTCGGGGTATATTAGATATTCGC GAGCTACTAAGTTGAACTTAATTTGATGATTTCTTTTTCTTTCTCTGTAGCCT ATCTATTCCCTAGCAGCTTAAATCCTGAGATTCTAGAAGGTCTACTTGGATC AGTCGGTGGAGTTATGGCTTTTCTAACCCTTCATGAAATGCTGCCATTGGCTT TTGATTATGCTGGACAGTAGCAATCAGTCAAGGCTGTATTTTTTGGGATGGC TCTCATGTCTGCAAGTCTGTATTTTCTAAGTGTCAGCTTACCAAAGGAAATA AGCTTGTAGATGGAAACAATCTCTTTTCTTTTCTTAATTCACGAACTCCACAG TATCATTGTGGTGTGTATCTTCCGATTCTATCATCAGTTGCAGCAAAATTTCT TCATATAGAGTCTCACAAACTGTCCAATGACAATAA***CATACAGCATTTAGTG AGAGTTAAT*** **Forward frame 2, 161 amino acids** FMLSDIFFFTLGVY*IFASY*VELNLMISFSFSVAYLFPSSLNPEILEGLLGSVGGV MAFLTLHEMLPLAFDYAGQ*QSVKAVFFGMALMSASLYFLSVSLPKEISL*

124 1e-27 **CONSERVED DOMAIN:** PRK04201, PRK04201, zinc transporter ZupT. 81323 No 6e-06	
EST NO: 1476a ↓ 1476a (D13-1), P7/T3; C1,C2,C3 **DETAILS: P7/T3** U_HW-070116_Plate6b02, **sequence size (bp):** 300 **BLASTX:** ref|YP_173415.1| hypothetical protein NitaMp073 [Nicotiana ta.. 136 9e-36 emb|CAO46934.1| unnamed protein product [Vitis vinifera] 123 3e-27 gb|ABR26094.1| retrotransposon protein [Oryza sativa (indica cul 110 4e-23	**CATTATGCTGAGTGATATCTTTTTTTTTAG**GTCATATCTAGTATTCAGAGT TTGCCTCGATTTGGTACCGCTCGCGCAGCCCGCACCGAAACAGTGCTTTACC CCTAGATGTCCAGTCAACTGCTGCGCCTCAACGCATTTCGGGGAGAACCAGC TAGCTCTGGGTTCGAGTGGCATTTCACCCCTAACCACAACTCATCCGCTGATT CTTCAACATCAGTCGGTTCGGACCTCTGCTTAGTTTCATCCAAGCTTCATCCT GGTCATGGATAGAT**CATACAGCATTTAGTGAGGGTTAAT** **Forward frame 2, 99 amino acids** IMLSDIFFFRSYLVFRVCLDLVPLAQPAPKQCFTPRCPVNCCASTHFGENQLALG SSGISPLTTTHPLILQHQSVRTSA **Reverse frame 1, 100 amino acids** INPH*MLYDLSMTRMKLG*N*AEVRTD*C*RISG*VVVRGEMPLEPRASWFSPKC VEAQQLTGHLGVKHCFGAGCASGTKSRQTLNTRYDLKKKISLSIM
EST NO: 1477a-a (P7) ↓ 1477a-a; P7/T3; more in C1;C2;C3, less in H1, H2, H3 **DETAILS:** P7/P7; e U_HW-070116_Plate6a07 **sequence size (bp):** 298 **BLASTX:** emb|CAO21131.1| unnamed protein product [Vitis vinifera] 48.5 1e-04 ref|NP_568392.1| unknown protein [Arabidopsis thaliana] >gb|A.. 46.2 7e-04	***ATTAACCCTCACTAAATGCTGTATG***GAGCTAAGGTGATGTAGAACCAACAAT GAAGGGTAATATAAATGACATTCTGCTCTAAATGTGACGATGCCAAACCTGT GACAAACAAGTTATCCACTAATCACTACCCCATTTTCTTACAACGTTCTCTTT AGCAGAAGCACTCTTGTAACCTTTCTTCAAATCAAAATCAGGCTGATCACCG TTAGAAGTATTTTTCATGACTTTTGATGTTGTGAGGCCAAATCCATTCTCAC ACCCCGAAGTG***CATACAGCATTTAGTGAGGGTTAAT*** **Reverse frame 2, 99 amino acids** INPH*MLYALRGVRNGFGLTTSKVMKNTSNGDQPDFDLKKGYKSASAKENVVR KWGSD
EST NO: 1486 (P7) (P7/T7), **DETAILS:** P7/P7**,** U_HW-070501-Plate5e05, **Sequence size (bp):** 298 **BLASTX:** ref|YP_001023713.1| chloroplast envelope membrane protein [An.. 32.7 7.6 Reverse sequence of 1477a-a (P7/P7) **:** Optimal Global aligment Alignment score: 578 Identities: 0.9798658	***ATTAACCCTCACTAAATGCTGTATG***CACTTCGGGGTGTGAGAAATGGATTTGG CCTCACAACATCAAAAGTCATGAAAAATACTTCTAACGGTGATCAGCCTGAT TTTGATTTGAAAAAAGGTTACAAGAGTGCTTCTGCTAAAAAGAACGTTGTAA AAAAATGGGGTAGTGATTAGTGGATAACTTGTTTGTCACAGGTTTGGCATCG TCACATTTAAAGCAAAATGTCATTTATATTACCCTTCATTGTTGGTTCTACAT CACCTTACCTC***CATACAGCATTTAGTGAGGGTTAAT***
EST NO: 1479 ↑ 1479 (D5-1); P7/T7 H2, H3 **DETAILS:** P7/T7 e U_HW-070116_Plate5f06, **sequence size (bp):** 501 **BLASTX:** gb|ABN08405.1| Peptidase aspartic, active site [Medicago truncat 65.5 1e-09 gb|ABN08407.1| Peptidase aspartic, active site [Medicago truncat 65.5 1e-09 gb|ABN06064.1| RNA-directed DNA polymerase (Reverse transcrip.. 64.7 2e-09	***ATTAACCCTCACTAAATGCTGTATG***TTGGGTGCTCTCCCTCTGTCAACTCAGA AACTCTCCCCTTATTCCTTCTGGTGTTAATCTTCCATACTTTTGGATCTAGCCC TATTGGATCCAAGTTCAATTGTTACTGGGTTATATCAGTGTCAATAATCAAG ATGTTGAAGGATTGTTGGGGTATGGTTCTTTCAACTAAAGCAGGCAACGGCA TCTCTCACTGCTGCAGCAAAGTGAATTAAATTAATGGTTGGACAGTTTAGTG GAAGTATTTAAGGATGTTATGGGCCTTCTCTCTCTTTGGAACACAGTCCATCC AATTGTGCTAAAAGAATGCACTAGTTCTGTAAGTTTCAAACCATATAGCTAC CCTCACCACCATTAAATTGAAATTGAGAGGTAGGTTTGAGACATGCTGAAAT AGGGGATCATATGGCCCAGCTCAAGTTATCTCTCCATCCGAGTCATTTTGGT ***CAAAAAAAAAGATATCACTCAGCATAATG*** **Forward frame 3, 166 amino acids** *PSLNAVCWVLSLCQLRNSPLIPSGVNLPYFWI*PYWIQVQLLLGYISVNNQDVE GLLGYGSFN*SRQRHLSLLQQSELN*WLDSLVEVFKDVMGLLSLWNTVHPIVLK ECTSSVSFKPYSYPHHH*IEIER*V*DMLK*GIIWPSSSYLSIRVILVKKKDITQHN
EST NO: 1480 ↓ 1480-2; P7/T7, C2, C3 **DETAILS:** P7/T7; U_HW-Plate3b12.b1, **sequence size (bp):** 443 **BLASTX:** emb|CAO65523.1| unnamed protein product [Vitis vinifera] 59.7 6e-08 gb|EAZ11112.1| hypothetical protein OsJ_000937 [Oryza sativa .. 56.6 5e-07 gb|EAY73105.1| hypothetical protein OsI_000952 [Oryza sativa .. 56.6 5e-07 ref|NP_001042468.1| Os01g0227100 [Oryza sativa (japonica cult.. 56.6 5e-07	***ATTAACCCTCACTAAATGCTGTATG***GACACCTACTTTCGATCGTTTGCATTCCT TCAATAGCGATCCCTGAAATTGTCTAAGGCCACACTTTGTAGAACCATAGCT ACACACCGAACGAAAACGAAGTGAGCATCATTGGCAATCATCATCCACCAT GACTTTTGAAGAGCACTCGCTCATATATACGTGCAATCACACATTTTTCGGA TAACATAAATAACGCATTGTGCACAGACACTTTAGATTGAAGGCGCGTTTGA TGTCTAACACCAACACCGAATCATGTTACATTCAATTTCTTCCATTTTTCAAA TTATTATTGGTGTGAATGTGTC*A*GTGTAAGTGTTTTGTCTAGTGTCGATATTT ATGGAATTATTAACATTTTTTCCCATGTGATATGAAACTACAACTC***TCAAAAA*** ***AAAAAA****TAT****CACTCAGCATAATG*** **Reverse frame 2, 147 amino acids** IMLSDIFFFESCSFISHGKKC**FHKYRH*TKHLH*HIHTNNNLKNGRN*M*HDSV LVLDIKRAFNLKCLCTMRYLCYPKNV*LHVYMSECSSKVMVDDDCQ*CSLRFR SVCSYGSTKCGLRQFQGSLLKECKRSKVGVHTAFSEG*
EST NO: 1486 ↓ 1486-4 ; P7/T7; C1, C2 **DETAILS:** P?/T7 U_HW-Plate4f05.b1, **Sequence size (bp):** 186 **BLASTX:** gb|EDK98054.1| mCG128709 [Mus musculus] 33.1 5.9 ref|NP_996258.1| Cd200 receptor 2 [Mus musculus] >gb|AAO84052.. 33.1 5.9 **Reverse frame 3, BLASTP** ref|XP_535637.2| PREDICTED: similar to CG3209-PB, isoform B [Can 32.7 7.7 ref|XP_001493550.1| PREDICTED: hypothetical protein [Equus cabal 32.3 9.7	***ATTAACCCTCACTAAA***GGGGGAGAAACAACGCAGAGTACATTTCTTTCATGC TGCTGTAAAAAACACGATTCCAAGAGGTCGGGTCTGTTGATGTAAATCGAAA TCCAAGTCGTGTTTGTATAAACTGATGGATGATTGATTGATTTCCTGTGGCC***G*** ***TAAAAAAAAAGATATCACTCAGCATAATG*** **Reverse frame 2, 62 amino acids** HYAE*YLFFYGHRKSINHPSVYTNTTWISIYINRPDLLESCFLQQHERNVLCVVSP PLVRVN **Reverse frame 3, 61 amino acids** IMLSDIFFFTATGNQSIIHQFIQTRLGFRFTSTDPTSWNRVFYSSMKEMYSALFLPL **
EST NO: 1490 ↑ 1490-3; P7/T9; H1, H2 **DETAILS:** P7/T9, U_HW-Plate3c01.b1, **sequence size (bp):** 329 **BLASTX:** emb|CAC44123.1| N3 like protein [Medicago truncatula] 77.8 2e-13	***ATTAACCCTCACTAAATGG***AGGAGGTGGACCTTCAATGGTCTTCTCCCCGAGA TTATAAAGATGCTCTTCCAAATACACTTGGATTTGAATTCAGCTTAATTCATA TGATGATGCATTTAATAGATAGAAATGCCCCACCAATGACACTAAATGGGCC TATGAAGGGTCAAGAATTGAGTGGGGGCCTTATTGTTGATGTTGTGAAGATT GGATCCCACCCCCCCCGTGGAGGAGGTCCACTTAGCAAACTTTGATTTTACC AATGATAGTATTTATGCAAAAATTAAGGGATCCCTGA***CCAAAAAAA****CATTAAT* *CA****CTCACGATAATG*** **Forward frame 1, 109 amino acids** INPH*MEEVDLQWSSPRDYKDALPNTLGFEFSLIHMMMHLIDRNAPPMTLNGPM KGQELSGGLIVDVVKIGSHPPRGGGPLSKL
EST NO: 1931 ↓ 1931-3; P7/T7; more in C3; less in H2, H1 **DETAILS:** P7/T7 U_HW-Plate3e03.b1, **sequence size (bp):** 336 **BLASTX:** gb|ABB29467.1| salt-tolerance protein [Glycine max] 37.4 0.29	***ATTAACCCTCACTAGATGCTGTATG***TCTTACAAAAAGCCTAGGATTGAACTCC TAAAACAAAATGATGATGATGATGACCACCCCACAATGCCTGATCTTGGTTA ATGCACGACAATCTGAACTGGTCCGGTCCATGAATCTGTTGATCAATTAGTT TATATGCATACTTCCCTAATCATGCTACGTATTCCTGGTATGTTTACTACCTG TGTGACTTACCACATTTAAGAAGATTCAAGTCATTTCGAAATTAAAAAAAAC GTAAAATTATACCTTAAGGTGTTAAATTTAACATGGCGTGAGTGA***TCAAAAA*** ***AAAACATATCACTCACAAAAGG*** **Forward frame 2, 110 amino acids** LTLTRCCMSYKKPRIELLKQNDDDDDHPTMPDLG*
EST NO: 1934 ↓	***CATTATGCTGAGTGATATTTTTTTTTTGG***TATTAAAATAAAAATCATTCAAC AAGTCATAACAATAACAAAAATTGTCTACCCCACTATTCCAAATTCATAAAT

P7/T9 C1, C2 **DETAILS:** P7/T9 U_HW- U Plate3e04.b1, **sequence size (bp):** 294 **BLASTX:** emb|CAA80334.1| ubiquitin extension protein [Lupinus albus] 72.0 1e-11 gb|ABI84265.1| ubiquitin/ribosomal protein S27a [Arachis hypogae 71.2 2e-11 **CONSERVED DOMAIN:** pfam01599, Ribosomal_S27, Ribosomal protein S27a. This family of ribosomal proteins co.. 65402 No	ACCAAATCTTAAAAAACTAAAATACCAAAAATAAAAAACATTTAGGGTAGC ATTAAAAAAACAGAAATCAAAATCAAGCATCGGTTTTCTGGTAAACATAAG TCAAACCACACTTGCCACAGTAATGACGATCAAAGTGATTAGCCATGAAAGT TCCAGCACCA***CATACAGCATTTAGTGAGGGTTAAT*** **Reverse frame 1, 98 amino acids** Y*PSLNAVCGAGTFMANHFDRHYCGKCGLTYVYQKTDA
EST NO: 1937 ↓ 1937 (D2-1), P7/T7 C3, C2, C1 **DETAILS**: P7/T7 e U_HW-070116_Plate5g06 **sequence size (bp):** 386 **BLASTX:** emb|CAO67101.1| unnamed protein product [Vitis vinifera] 65.1 1e-09 ref|NP_001052878.1| Os04g0441900 [Oryza sativa (japonica cult.. 61.2 2e-08 emb|CAE02511.1| P0076O17.9 [Oryza sativa (japonica cultivar-g.. 61.2 2e-08 emb|CAE04225.2| OSJNBa0064D20.9 [Oryza sativa (japonica cultivar 61.2 2e-08 dbj|BAD95341.1| hypothetical protein [Arabidopsis thaliana] 60.1 4e-08 ref|NP_680213.1| transmembrane protein, putative [Arabidopsis.. 60.1 4e-08 **CONSERVED DOMAIN:** pfam05602, CLPTM1, Cleft lip and palate transmembrane protein 1 (CLPTM1). This family ..	***ATTAACCCTCACTAAATGCTGTATG***ACCATCACCGACTGCCGTGTAGTAGCCA GATACCCTTCATCTTTCTGTCCTAGTAAAACTTCCAGGAGATTAAGGTCAAG ATTTCTTCAAACAAAAACCTAACAGCAAATGTATACCTCAAAGAGAGAGTA AACACAAATTAATCCTCATTGTATTTAGTATCAACTCCCTCAAGCGTTTGAG AGAAAACCAAATCAATTGGTTTTCTTCTCTTCTTCCTTTGCAGCATCCGACTC TGCAGAAACCACGGCCTGATCATCCTCACCTCCAAAACCAAATTCATTTACA CGCTTCTTATCCACTGGATAAATCCACCTTTGGTATACATATA***TCAAAAAAAA*** ***AGATATCACTCAGCATAATG*** **Reverse frame 3, 128 amino acids** LC*VISFFLIYVYQRWIYPVDKKRVNEFGFGGEDDQAVVSAESDAAKEEEKKTN
ESTs in DDRT-PCR AUTORADIOGRAPH FILM-2	
EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers***
EST NO: 1501 ↓ P9/T1 C1, C2, C3 **DETAILS:** P9/T1; U_HW-070501-Plate5e06.b1, **sequence size (bp):** 329 **BLASTX:** ref|NP_779104.1| potassium uptake protein [Xylella fastidiosa.. 34.3 2.4 ref|NP_299189.1| potassium uptake protein [Xylella fastidiosa.. 34.3 2.4 ref|ZP_00684418.1| K+ potassium transporter [Xylella fastidio.. 34.3 2.4 gb|AAS55400.1| ASPM protein [Cercopithecus aethiops] 33.9 3.2 sp|P62292|ASPM_MACMU Abnormal spindle-like microcephaly-assoc.. 33.5 4.2 gb|EAY93669.1| hypothetical protein OsI_014902 [Oryza sativa .. 27.3 9.6 ref|NP_001052526.1| Os04g0349500 [Oryza sativa (japonica cult.. 27.3 9.6 gb|ABR25841.1| 40S ribosomal protein S8 [Oryza sativa (indica cu 27.3 9.8	***ATTATAACCCTCCCAAACGAATGG***GGGCTGATGGGTAATCCTTGAAAGTAAG GAACTGGAATTTTACATGAAAATTTACAAAGGAAAAAGGGAAAGGGTGCTG CTTAATTGTAGTGTTTTCCGTCTTATATAAGATTTCATTTGTTTTTAATAGTGC AACAATATTTGGTACCTTTCAAGTTGATGTCTTTTGTTTTGAATGAGACAATG TTTATTGGACAACCTTTAGGTTGATGTCCTAAAACTTCTTTAGTGTCAAACCT TCACTAGTTTTGTGACACTTTAAAAAATTTTTTGTC***TTAAAAAAAAAGATATCA*** ***CTCAGCATAATG*** **Reverse frame 2, 109 amino acids** IMLSDIFFFKTKNFLKCHKTSEGLTLKKF*DINLKVVQ*TLSHSKQKTST*KVPNI VALLKTNEILYKTENTTIKQHPFPFSFVNFHVKFQFLTFKDYPSAPIRLGGL*
EST NO: 1508 ↑ 1508-1-2; more in H1, H2, H3; less in C2, C3 **DETAILS:** Primers found: ?/T7; U_HW-Plate3c04.b1 **sequence size (bp):** > 549 **BLASTX:** emb|CAO15460.1| unnamed protein product [Vitis vinifera] 97.4 4e-19 gb|ABF94173.1| ReMembR-H2 protein JR702, putative, expressed .. 89.0 1e-16 ref|NP_001049082.1| Os03g0167500 [Oryza sativa (japonica cult.. 89.0 1e-16 ref|NP_177343.2| protease-associated zinc finger (C3HC4-type .. 87.8 3e-16 **CONSERVED DOMAIN:** cd02123, PA_C_RZF_like, PA_C-RZF_ like: Protease-associated (PA) domain C_RZF-like. Th.. 80345 No 1e-04	***TTATGCTGAGTGATATCTTTTTTTTTGA***TGATTATAGGGGGGTGTCGCTTCATT TTTATGTTACAATGGCATATTGGCTGGCTACACTGCTGCTGTTGTCTTTGACA ATGAAAATGGCGGCGTCCTGGTTGCACTGCCAAGAAATTCTGCTGGGATATG ATTACACGCGGTATTTGAATCAAAACCTTCGGGTAAAATACTTCATAGATAC CTGGGTTTCACCAATGTGGAACCGCGCCCCATTCCATCTATGGAAACCTCGA GATGTCCAATCGGGCACATTTCTTTTTTTTCCCCGCCAGGGGCGTCTGCGTTG CTGGCGACTTGTTTCTATCCCCTCACGCTTCCCATGAGAAAAAAAAGCCCAC GAGCTTCTCAATTCTTGAATGAGACGGAAGGAGGAGCCACCAAAAGAAAAC AGCGCTAAGTTCGATTCTCGCTTTCGCTTAGGAAACTCAGGGTACATAAATG ATTTGTGCAATCTGCCAAAGAAATCTATAGTGTGGGAAAGAA TTTCGGGATCCTTCCCTGTTATTACAGAAACACCTC **Forward frame 2, 182 amino acids** YAE*YLFF**L*GGVASFLCYNGILAGYTAAVVFDNENGGVLVALPRNSAGI*LH AVFESKPSGKILHRYLGFTNVEPRPIPSMETSRCPIGHISFFSPPGASALLATCFYPL TLPMRKKPRASQFLNETEGGATKRKQR*VRFSLSLRKLRVHK*FVQSAKEIYSV GKNFGILPCYYRNT
EST NO:1940 ↓ 1940-2, P9/T7 C1, less in H3 **DETAILS:** P9/T7 U_HW-Plate3e06.b1, **sequence size (bp):** 398 **BLASTX:** dbj|BAA02117.1| GTP-binding protein [Pisum sativum] >prf||200.. 75.5 1e-12 emb|CAA98161.1| RAB1D [Lotus japonicus] 69.7 6e-11 **BlastN:** D12549.1 Pisum sativum mRNA for GTP-binding protein, complete cds, clone:pra9B 389 8e-106	***ATTAACCCTCACTAAATGTGGCAGG***GGCATTCATGGCCATGGCTGCTGCCATC AAGGATAGAATGGCAAGCCAACCGTCCGCAAACAATGCAAGGCCTCCGACG GTGCAAATTAAAGGACAACCAGTTGGGCAAAAAGGTGGTTGCTGCTCTTCTT AGCCAAATGACATGGCATTTCACCTGGTCTGTTGCTTGACCCTTTTCATGTAA AATTTGTTATGTCACTAAATAGTTGACCAATGTCGCATAATCTGTCAAATGG TTTGGGAATTTGGCTTAAATGATCCCATTCTTTACATACACTTGAATGGTATG CTTGTGCTAAATATAGTAAGTACTTGTGTTGATGATATATATCTATGTTTCTG C***TCAAAAAAAAAGATATCACTCAGCATAATG*** **Forward frame 3, 132 amino acids** *PSLNVAGAFMAMAAAIKDRMASQPSANNARPPTVQIKGQPVGQKGGCCSS*
EST NO: 1943-D5 ↑ 1943 (D5-5); P9/T9 more in H3, H2; less in C1 **DETAILS:** P9/T9 U_HW-070501-Plate5c08.b1, **sequence size (bp):** 473 **BLASTX:** ref|NP_563986.1| unknown protein [Arabidopsis thaliana] >gb|A.. 112 5e-24 gb|EAZ42142.1| hypothetical protein OsJ_025625 [Oryza sativa ..	***CATTATGCTGAGTGATATCTTTTTTTTTGG***AGTTTTAAAGAGTAGATACTATG ACATGGTGTTAAGCACAAAATTGGCAGGCATCGGGCATGCATCATATTTATT CATGACAACTGCAAAAGATAGAGTTAGTTATGTCTACCCAAATGTGAATGCT GCAGGAGCAGGATTGCTTTTATCTGAAACATTCACACCTGATTCTCTAAATC TTTCAAAAGGAGGATACCATATGTGAGTTAATGTAACAACACAAATGATTTA GTGTGTTTTTTGTTTCTGCGCTAACAAATATTGATTTTGAACCAATTGATTTT GGAAAATATATTTTAGTCGAGTTGAAAGCAAAAAGAGGTTTATGTTTAGATA ATAAATTCATGAAAAAATTTTTTTTTTTTCATTTCGGGTCGAATAGTTTAAAAT

102 1e-20	CAAAATTTGCTTTTGAGTATATATATAC***CCTGCCCACATTTAGTGAGGGTTAAT*** **Forward frame 2, 157 amino acids** IMLSDIFFFGVLKSRYYDMVLSTKLAGIGHASYLFMTTAKDRVSYVYPNVNAAG AGLLLSETFTPDSLNLSKGGYHM*
EST NO: 1943 (P9) ↑ P9/T9 more in H3, H2; less in C1 **DETAILS:** P9/P9 eU_HW-070116_Plate5c04, **sequence size (bp):** 416 **BLASTX:** emb|CAO49820.1| unnamed protein product [Vitis vinifera] 210 3e-53 ref|NP_566924.1| unknown protein [Arabidopsis thaliana] >ref|.. 196 3e-49	***ATTAACCCTCACTAAATGTGGCAGG***GTTTTATTAAGGTATTTTGGAGATAAGT AATCCAATGCATCCGACACAATGACAAGAGAAAATGACTTTGCACGGTAAG GTAGAGGAAACTTTATGTCAGCCACACGCACTATGCCCTTGCGTACAAGACT TTTACACTTTGCACCAACATCATCTAATTCGTATGGTTCTATCCCCCAAGCCT CAACATCCTCTTCTGCTAACAAACTAGATACCAAAGAACAAGAATCGGGGC CGACATGCAAAACCTTATGCATACTGTCTCCATATGCTTTCTTCAAAATGGG CAATGCTTGTAGTACTTCTAATGTACATGATGAACCCCCTTCAAGTTTGCTTA AATCCTTAATATCATTACTAGCTCCA***CCTGCCACATTTAGTGAGGGTTAAT*** **Reverse frame 3, 138 amino acids** *PSLNVAGGASNDIKDLSKLEGGSSCTLEVLQALPILKKAYGDSMHKVLHVGPD SCSLVSSLLAEEDVEAWGIEPYELDDVGAKCKSLVRKGIVRVADIKFPLPYRAKS FSLVIVSDALDYLSPKYLNKTLPHLVRVN
ESTs in DDRT-PCR AUTORADIOGRAPH FILM-3	
EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 1517 ↑ 1517-1a, P3/T3 H1;H2;H3 **DETAILS: P3/P3** U_HW-070116_Plate6a05, **sequence size (bp):** 311 **BLASTX:** ref|XP_001018963.1| Leucine Rich Repeat family protein [Tetra.. 37.7 0.23 ref|XP_001599246.1| PREDICTED: similar to paired [Nasonia vitrip 37.0 0.40 ref|XP_001313628.1| Immuno-dominant variable surface antigen-.. 34.7 2.0	***ATTAACCCTCACTAAATGCTGGTGG***TGTTGGGGCGCTTGTTGATAGTCTAACC TCAGAAGAGATTCCCAAAGAGAAGTGTAGGTTCGATAGTGTTGATAAAGC ATATCTGCGACACATTCTGATTCTGATAGCCAAAATTTGAATTCCATAGAAG GACAACATAGGAGTGTTCCTGTGGCAGTGGAGTCTCAATGTACCGATAAATC TCCTAAGTCCTTTCCATCCCCAAATCACATGCCTCAAGAAATAAATGGTTAC AGCGGAGCAACTCATATACAGGGTAG***CCACCAGCATTTAGTGAGGGTTAAT*** **Forward frame 3, 103 amino acids** *PSLNAGGVGALVDSLTSEEIPKEKCRFDSVDKSISATHSDSDSQNLNSIEGQHRS VPVAVESQCTDKSPKSFPSPNHMPQEINGYSGATHIQGSHQHLVRVN
ESTs in DDRT-PCR AUTORADIOGRAPH FILM-5	
EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 1528 ↑ P4.T1; H2, H3 **DETAILS:** P4/P4, U_HW-070116_Plate5d04 **sequence size (bp):** 508 **BLASTX:** emb|CAO65716.1| unnamed protein product [Vitis vinifera] 227 2e-58 gb|AAW28145.1| hAT-like transposase [Arabidopsis thaliana] 225 7e-58 emb|CAB68118.1| putative transposase [Arabidopsis thaliana] >.. 225 7e-58	***ATTAACCCTCACTAAATGCTGGTAG***CAGCATCTGAGCTAAAGGAAGTGTTTTC TTGTTTGGATACTTCGGATCCAGATTACAAGCGAACCCCATCTATGCAAGAT TGGAAGGTAGTTGAGACCCTCTGCACTTATTTGAAGCCCCTTTATGATGCAG CAAACATTCTTACCACAACAACTCAACCCACTGCTATTACCTTCTTTCATGAA GTTTGGAAATTGCAGTTGGATCTTGCTCGTGCTGTTACAAATGAAGAAGATC CCTTTATTAGCAACCTTACTAAGCCCATGCAAGAAAAGATTGACAAGTACTG GAGAGATTGTAGTTTTGTTTTGGCAATTGCAGTTGTTATGGATCCTCGTTTCA AGATGAAGCTTGTAGAATTTAGCTTCACAAAAATCTATGGTGATGATGCTCA TGAATATGTCAAGATTGTTGAAGATGGAGTACATGAGTTGTTCAATGAGTAT GTGGCCCTTCCC***CTACCAGCATTTAGTGAGGGTTAAT*** **Forward frame 2, 169 amino acids** INPH*MLVAASELKEVFSCLDTSDPDYKRTPSMQDWKVVETLCTYLKPLYDAA NILTTTQPTAITFFHEVWKLQLDLARAVTNEEDPFISNLTKPMQEKIDKYWRDC SFVLAIAVVMDPRFKMKLVEFSFTKIYGDDAHEYVKIVEDGVHELFNEYVALPL PAFSEG*
EST NO: **1531** ↓ C1, C2 P4/T1 **DETAILS:** P4/T1 U_HW-Plate3g07.b1 **sequence size (bp):** 212 **BLASTX:** gb|EAY75272.1| hypothetical protein OsI_003119 [Oryza sativa .. 33.9 3.3 ref|NP_174274.2| dehydratase family [Arabidopsis thaliana] >g.. 32.7 7.4	***ATTAACCCTCACTAAATGCTGGTAG***GCTGACATAGAATGACTTCTTACTTGCG GCTAATATCAATGAGCTTAATGTGCATATCTTACTAGTAAGGAAAGCTTCTG ACTGATTTATCTATATGAAGAGGAACTAAAAACTTGTAAATATTCTAGAGTT ATCACTTTTTCTGTCCATTTTTCCA***TTAAAAAAAAAGATATCACTCAGCATAATG*** **Forward frame 3, 71 amino acids** Y*PSLNAGRLT*NDFLLAANINELNVHILLVRKASD*
EST NO: 1528-D6 ↑ 1528 (D6-4-1); P4.T1; H2, H3 **DETAILS:** P4/P4 U_HW-070501-Plate5c09.b1, **sequence size (bp):** 508 **BLASTX:** No significant similarity found **BlastN:** AC123899.15 Medicago truncatula clone mth2-33n6, complete sequence 376 6e-102	***ATTAACCCTCACTAAATGCTGGTAG***TGTTGCGGATGCTGATTCTGCATCATGT GTCTCTGCACATACTGTTGTTGTTGGTCTCTGTTGAAACCCAGGGTTCGAG TTCAAACCACCACCACCACCTCGCCCTAACCCAATCCCAGGTGGGTATCTCG CTCTATTGTTCATCCTACAAATTCAAAAACAAAAGAATTTCTACCCGAAAAA ATGTACAAAAAGCTAAACTCAAACCTAAGAAGCTAGGGTTTCTTCTGGGTCA CTGAAAAGGGAACTAGACCCAAACCCCACGAAACGATGATTTCTCGAAATG GGGTTAAAATGAAAGGCTAAACTAACGAAATCTGAAAAGAAAAAAGTGAA ATTTCTACGGGTATCATACAACTACAAGGATCCCCCAAAAAAACTGGGTAGG GTTGGGACTTTGACAGAAAATTTGGAGGAGGAAAATTTGAGGAGGATGAGA GGGTGTTTTGTACAGAT***CTACCAGCATTTAGTGAGGGTTAAT***
EST NO: 1536 ↓ P4/T2; C1, C2, C3, less in H1, H2, H3 **DETAILS:** P4/T2 e U_HW-070116_Plate6g07, **sequence size (bp):** 447 **BLASTX:** gb|ABD32724.1| Helicase, C-terminal; Argonaute and Dicer prot.. 229 3e-59 emb|CAO24342.1| unnamed protein product [Vitis vinifera] 201 1e-50 gb|EAZ25399.1| hypothetical protein OsJ_008882 [Oryza sativa .. 191 1e-47 gb|EAY88320.1| hypothetical protein OsI_009553 [Oryza sativa .. 191 1e-47 ref|NP_001048796.1| Os03g0121800 [Oryza sativa (japonica cult.. 191 1e-47	***ATTAACCCTCACTAAATGCTGGTAG***AATGGCATAGGCCAATTTCTTCGCAAAC AGATGTCATTTAATGTCTGTCTAGTAAATGTCTGGTTCCCATTCTTTGTCTCA TTCTCATCATTCTTCCCCTGAATTTTCTCCACTTCCTTCTCTTTCAAAGCTGCA AGAGCATTTCTGGCAGCTAATTTCTGTGCCATTTTTTTCTGTGGATTTTGAGC AGCCCCTACTTGTACCCCATCAATGAATACTTCAACAGTGGCCAAATTGCCA ATACGACTTGCTCTATACTCCAAGCCTTCGGCCTGCTGCTGGCATCGCTCTTG CAGCTCACGCACAGGATGCATTGGCAAAGTCTCTGGGGTAACCATAGGATG CAAAAGAGGCTGAAAAACCTTCCAAACAACAGCAGTATTCCTCCCACT***GTAA AAAAAAGATATCACTCAGCATAATG*** **Reverse frame 1, 149 amino acids** HYAE*YLFFYSGRNTAVVWKVFQPLLHPMVTPETLPMHPVRELQERCQQQAEG LEYRASRIGNLATVEVFIDGVQVGAAQNPQKKMAQKLAARNALAALKEKEVE KIQGKNDENETKNGNQTFTRQTLNDICLRRNWPMPFYQHLVRVN
EST NO: 1538 ↑ P4/T2; H2, H3 **DETAILS:** P4/T2, U_HW-070116_Plate5d03.g1	***CATTATGCTGAGTGATATCTTTTTTTTTAC***AAGTAAAGTAAATATTTTAAAAT GTTAACTTTGGCAAATTGTCGTTAACATGATTATTTTGTTTTATTACTGAAAA TAAAAATTGACAAACCAAAATCTTATATTACCCGAGTGAAATGAATAAAAA TGCTAGTAATTAAAAAATCATAATTAACTTCTTAGTATCTGATTCCTTTTGCA

sequence size (bp): 313 **BLASTX:** pdb|1J93|A Chain A, Crystal Structure And Substrate Binding M.. 69.7 5e-11 sp|Q42967|DCUP_TOBAC Uroporphyrinogen decarboxylase, chloropl.. 69.7 5e-11 gb|EAZ26846.1| hypothetical protein OsJ_010329 [Oryza sativa .. 66.6 5e-10 gb|EAY89926.1| hypothetical protein OsI_011159 [Oryza sativa .. 66.6 5e-10 ref|NP_001050049.1| Os03g0337600 [Oryza sativa (japonica cult.. 66.6 5e-10 ref|NP_181581.1| HEME2; uroporphyrinogen decarboxylase [Arabi.. 66.6 5e-10 **CONSERVED DOMAIN:** cd00717, URO-D, Uroporphyrinogen decarboxylase (URO-D) is a dimeric cytosolic enzyme t.. 48141 No 7e-07	ACCTCAAAAAAATGTGCTACATTCTCCTCAGGTGTACCTACTACAATGCCAT GACCAAGATTCAAGATATGTTTCCCT***CTACCAGCATTTAGTGAGGGTTAAT*** **Reverse frame 3, 103 amino acids** *PSLNAGRGKHILNLGHGIVVGTPEENVAHFFEVAKGIRY*
EST NO: 1540 ↑ 1540-4, P4/T2; H1,H2 **DETAILS**: P4/T2 U_HW-Plate3c08.b1 and U_HW-Plate3c07.b1, **sequence size (bp):** 186 **BLASTX:** emb|CAD23614.1| tri m 2 allergen [Arthroderma benhamiae] 33.1 4.6	***CATTATGCTGAGTGATATCTTTTTTTTTAC***GGCCACAGGAAATCAATCAATCA TCCATCATTTTATACAAACACGACTTGGATTTCGACTTACATCAACAGACGC GACCTCTTGGAATCAGGTACTAAAACAGCAGCATGGAAGAAATGTACTCTG CGTTG***CTACCAGCATTTAGTGAGGGTTAAT*** **Forward frame 2, 62 amino acids** HYAE*YLFFYGHRKSINHPSFYTNTTWISTYINRRDLLESGTKTAAWKKCTLRCY QHLVRVN
EST NO: 1544 ↑ 1544-2, P4/T3; H1, H3; less in C1 **DETAILS:** P4/T?, U_HW-Plate3c09.b1, **sequence size (bp):** 169 **BLASTX:** No significant similarity found **BlastN:** AC126780.18 Medicago truncatula chromosome 8 clone mth2-10n4, complete sequence 132 4e-29	***ATTAACCCTCACTAAATGCTGGTAG***GGATGGATAAATGTGCAGTGAGCTTTTA GGTCCATTTTTTCACATATGTATTCGGAATCGTCATCCATTGTAAATCTCTAT GAAACTGTTGCGTATGCAATGGTCTCTTTGTGCTGA***AAAAAAAAGATATCACT CAGCATAATG***
EST NO: 1550 ↑ 1550-1-2, P4/T4, H1, H2 **DETAILS:** P4/T4 U_HW-Plate4f09.b1, **sequence size (bp):** 169 **BLASTX:** No significant similarity found **BlastN:** AC126780.18 Medicago truncatula chromosome 8 clone mth2-10n4, complete sequence 123 2e-25	***CATTATGCTGAGTGATAT****GGAA****TTTTTTCA***GCACAAAGAGACCATTGCATACG CAACAGTAACATAGAGATTTACAATGGATGACGATTCCGAATACATATGTGA AAAAATGGACCTAAAAGCTCACTGCACATTTATCCATCC***CTACCAGCATTTA GTGAGGGTTAAT***
EST NO: 1555 ↑ P4/T4; H2, H3 **DETAILS:** P4/T4 eU_HW-070116_Plate5d02, **sequence size (bp):** 226 **BLASTX:** emb|CAO50143.1| unnamed protein product [Vitis vinifera] 47.0 4e-04 ref|NP_195760.1| ESP4 (ENHANCED SILENCING PHENOTYPE 4); bindi.. 34.7 2.0	**ATTAACCCTCACTAAATGCTGGTAG**AGCTGAAGGAGAGTCATCCAGTGTG TTAGCAGTACTTTCATCTAACTCATTTTTAGGTTCTTTGAAACCTTCGGTTGTT GTTCTAACACGTTTACCAGGGACATCTGCATCTCCAGTTAAATCGCCTCCATT TTCTGATCCTGACCGTTTCCTTACCAAATTAACGTGCCC**TGAAAAAAAAAGA TATCACTCAGCATAATG** **Reverse frame 1, 75 amino acids** HYAE*YLFFSGHVNLVRKRSGSENGGDLTGDADVPGKRVRTTTEGFKEPKNEL DESTANTLDDSPSALPAFSEG*
EST NO: 1558 ↑ 1558-1-1, P4/T4; H1, H2, H3; less in C3 **DETAILS:** P4/T4 U_HW-Plate2b06.b1, **sequence size (bp):** 183 **BLASTX:** gb|EDP19348.1| hypothetical protein CLOBOL_00185 [Clostridium.. 33.1 5.9 ref|XP_001660818.1| conserved hypothetical protein [Aedes aeg.. 32.7 7.7	***ATTAACCCTCACTAAATGCGGGTAG***AGGTAGCATTGACAGGGAATTTGAAAC TGGTTCAAGCAGACACAGTGTGGATAAAAGAAAAAAATCCTATTATGATAG GCCAACTGGCTCGTATCTCGGAGATAACGTCGAACACAGTAGGGTCAAGA***T GAAAAAAAAAGATATCACTCAGCATAATG*** **Reverse frame 2, 60 amino acids** IMLSDIFFFHLDPTVFDVISEIRASWPIIIGFFSFIHTVSA*
EST NO: 1560 ↑ P4/T5; H2, H3 **DETAILS:** P4/T5 e U_HW-070116_Plate6g05, **sequence size (bp):** 410 **BLASTX:** emb|CAO45205.1| unnamed protein product [Vitis vinifera] 140 2e-32 ref|NP_001031934.1| AAP7 (amino acid permease 7) [Arabidopsis th 140 2e-32 ref|NP_197770.1| AAP7 (amino acid permease 7); amino acid per.. 140 2e-32 **CONSERVED DOMAIN:** pfam01490, Aa_trans, Transmembrane amino acid transporter protein. This transmembrane .. 79824 No 1e-16	***ATTAACCCTCACTAAATGCTGGTAG***CAGATGTAATGACATAGGCACATGTAG TCCCATATAAGCTCACATGTACCAACACTCCACACACCTTTCCCCACATCGTT CCTAAGTACAAGTTGACAGCATCCATGTATGAGGAGCTTGTAATGCTCCCAT GTTGAGGATGAGGGAATCTATAGCAATCAGAAAGAAGGTAGGTTGAAATAT AGGTAGTGACTGCAAAGAGTATAATACACACAGGACCTGCAATCCAACCTA ATTGTGCCAAAGACCATGCTAGTGACAATACACCTGCTCCTATCACCCCTGT TATTATATGTGCCACTGCACTCCATATATTACCTGTTTTCCTAAAAGGCTCTT TAACTGACACATCACC***GGAAAAAAAAAAGATATCACTCAGCATAATG*** **Reverse frame 1, 136 amino acids** HYAE*YLFFSGDVSVKEPFRKTGNIWSAVAHIITGVIGAGVLSLAWSLAQLGWIA GPVCIILFAVTTYISTYLLSDCYRFPHPQHGSITSSSYMDAVNLYLGTMWGKVCG VLVHVSLYGTTCAYVITSATSI**
EST NO: 1562 ↓ P4/T5; C1 C2 **DETAILS:** P4/T5/T1 eU_HW-070116_Plate5d01, **sequence size (bp):** 352 **BLASTX:** emb|CAO16829.1| unnamed protein product [Vitis vinifera] 89.0 2e-20 gb|AAB95218.1| putative serine-glyoxylate aminotransferase [F.. 89.7 4e-20 emb|CAN84001.1| hypothetical protein [Vitis vinifera] 89.0 4e-20 gb|AAZ94162.1| enzymatic resistance protein [Glycine max] 97.1 3e-19 gb|AAQ56194.1| aminotransferase 1 [Cucumis melo] 95.9 7e-19 **CONSERVED DOMAIN:** COG0075, COG0075, Serine-pyruvate aminotransferase/archaeal aspartate aminotransferase.. 30424 No 1e-06	***ATTATTGCTGATGATAT****CTT****TCTTTTTTTTT***AAACTGGGAACGAGTGCTTGGGA AAATGCTTGACAAACACATTGTCGCTGGAGATCGTACCGTATCGTTCTCGAT GGCCAATTCAGTTGCTTTGGATTGATCAGCAGCAACGCCTTAAATTCAACGT TGATGTTGTCGAAAGTGAATGGGGAAAAGGTGCCAATCTTGACATTCTGGAA TCAAAACTTGCTTCTGATTCTGCACACACTATAAAGGGCTGTTTTGCATTTGT TCATAATGAGACAGCAACTGGGTGTCACGAATAACTTGGCCAAAGTGAGAC AGATTCTTGATGC***CTACCAGCATTTAGTGAGGGTTAAT*** **Forward frame 1, 117 amino acids** IIADDIFLFF*TGNECLGKCLTNTLSLEIVPYRSRWPIQLLWIDQQQRLKFNVDVV ESEWGKGANLDILESKLASDSAHTIKGCFAFVHNETATGCHE
EST NO: 1565 ↓ 1565 (D3-3-1), P4.T5;C1,C2,C3; less in H1 **DETAILS:** P4.T5, U_HW-070501-Plate5c03.b1,	***CATTATGCTGAGTGATATCTTTTTTTTTCC***TATCTGCAGAGCTGGAGGAGTCC ACTACATGTACAGGGGAGATATGGGACTATGAATATATTCATTCTCAACAAA ACTTCACAACTAGGATGGTTATGTCTCAAATTTCTCAAGTAAAAGCAGCATG TCTACTCTTCCATATCATTGGAG***CTACCAGCATTTAGTGAGGGTTAAT***

sequence size (bp): 205 **BLASTX:** No significant similarity found **BlastN:** AC145449.8 Medicago truncatula clone mth2-20m15, complete sequence 183 3e-44	
EST NO: 1568 ↓ 1568-3, P4/T6; C1, C2, C3 **DETAILS:** P4/T6 U_HW-Plate4b10.b1, **sequence size (bp):** 179 **BLASTX:** emb|CAN79218.1| hypothetical protein [Vitis vinifera] 40.4 0.035	***ATTAACCCTCACTAAATG***GGGGGAAATAATTGTCCTTGAATTCCTGGTCCGAA GTTGACAAAGACTTCATTGCGATGAGCGTAGACAATACCTAAAGATACAAA ACACCAGTTGACTAAATGGAAATTCCTCTTTGCCATTTCGTATAT***CGAAAAAA*** ***AAAGATATCACTCAGCATAATG*** **Reverse frame 1, 59 amino acids** HYAE*YLFFSIYEMAKRNFHLVNWCFVSLGIVYAHRNEVFVNFGPGIQGQLFPPI **
EST NO: 1642 ↑ P4/T5; H1, H2, H3 **DETAILS:** P4/T5 eU_HW-070116_Plate5c08, **sequence size (bp):** 245 **BLASTX:** emb|CAE00491.2| 1-deoxy-D-xylulose-5-phosphate reductoisomera.. 52.0 1e-05	***CATTATGCTGAGTGATATCTTTTTTTTTCC***AACTCATTAAGTTGTTACCATGGC TCTCAATTTATGTTCTCCAACTGAACTCAACTCCATTTTTTTCACTGATCCCTT CAACTCTAACCGTCTCACGCCTAGACTCTCAGGTGGTCTTTCTTTGAGAAGA AAAGAATGTGGTGGAACAGTTTCTAGAAGAAGAGTTTCTTGCTCAGTGCAGT CACAACCA***CTACCAGCATTTAGTGAGGGTTAAT*** **Forward frame 2, 81 amino acids** IMLSDIFFFPTH*VVTMALNLCSPTELNSIFFTDPFNSNRLTPRLSGGLSLRRKECG GTVSRRRVSCSVQSQPLPAFSEG*

ESTs in DDRT-PCR AUTORADIOGRAPH FILM-6

EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 1571 ↓ 1571-1-2, P3/T4; C1, C2, C3, less in H1, H2, H3 **DETAILS:** P3/? U_HW-Plate3c10.b1, **sequence size (bp):** About 400bp **BLASTX:** emb|CAA04767.1| ripening-induced protein [Fragaria vesca] 54.3 6e-06 gb|AAO22131.1| quinone oxidoreductase [Fragaria x ananassa] 52.0 3e-05	***ATTATTAACCCTCACTAAATGCTGG***TGGAGATGGAACCCTTGTTATTCCGCTT GCCAACATGTTTTTGGCGCTTCCAAGGTGGCCCCTACTTCTTCTAGTGGGAA ACTGGGATTGTTGAGAAAGTTGGGAACTGATTTGGCTAAAAATTATACAAAG GACAATTTTGAATGCTTGCCGGATGAGTTTGATGTTGCGTTTGATACTGCACT CAAAACTGACTTTCCATTGGGGGTTCTGTCGTGTGTGTTGTATTTATGACCCC CCCACCACCCGGGGATAGGGGGGGTGAGTATTGGTTCCTGTGCGCCTGCCCC CCTCCCTGACTCCCTGCGGTCGGTCCTCCCAACGCGGCGAGC GCTTTCATCTCACATGGGGGAGGGAATCCAGTTATCCATGGAGT **Forward frame 2, 222 amino acids** LLTLTKCWWRWNPCYSACQHVFGASKVAPTSSSGKLGLLRKLGTDLAKNYTK DNFECLPDEFDVAFDTALKTDFPLGVLSCVLYL*
EST NO: 1633 ↓ P3/T5; C1, C2, C3 less im H1, H2, H3 **DETAILS:** P3/T5 U_HW-070116_Plate6f12, **sequence size (bp):** 445 **BLASTX:** gb|AAO33590.1|AF479308_1 putative caffeic acid methyl transferas 80.9 2e-14 dbj|BAC78828.1| caffeic acid O-methyltransferase [Rosa chinen.. 58.2 2e-07	***CATTATGCTGAGTGATATCTTTTTTTTTCC***CACCAATTAATTAATAATTCATGA AACTGATTAACAATAGTACTACATTCTCAACACTCACACAACACAAGACTCT GCTGTCTGACCTGATGGAACCAAAATCCCATTCACGAATCATAGCTTAATTA GAGTGACCCACCAACAATGTTTATTCAAATTATCATACAAAAAGGAACACTT GTCTAGCATAAACCAACAACAAAACTCCCATTAAAGCCACACATAACACGA AACCATATCATAAAACCAAAGTAACATCTCATGAATTCAGATTATTAGATTG GAAACGCCTCAATAATTGATGGCAGAGCATTGATTTTGATGATGTTGTAACG AGGGAATCCTGTCTCTTTAAAGAGATATTTCCAATTCTCTTCAGTTCTTTCTT TA***CCACCAGCATTTAGTGAGGGTTAAT*** **Reverse frame 3, 147 amino acids** *PSLNAGGKERTEENWKYLFKETGFPRYNIIKINALPSIIEAFPI**

ESTs in DDRT-PCR AUTORADIOGRAPH FILM-7

EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 1595 ↑ 1595 (D3-3-1), P8/T1; H2, H3 **DETAILS:** P8/P8; U_HW-070501-Plate5c04.b1, **sequence size (bp):** 611 **BLASTX:** dbj|BAE71297.1| hypothetical protein [Trifolium pratense] 308 2e-82 emb|CAA66481.1| transcription factor [Vicia faba var. minor] 285 1e-75 **CONSERVED DOMAIN:** pfam04774, HABP4_PAI-RBP1, Hyaluronan / mRNA binding family. This family includes the .. 68350 No 1e-09	***ATTAACCCTCACTAAATGGAGCTGG***TGAAGAGGGACGTCCACGAAGACCTTT TGATCGCCACAGCGGGACTGGGCGCGGAAGTGGATTCAAACGTGAAGGTGC TGGACGAGGCAATTGGGGAACCCAATCTGATGAAATTGCTCAGGTGACTGA GGAAGTTGCTAATGAAACTGAAAAGAATTTGGGTGAAAAGAAGCCTGCTGG TGAGGAAAAAGCAGCAAATGTTAGCAAGGAAACTCCTGCTAATGAAGCTGA AAAAAAGGAACCTGAGGATAAGGAGATGACACTGGAAAAGTATGAGAAAG TGCTGGAAAAAAAAAGGAAAGCCCTGCAGGCACTTAAAACTGAAGGAAGA AAGGTGGATGCTAAAGAGTTTGAATCCATGAAGCCATTGTCGTGCAAAAAA GACAATGTTGAGATCTTTGCTAAATTGGGATCTGACAAGGATAAGCGCAAA GAAGCTTTTGATAAGGAGAAGTCAAAGAAGGCTCTTAGCATTAACGAGTTTC TGAAGCCTGCTGATGGAGAGAAGTTCTACAACCCAGGTGGTCGTGGTGGAC GTGGTGGTCGCGGTGGCCGTGGTTCAAGGGGAGGAGGTTTTGGTGAAAATG CATACAGCAATGTC***CCAGCTCCATTTAGTGAGGGTTAAT*** **Forward frame 3, 216 amino acids** *PSLNGAGEEGRPRRPFDRHSGTGRGSGFKREGAGRGNWGTQSDEIAQVTEEVA NETEKNLGEKKPAGEEKAANVSKETPANEAEKKEPEDKEMTLEKYEKVLEKKR KALQALKTEGRKVDAKEFESMKPLSCKKDNVEIFAKLGSDKDKRKEAFDKEKS KKALSINEFLKPADGEKFYNPGGRGGRGGRGGRGSRGGGFGENAYSNVPAPFSE G*
EST NO: 1597 ↓ 1597-2a, P8/T1; C1, C2 **DETAILS:** P8/T1 U_HW-070116_Plate6g04, **sequence size (bp):** 173 **BLASTX:** ref|XP_001538725.1| predicted protein [Ajellomyces capsulatus.. 35.0 1.5 gb|AAU43741.1| EMP70 [Saccharomyces kudriavzevii IFO 1802] 33.1 5.6 ref|NP_013184.1| Emp70p [Saccharomyces cerevisiae] >sp|P32802.. 33.1 5.6	*T****ATTAACCCTCACTAAATGGAGCTGG***AGGATGCGGGGGATGGAGCTGCTGCA CGTGCACCTGAAGTCCCGCATGTCGTGGCTGTCAATTTGTAATTGTTCATTCT CTCTGAAATTTCTTCAGTTGGTTATTGTTCACTCCCCTC***TTAAAAAAAAAGATA TCACTCAGCATAATG*** **Forward frame 2, 57 amino acids** LTLTKWSWRMRGMELLHVHLKSRMSWLSICNCSFSLKFLQLVIVHSPLKKKDIT QHN
EST NO: 1597a ↑ 1597a-2a, P8/T1; H2, H3 **DETAILS:** P8/T1 U_HW-70116_Plate5h09 **sequence size (bp):** 304 **BLASTX:** gb|EAZ37628.1| hypothetical protein OsJ_021111 [Oryza sativa .. 75.9 8e-13 gb|EAZ01654.1| hypothetical protein OsI_022886 [Oryza sativa .. 75.9 8e-13	***ATTAACCCTCACTAAATGGAGCTGG***CAGAGATAAAGTTTCAGTATTCAATATC ATCTTGGCACCATTGGAGTGAGTCCAAGATGGTAGTCGAAAGTTCAATGTTG ACAAAGTTCCATTTTTCTCAATAGGAGAAAAGGTAAATGTAACTCGTAGGTA AGGATCCGATGAAGCAGCTGGAACAACTGTCTGAGTGAGCAAAATATTCCC AGATTTCCAATTAATTGAGCTTGATATGTACTGAATGATGTATAGACTAGGA TCATTCCCTTCCTC***TTAAAAAAAAAGATATCACTCAGCATAATG*** **Reverse frame 2, 95 amino acids** LFF*EEGNDPSLYIIQYISSSINWKSGNILLTQTVVPAASSDPYLRVTFTFSPIEKNG TLSTLNFRLPSWTHSNGAKMILNTETLSLPAPFSEG*

gb|EAZ01658.1| hypothetical protein OsI_022890 [Oryza sativa .. 75.9 8e-13 ref|NP_001058067.1| Os06g0612900 [Oryza sativa (japonica cult.. 75.9 8e-13 dbj|BAD35522.1| hypothetical protein [Oryza sativa (japonica .. 75.9 8e-13 emb|CAB88260.1| putative protein [Arabidopsis thaliana] 68.9 9e-11 ref|NP_196800.2| unknown protein [Arabidopsis thaliana] >gb|A.. 68.9 9e-11	
EST NO: 1598 ↓ P8/T1; C3, less in H3 **DETAILS:** P8/T1 U_HW-070116_Plate6g03, **sequence size (bp):** 214 **BLASTX:** ref|XP_001538725.1| predicted protein [Ajellomyces capsulatus.. 40.0 0.046 gb|EAY87847.1| hypothetical protein OsI_009080 [Oryza sativa .. 34.3 2.5	***ATTAACCCTCACTAAATGGAGCTGG***AGGATGCGGGGGATGGAGCTGCTGCAC GTGCATCTGAAGTCCCGCATGTCGTGGCTGTCAATTTGTAATTGTTCATTCTC TCTGAAATTTCTTCAGTTGGTAATTGTTCACTCCCCTCTGAACTTTCTTCAGTT TCAGTTGGTATTTGTTCACTCCGTC***TTAAAAAAAAAGATATCACTCAGCATAAT G*** **Forward frame 2, 71 amino acids** LTLTKWSWRMRGMELLHVHLKSRMSWLSICNCSFSLKFLQLVIVHSPLNFLQFQ LVFVHSVLKKKISLSIM Similar to 1597-2a: Optimal Global aligment Alignment score: 293 Identities: 0,7953488
EST NO: 1606 ↑ P8/T3; H1, H2, H3 **DETAILS:** P8/ T4/T5/T6? e U_HW-070116_Plate6g02, **sequence size (bp):** 337 **BLASTX:** sp|Q41001|BCP_PEA Blue copper protein precursor >emb|CAA80963.. 107 2e-22 ref|NP_177368.1| plastocyanin-like domain-containing protein .. 99.4 6e-20 ref|NP_173664.1| plastocyanin-like domain-containing protein .. 92.4 8e-18 **CONSERVED DOMAIN:** pfam02298, Cu_bind_like, Plastocyanin-like domain. This family represents a domain fou.. 66026 No 3e-14	***ATTAACCCTCACTAAATGGAGCTGG***ACACACAGCGGATGAAGTTAAAGAAAG TGATTACAAATCATGCAAAACAGGAAACTCAATAAGTACAGACAGCAGTGG TGCCACAACCATTCCTCTAAAGAAATCAGGCACTCATTACTTCATATGTGCT GTTCCTGGACATTGCATTGGTGGCATGAAACTTTCTGTTAAAGTTGTTAGTAA ACCTTCTTCTTCTTCTTCTACTGCTCCTTCTGCAACACCATCATCATCAGGGA AACCTTCACCTTCTGATTCCACTCCTACCACTACTACTACACCAACTA***GAAAA AAAAGATATCACTCAGCATAATG*** **Forward frame 3, 111 amino acids** *PSLNGAGHTADEVKESDYKSCKTGNSISTDSSGATTIPLKKSGTHYFICAVPGH CIGGMKLSVKVVSKPSSSSTAPSATPSSSGKPSPSDSTPTTTTTPTRKKRYHSA
EST NO: 1601 ↓ 1601-1-2, P8/T2; C1, C2, C3; less in H1, H2 **DETAILS:** P?/? U_HW-Plate3d03.b1, **sequence size (bp):** > 400 **BLASTX:** gb|AAN32497.1| ATP synthase beta subunit [Cypripedium passerinum 207 2e-52 gb|AAG43915.1|AF213784_1 ATP synthase beta subunit [Primula gaub 207 2e-52 gb|AAL37115.1| ATP synthase beta subunit [Aletris farinosa] 207 3e-52 ref|YP_001381743.1| ATP synthase CF1 beta subunit [Medicago trun 206 3e-52 gb|ABN08803.1| H+-transporting two-sector ATPase, alpha/beta .. 206 3e-52 **CONSERVED DOMAIN:** cd01133, F1-ATPase_beta, F1 ATP synthase beta subunit, nucleotide-binding domain. The .. 29999 No 2e-51	***ATTAACCCTCACT***AGATGGTTCTGGATCGGTCAAATCGTCGGCAGGTACATA AACTGCTTGAATAAAAGTTATAGACCCTTCTTTGGTAAAAGTAATTCTTTCTT GTAAAGAACCCATTTCAGTACCCAGGGGGGGTTGATAACCCACAGCACAAG GCATTCGGCCCAATAAGGCGGATACTTCGGATCCTGCTTGGACAAAGCGAA AAATATTGTCAATAAATAAAATTACGTCTGGTTCATTGACCTCTCGGAAATA TTCCGCCATATTTAGGGCAGTTAAACCAACTCTCTTGCTAGCTCCGGGCGGTT CTTTCTTTTGACCGTAAACTAGAGCTCCGTTTGATTCCCCAATATTTTTTCCAT CCGTAACTCCAGATTCTTTCGGCTGCGGAGGCGATGGTTTCCCTCACGAGTA TTTTTCTGCTACTCCGCCAACCCGTTTCCCCTTAGGGAGCCCGGCCAGGCTTA GTGAATTATTGATAGCGAACCGCTTTCAAATACGCAA **Reverse frame 1, 169 amino acids** LRI*KRFAINNSLSLAGLPKGKRVGGVAEKYS*GKPSPPQPKESGVTDGKNIGES NGALVYGQKKEPPGASKRVGLTALNMAEYFREVNEPDVILFIDNIFRFVQAGSE VSALLGRMPCAVGYQPPLGTEMGSLQERITFTKEGSITFIQAVYVPADDLTDPEP SSEG*
EST NO: 1609 ↑ 1609 (D4-2), P8/T3; C1, C2 **DETAILS**: P8/T3 U_HW-070501-Plate5c05.b1, **sequence size (bp):** 292 **BLASTX:** gb|**EAZ25827.1**| hypothetical protein OsJ_009310 [Oryza sativa .. 48.1 2e-04 gb|EAZ25827.1| hypothetical protein OsJ_009310 [Oryza sativa .. 48.1 2e-04	***ATTAACCCTCACTAAATGGAGCTGG***CCCAATCACTAGTGGAGGCACTTGTTTT TCCAACTATCACTCCATTGACAACATTTTCTGAAGAAAGGCCAGCAACTGGG GAGTTGGAAATTGATGACCTCTCGTATGGTTCTTCTTCACATGAGTTTCCTGA AAAAAATAGCCAATCCAGGCCCACAAAATTGGAGCAGCTGAAAGAATCTCC GTGCTCAAAATAGTAAATATGATGACTTTCAGCACAGCGCTCTCTCTGCATG T***CTAAAAAAAAAGATATCACTCAGCATAATG*** **Reverse frame 3, 96 amino acids** LC*VISFFLDMQRERCAESHHIYYFEHGDSFSCSNFVGLDWLFFSGNSCEEEPYE RSSISNSPVAGLSSENVVNGVIVGKTSASTSDWASSI
EST NO: 1611 ↑ 1611-1-2, P8/T5; H1, H2 **DETAILS:** P8/? U_HW-Plate3d05.b1, **sequence size (bp):** >370 **BLASTX:** gb|AAK37555.1|AF349572_1 SHOOT1 protein [Glycine max] 157 4e-37 emb|CAN75787.1| hypothetical protein [Vitis vinifera] 131 2e-29 **BlastN:** AC153124.20 Medicago truncatula clone mth2-21e10, complete sequence 336 6e-90 ..... NM_104423.3 Arabidopsis thaliana binding / protein binding (AT1G55480) mRNA, complete cds 136 1e-29	***ATTAACCCTCACTAAATGGAGCTGG***CCAAATTTCGGTCCCAAAAACAGCACT AGTGGCAAGAACTTTATCCCCAACATTGAAAACTCCAGCTTTGTCAGCTGAA CCTCCAGGTGCAATAGCATCAATGTAAGTTCCACCATCTCTTCCCTTAACAA ATTTGATTCCATAAGGTTGATCAATCTCTACCTCATACTCTCCATATGGTTCC TCATCTCCTTCACTCTCTTGTTTGGATGCATCGGTTGAACCCTTAACCACAAA AAGGAAGGGCCTTGACAAAAGGGTGTTAGAGAAAGAGTGGTTTCTTGAAAA AAACAACTTGGTGGCGGGAAAAAAATAATTTGGATTTTGCTTATAGTTTATT TTTGGAAATGAAAGAGAAAAGTAAAGAGACGAGTGAGTACACATAGATGAT TTTGACTTGGTTATTAGGCTAAACTAACAAAAAGAACATTACTTGGAGGAGT TGATTGAAAATTGTTGAATATATGAAAATAACAACACCCCCCCCCAACCTAA TGAACCACTTCGGAATTTCCAGCCCCGCCCGCCGCTCG **Reverse frame 3, 185 amino acids** SGGRGWKFRSGSLGWGGVLLFSYIQQFSINSSK*CSFC*FSLITKSKSSMCTHSSL YFSLSFPKINYKQNPNYFFPATKLFFSRNHSFSNTLLSRPFLFVVKGSTDASKQES EGDEEPYGEYEVEIDQPYGIKFVKGRDGGTYIDAIAPGGSADKAGVFNVGDKVL ATSAVFGTEIWPAPFSEG Query 403 THSSLY…… Sbjct 7 SYPSLY…
EST NO: 1612 ↑ 1612-2, P8/T5; H2, H3 **DETAILS:** P8/T5 U_HW-Plate3d06.b1, **sequence size (bp):** 198 **BLASTX:** ref|NP_192536.1| ATCSLC12 (Cellulose synthase-like C12); tran.. 111 1e-23	***ATTAACCCTCACTAAATGGAGCTG***GAAGGATGTTGAGAAATGACATGACGGC TGGGATGTAACAAACAACCCAGGCCGGAATCTCGGCCTCTGGTACGAACATT GTCATGGGAAGAATTATGCAGAATAGAGTGAATGAATAGAATGGTAACACC AGTTTTCTAAGAA***GGAAAAAAAAAGATATCACTCAGCATAATG*** **Reverse frame 3, 65 amino acids** LC*VISFFFLLRKLVLPFYSFTLFCIILPMTMFVPEAEIPAWVVCYIPAVMSFLNILP APFSEG*
EST NO: 1619 ↑ 1619-1-1, P8/T6; H1, H2, H3 **DETAILS:** P8/T7/T6, U_HW-Plate2b07.b1, **sequence size (bp):** 363 **BLASTX:** emb|CAO41131.1| unnamed protein product [Vitis vinifera] 110 4e-23	***ATTAACCCTCACTAAATGGAGCTGG***TAAATAAAGAAAAGTTGTTACCTGAAG GAAGTATTATTTCAATTCCAAAAGACCCTGTTTCCACCATTTTACTAATAAAC TCATCAATATGTACCATGCAAAGAATTGCAGTACTGGAGGATGACAAATTGG TTGAGTTACTATTGGAACCAGTTAAAACTAATGTGCAATGTGATAGTGTGTA TGTTGGGGTAATCACAAAGCTTCTTCCTTCTATGGGAGGAGCTTTTGTTGGTA TTGGGAATTCTAAACTCCCTCTTTTGGAGTTTAAGTCATACAAAGAACCATTT ATATTCCCTCCGTTTTG***TCAAAAAAAAAAGATATCACTCAGCATAATG***

gb|AAD27911.1| putative ribonuclease E [Arabidopsis thaliana] 106 5e-22 ref|NP_178508.2| glycoside hydrolase starch-binding domain-co.. 106 5e-22 **CONSERVED DOMAIN:** COG1530, CafA, Ribonucleases G and E [Translation, ribosomal structure and biogenesis]. 31719 Yes 0.009	**Forward frame 1, 121 amino acids** INPH*MELVNKEKLLPEGSIISIPKDPVSTILLINSSICTMQRIAVLEDDKLVELLLE PVKTNVQCDSVYVGVITKLLPSMGGAFVGIGNSKLPLLEFKSYKEPFIFPPFCQK KKISLSIM
EST NO: 1631 ↑ 1631-2a, P8/T8; H1, H2 **DETAILS:** P8/T8 U_HW-070116_Plate5c09, **sequence size (bp):** 191 **BLASTX:** No significant similarity found **Forward frame 2** ref|ZP_01660568.1| two component transcriptional regulator, F.. 32.3 8.7	***ATTAACCCTCACTAAATGGAGCTGG***CCGAGTTGCGTTGCACAGAGTTCCTCCG GTGGCACTAAGCCACCTCCAGCACCAAGCCCACTAAGCATGCCCATCAAGGT GTCCAGGCCAACATTTCCCCGCGTGCCTGTGCCATTGCCCCCTAGGTTACCA GCCC***GCAAAAAAAAAGATATCACTCAGCATAATG*** **Forward frame 2, 63 amino acids** LTLTKWSWPSCVAQSSSGGTKPPPAPSPLSMPIKVSRPTFPRVPVPLPPRLPARKK KDITQHN
EST NO: 1623 ↑ P8/T7; H1, H2, H3 less in C1, C2, C3 **DETAILS:** T7/T7 U_HW-070501-Plate6b12.b1 **sequence size (bp):** 384 **BLASTX:** emb|CAO47145.1| unnamed protein product [Vitis vinifera] 180 3e-44 ref|NP_051110.1| photosystem I subunit VII [Arabidopsis thali.. 178 1e-43 **CONSERVED DOMAIN:** cd01916, ACS_1, Acetyl-CoA synthase (ACS), also known as acetyl-CoA decarbonylase, is .. 29678 Yes 6e-07 CHL00065, psaC, photosystem I subunit VII. 79282 Yes 3e-37	***CATTATGCTGAGTGATATCTTTTTTTTTG***AGCACGGGTTTTTGCCAGTAAAAA AACGTATTCGTGTGGTGTTTTTTGAAACGTATCAATAAGCTAGACCCATACTT CGAGTTGTTTCATGCCATAAATAAACTCGAACACTTAAAAAATCCGTTGGAC AAGCGGACTCACACCTCTTACAACCAACACAGTCCTCTGTTCTTGGGGCAAA AGCTATTTGCTTGGCTTTACATCCATCCCAGGGTATCATTTCTAATACATCTG TGGGACAGGCTCGGACACATTGAGTACATCCTATACATGTATCATAAATCTT TACTGAATGTGACATTGTATCTATAGTAATTTTTTAACA***TCAAAAAAAAAGAT ATCACTCAGCATAATG*** **Reverse frame 1, 128 amino acids** HYAE*YLFF*C*KITIDTMSHSVKIYDTCIGCTQCVRACPTDVLEMIPWDGCKAK QIAFAPRTEDCVGCKRCESACPTDFLSVRVYLWHETTRSMGLAY*YVSKNTTRI RFFTGKNPCSKKKISLSIM Query 330 MSHSVK Sbjct 1 MSHSVK
EST NO: 1626 ↓ 1626 (D1-2), P8/T8; C1, C2, C3 ; less in H2, H3 **DETAILS:** P8/T8, U_HW-070116_Plate6a09, **sequence size (bp):** 394 **BLASTX:** gb|ABD33010.1| Fumarylacetoacetase [Medicago truncatula] 225 8e-58 emb|CAO21140.1| unnamed protein product [Vitis vinifera] 202 4e-51 emb|CAN77935.1| hypothetical protein [Vitis vinifera] 202 4e-51 **CONSERVED DOMAIN:** pfam01557, FAA_hydrolase, Fumarylacetoacetate (FAA) hydrolase family. This family cons.. 79833 No 2e-07	***CATTATGCTGAGTGATATCTTTTTTTTTG***CTGCAAGGTTCACATTAAACCTGTT GATCACAAAGATTCGACCGTTGTGACACGGAGTAACCTCAAACACTTATATT GGACATTGACTCAACAACTTGCTCACCACACAATCAATGGTTGCAACCTGAG GCCAGGCGATCTCCTTGGAACCGGGACAATTAGTGGCCCTGAGCCAGAGTC GCGTGGATGCTTGTTAGAGTTAACCTGGAACGGACAAAATTCGCTGAATGGT TTAGATAGAAAATTTCTTGAAGATGGAGATGAAGTCGTCTTAACTGGATTTT GCAAGGGAAATGGTTACACTGTTGGGTTTGGCACCTGCTCAGGCAAGATTGC TCCA***CCAGCTCCATTTAGTGAGGGTTAAT*** **Forward frame 1, 131 amino acids** HYAE*YLFFCCKVHIKPVDHKDSTVVTRSNLKHLYWTLTQQLAHHTINGCNLRP GDLLGTGTISGPEPESRGCLLELTWNGQNSLNGLDRKFLEDGDEVVLTGFCKGN GYTVGFGTCSGKIAPPAPFSEG*

ESTs in DDRT-PCR AUTORADIOGRAPH FILM-12

EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 1644a ↑ 1644a-1-2, P10/T7; H1, H2, **H3** **DETAILS:** P10/T7 U_HW-Plate3d07.b1, **sequence size (bp):** 431 **BLASTX:** ref|NP_001092806.1| hypothetical protein LOC374470 [Homo sapi.. 37.0 0.40	***CATTATGCTGAGTGATATCTTTTTTTTTG***AGCATCTATTTCAAGGTTTCATCTT TTTTCTTTATTGTTGGATTCTTACATAGCTCACACATCCTCGATTTTCTCAACC TGTTTTCCTCTTCCGTTCTGTTTCAATAGGGTTTCTATTTTTTTCCCTTTCCCTC TCCGTACCTTATTTATTTTTTCCCCACAAATTCTTAAATTCGTCGACGTAAAA CCTCGATTCATTTGTGCATTGAATTGGTGCTGCATTTGCTCGATTTTGCTCTCT GCGAATTGAACTTGATGAAAGGATGCTATGAAGTATAGTTCTTGGAAATAAC TCTGAGTGTAGCACTCTGAATTTTGGAATATAATGTTCACTCCGAAAATGGA TTCTGCAAGGGATGCTGGTGGTGTTGCAGCAG***GGACGGTGCTTTAGTGAGGG TTAAT*** **Reverse frame 3, 143 amino acids** *PSLKHRPCCNTTSIPCRIHFRSEHYIPKFRVLHSELFPRTILHSILSSSSSIRREQNRA NAAPIQCTNESRFYVDEFKNLWGKNK*

ESTs in RGA-DDRT-PCR AUTORADIOGRAPH FILM-1

EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 1990 ↓ 1990-2, PtoFenS/T6; C3, U **DETAILS:** PtoFenS/T6 U_HW-Plate3e08.b1 **sequence size (bp):** 459 **BLASTX:** dbj|BAE99990.1| peptidylprolyl isomerase [Arabidopsis thaliana] 195 7e-49 ref|NP_199668.1| peptidyl-prolyl cis-trans isomerase, putativ.. 195 7e-49 emb|CAO15270.1| unnamed protein product [Vitis vinifera] 192 7e-48 emb|CAO46497.1| unnamed protein product [Vitis vinifera] 192 7e-48 dbj|BAB02082.1| peptidylprolyl isomerase; FK506-binding prote.. 182 4e-45 ref|NP_189160.3| ROF1 (ROTAMASE FKBP 1); FK506 binding / calm.. 182 4e-45 **CONSERVED DOMAIN:** pfam00254, FKBP_C, FKBP-type peptidyl-prolyl cis-trans isomerase. 79537 No 3e-14	***ATGGGAAGCAAGTATTCAAGGC***CTTTGATACCTTTTTCTCCTCCTCGCTGAAG GAGGAATCATACTCAATGTACTTTACAGCCTTCTCATATCTTTTGGAAGCTCT TGCATATTTACCGGCTTTAAACAAAACATTCCCTTCTTCTTTCTTCTTACCAG CAGCTTCAATTTTCTCTTGAGTGTTCATATCCCAAGATTCCTTCTCCTTGTCA AATGACACTAGCTCAACTTCATAATAGACAGTTGAATTAGGAGGAATAACA GCCAACTCCTGCTGAGACTCTGATGAACCAAATGCATATTCTGGAGCAATAG TCAATAATGCTACCTCACCCTTCTTCATAGTCATTACAGCTTTATCAAGTCCA TCAATGACTTGCTCCTCATCTGTTTTGAATTCAAACAGCTTCTCTTCTTCATC GCGGCCCTTCT***CAAAAAAAAAAGATATCACTAGTGAAT*** **Reverse frame 3, 152 amino acids** SLVISFFFEKGRDEEEKLFEFKTDEEQVIDGLDKAVMTMKKGEVALLTIAPEYAF GSSESQQELAVIPPNSTVYYEVELVSFDKEKESWDMNTQEKIEAAGKKKEEGNV LFKAGKYARASKRYEKAVKYIEYDSSFSEEEKKVSKALNTCFP
NO: 1998 PtoFen-S/T3, H3,C3,I,U **DETAILS:** PtoFenS/?, **sequence size (bp):** > 473 **BLASTX**	***ATGGGAAGCAAGTATTCAAGGC***CACATTCTACCCTAATGGGTATTCAATTGA AGCAAATATCACATCCAAACCCCAAATATTGGATTCATCATTAAAATCCACT ACTACTGCAGGTCAAGATGGTGATAAGTTGAGTTTGATTAAACTTGCTAGCA TAATTGAGGTTTCGGCAAAGAAAGGAACTCGCGATCTCAAGCTCCAAAACA

emb|CAO71692.1| unnamed protein product [Vitis vinifera] 187 2e-46 ref|NP_179336.1| leucine-rich repeat family protein [Arabidop.. 155 8e-37 **CONSERVED DOMAIN** COG4886, COG4886, Leucine-rich repeat (LRR) protein [Function unknown]. 1e-04	AGTTAATGGACCGAGTCGATTGGCTACCTGATTCGATAGGAAAGTTATCTAG TTTGGTCACCCTTGATTTATCAAAGAATAGGATTGTTGCTTTACCTTCCACAA TTGGTGGCCTTTCCTCACTGACCAAATTGGACTTGCATTCCAATAGGATCAC AAAAATTCCTGATTCTGTTGCAAATCCCCTAAGCCTTGTCAACTACTTCTGAT GTTTATTACACTTAACCCCTTACCTGCTTCTTTAGACCAAAAAGAACCCCGTG AAT **Forward frame 3, 157 amino acids** GKQVFKATFYPNGYSIEANITSKPQILDSSLKSTTTAGQDGDKLSLIKLASIIEVSA KKGTRDLKLQNKLMDRVDWLPDSIGKLSSLVTLDLSKNRIVALPSTIGGLSSLTK LDLHSNRI TKIPDSVANPLSLVNYF Similar to 262: Optimal Global aligment Alignment score: 824 Identities: 0,9059305
EST NO: 2007 ↓ S1/T2; C3, U **DETAILS:** S1/T2 U_HW-070501-Plate5f10.b1 **sequence size (bp):** 549 **BLASTX:** gb|ABV82363.1| IP20188p [Drosophila melanogaster] 33.5 6.6 gb|AAY85542.1| male accessory gland protein [Drosophila simulans 33.5 6.6 ref|NP_648459.1| CG6168-PB [Drosophila melanogaster] >gb|AAF5.. 33.5 6.6 **BLASTP: Forward frame 1, 177 amino acids** emb|CAB10321.1| UFD1 like protein [Arabidopsis thaliana] >emb.. 34.7 1.6	***GGGGGGGTGGGGAAGACGGC***AAAAAAGATGATGATGGAGATGGTGCATTTG GAGAAGGAGAAGAGGAATTGTCATCTGAAGATGGAGGTGGATATGGCAACA ATTCCAACAATAAGAGCAACTCAAAGAAGGCTCCTGAAGGTGGTGCTGGTG GGGCAAATGAAAATGGAAAAAAGGAAAACAATGAAGATGGTGAAGGTGAT GACCATGATGAAGATGACGATGAGGACGAGGACAACAAAAAAGAGGATGA GGTCGGAAAAAAGGACAAAAAAGATGGTGTTGAAAAAGAGAATGAAGAGG AAAAAAAAAACGAAGATGAGGAACCCTTGCACCCACCAAAGAAAAGGAAG AAGTGAATTTAGCTTAGTCAAGCCTCCTTTTGTATTGTTGTGTACGAATTTGG CTTCTAACTTCCCATGATGTTTTTTTTAAGTATTAAACTTCTATGTTCTTGTTT TCTTTATAAGGAGGCCTATGTAGGACATGTTTTAATTGATATCTGTGTTTACC ATTGC***GTAAAAAAAAAAGATATCACTCAGCATAATG*** **Reverse frame 3, 177 amino acids** LFFLRNGKHRYQLKHVLHRPPYKENKNIEV*YLKKTSWEVRSQIRTQQYKRRLD * **Forward frame 1, 177 amino acids** GGVGKTAKKMMMEMVHLEKEKRNCHLKMEVDMATIPTIRATQRRLLKVVLV GQMKMEKRKTMKMVKVMTMMKMTMRTRTTKKRMRSEKRTKKMVLKKRM KRKKKTKMRNPCTHQRKGRSEFSLVKPPFVLLCTNLASNFP*
EST NO: 2010 ↑ S1/T3, H3, I **DETAILS:** S2/?, U_HW-070116_Plate6f05, **sequence size (bp):** 414 **BLASTX:** ref|NP_744217.1| drug resistance transporter, EmrB/QacA famil.. 33.5 4.5 ref|XP_001631372.1| predicted protein [Nematostella vectensis.. 32.7 7.7 ref|XP_784522.1| PREDICTED: hypothetical protein [Strongyloce.. 32.7 7.7 ref|XP_380213.1| hypothetical protein FG00037.1 [Gibberella zeae 32.7 7.7	***GGGGGGGTGGGGAAGACGAC***TGATGATTTCCACCTGGGGTGCCATACCTGTA AGGAAATGGGCTGGTTGGTCCTGATCGGGATATACCGCGCGTGACTACTTCT AAGATACCAACGAAAGCGATTGTAACCTATGTTAGATTTTTTCTGTCTCCAA ACAACGAATAAGGTGAGAAGAACCTTTTACTTATGGACCCATGTCCTATGGA CTTAATGATTCTGATGATAACTCGGTCTTTCAACCTTACAGATCCGTTTCCTG CGTGGGGAAAGAACATGAACTAGTTATCCTCTGTGTTGCTACATTGCTGGAC TCCTTAATCGATATTTCTCCCATAAAGCTCAACACATGCTTCAGTGGTGAGA ATCAAATTCTCGCGAAAAATCATCTTCATCGGCATCATGCTACTTTACA **Forward frame 2, 137 amino acids** GGWGRRLMISTWGAIPVRKWAGWS*SGYTARDYF*DTNESDCNLC*IFSVSKQR IR*EEPFTYGPMSYGLNDSDDNSVFQPYRSVSCVGKEHELVILCVATLLDSLIDIS PIKLNTCFSGENQILAKNHLHRHHATL

ESTs in RGA-DDRT-PCR AUTORADIOGRAPH FILM-3

EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 2132 ↓ 2132-1, Ptokin-1/T2; C3, U3 **DETAILS:** U_HW-Plate2d07.b1, **sequence size (bp):** 287 **BLASTX:** ref|NP_001054720.1| Os05g0160200 [Oryza sativa (japonica cult.. 140 3e-32 gb|ABK42077.1| ubiquitin extension protein [Capsicum annuum] 138 1e-31 pir||S42643 ubiquitin / ribosomal protein S27a - potato (fragmen 138 1e-31 ref|XP_001271126.1| ubiquitin [Aspergillus clavatus NRRL 1] >.. 137 2e-31 **CONSERVED DOMAIN:** **Ubiquitin (includes Ubq/RPL40e and Ubq/RPS27a** cd01803, Ubiquitin, Ubiquitin (includes Ubq/RPL40e and Ubq/RPS27a fusions as well as h.. 29205 No 2e-32 **Similar to 645 and 1934**	***GCATTGGAACAAGGTGAA***GAGTTGATTCCTTCTGGATGTTGTAGTCGGCGAG AGTGCGGCCGTCTTCGAGTTGTTTTCCGGCGAAGATGAGTCGTTGTTGGTCC GGTGGAATCCCTTCCTTGTCTTGGATTTTGGCTTTGACGTTGTCGATTGTGTC AAAGGATTCAACCTCGAGGGTGATTGTTTTACCCGTTAGGGTTTTCACGAAG ATCTGCATCTTCGGTCGATTAGGGTTTGCGCCGAGGGAAGGAAAATGG***GTAA AAAAAAAGATATCCCTCAGCATAATG*** **Reverse frame 3, 95 amino acids** IMLRDIFFFTHFPSLGANPNRPKMQIFVKTLTGKTITLEVESFDTIDNVKAKIQDK EGIPPDQQRLIFAGKQLEDGRTLADYNIQKESTLHLVPM

ESTs in RGA-DDRT-PCR AUTORADIOGRAPH FILM-4

EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 2150 ↓ 2150-1-1, RLRRfwd/T5; C3, U **DETAILS:** RLRRfwd/T5 U_HW-Plate2b12.b1 **sequence size (bp):** 364 **BLASTX:** gb|ABQ41357.1| mitochondrial dihydroorotase [Vicia faba] 127 3e-28 dbj|BAF00239.1| dihydroorotase [Arabidopsis thaliana] 112 6e-24 ref|NP_194024.1| PYR4 (DIHYDROOROTASE, PYRIMIDIN 4, dihydroor.. 112 6e-24 **CONSERVED DOMAIN:** cd01294, DHOase, Dihydroorotase (DHOase) catalyzes the reversible interconversion of c.. 73253 No 4e-19	***CGCAACCACTAGAGTAAC***AGACTCCAATAAATGGTACCTAACGAACAAACTG CGGAGAGTTGGAGACCAGTCAGACAAGCATTGCCTCCCATTCAAGTGTTTCA CCGGCAAACATGGGAATAATATCTCCAAATGGAAATGAAAAACACTCGGGT ACTTTCCAAGGAGATTTCCTCAGTTTTATCTTTGACTTGTTTCTGGGGATATC ATAGAAGTCGGGTCCATTAAAACTTGTAAAAGCCTCAAGCTTGTCAAGTGCA CCAGCCTAAAACAGGGAACAATTTCATAAAACATTAAACCCCACAACTTTCC AAGGGAAGCAAGCAAAATATGAA***GGAAAAAAAAAGATATCACTCCCATAATG*** **Reverse frame 1, 121 amino acids** HYGSDIFFFPSYFACFPWKVVGFNVL*NCSLF*AGALDKLEAFTSFNGPDFYDIPR NKSKIKLRKSPWKVPECFSFPFGDIIPMFAGETLEWEAMLV*
EST NO: 2163 ↓ 2163- D23-1, RLRRfwd/T7; C3, U **DETAILS:** RLRRfwd/T7 U_HW-070116_Plate5g03 **sequence size (bp):** 215 **BLASTX:** No significant similarity found	***CATTATGCTGAGTGATATCTTTTTTTTTGA***TTAAATCTAAATTTTTAATGAAGT GAATAACCCACCTTTCCCACGAATGGGGAGGAGTCATATGGCATCAGTATAT ATGTAGTATAGAGTATCAGTTATGTTCGCCAACGCCTGTTAATTTTAACAGCT ACTGTAAACAAGCACTTCAATCAACTCACTCCGTACAAT***GTTACTCTAGTGG TTGC***

BlastN: AF100336.1 Dendrobium grex Madame Thong-IN putative phenylalanine ammonia-lyase (ovg43) mRNA, partial cds 60.8 2e-06	
EST NO: 2166 ↓ B28 (2161, RLRRfwd/T7; 2166, RLRRfwd/T8; 2163, RLRRfwd/T7); C3, U **DETAILS:** Primers found: RLRRfwd/T7, **sequence size (bp): 240** **BLASTX:** gb|AAL47004.1| unknown [Davidia involucrata] 134 2e-30 gb|ABV89652.1| early-responsive to dehydration 4 [Brassica rapa] 128 1e-28 ref|NP_564354.1| ERD4 (EARLY-RESPONSIVE TO DEHYDRATION 4) [Ar.. 126 4e-28 **CONSERVED DOMAIN:** pfam02714, DUF221, Domain of unknown function DUF221. This family consists of hypothet.. 66403 No 6e-09	***CGCAACCACTAGAGTAAC***ATAAAACAATTGTGACAATCAGCATGTCACTGGG AATTCTTGTTGCATAACCAGGATCTCCGGGAGCCCAAGCTTCTTTCAACTCG GCCTCGGTCTTACAAAGATACTTTTTCTTCAAATGGTACATAATAAGAGGAA CTAATCGGGACAGCTCGAGGCCATAGCCAACAAAAAATTTCAGGGCCACGT AGG***TCAAAAAAAAAGATATCACTCAGCATAATG*** **Reverse frame 3, 79 amino acids** LC*VISFFLTYVALKFFVGYGLELSRLVPLIMYHLKKKYLCKTEAELKEAWAPG DPGYATRIPSDMLIVTIVLCYSSGC Query 223 SFFLTYVAL Sbjct 7 TFFLTYVAL
EST NO: 2180 ↑ 2180-D22-1; RLLfwd/AS3; H3, I **DETAILS:** RLRRfwd/AS3 U_HW-070116_Plate5g04, **sequence size (bp):** 241 **BLASTX:** No significant similarity found **BlastN:** XM_001018177.1 Tetrahymena thermophila SB210 hypothetical protein (TTHERM_00283210) mRNA, complete cds 50.0 0.004	***CGCAACCACTAGAGTAAC***AAAAGCATAACACCAAAGCACACTATTTTTTTAA GGACCAAATTATTATATAATAACTTATTTTATTTACGTCTACCAAGTAATTGA TAATATGAAAATTAATCAAGGCCACAAAAAACCCATAAAAATGTCACTATT ATATACATTCCATAAAATCATCACATAACACCTTGATTTGTGTCATCTACCAC CGGAATTGAAGTTC***GGCCTCCCCTTCACCCTC***
EST NO: 2191 ↓ 2191- D23-1, RLRRfwd/AS7; C3, U **DETAILS:** RLRRfwd/AS7 U_HW-070116_Plate5g02 **sequence size (bp):** 511 **BLASTX:** gb|ABC59094.1| cytochrome P450 monooxygenase CYP704G9 [Medicago 250 4e-66 **CONSERVED DOMAIN:** pfam00067, p450, Cytochrome P450. Cytochrome P450s are haem-thiolate proteins involved.. 79443 No 4e-23	***GAAGGCGAAGGGGAGGCC***ATTAATATTTGTCTCTCTGAAACGCATACACATG CAGGCCACCATCCATTTAGTAGAGTGAGCGTGGTTCTATATCTTACCAATTT ATTTTTGGTCTGCTAATTTGAAGCTGTGGCTACCTAACAGGATTGCAGCAAA TACTTTCATCTGTCTGTATGCAAACTCCTTGCTAAGGCAAATTCTTGGACCCG CCTGGAAGGCTGTAAACTTAAAAGGGCTTTCTTTCTGAAATTTTCCATTTTCA TCAATCCATCTCTCTGGCCTGAATTTCTCAGCATCTTCACCCCACAAGAATTT CATCCTTCCCATAACATAAGGTTGAAATGACACAAGATCACCTTTCCTCACA CTAAATCCATCTGGCCACTTGTCATCTGAAAAACAATACTTGCTTTCCACTGG AAGGGCTGGATGGAGTCTGAGTGTTTCAGTCAAAGCTGCATGCAGATACTGC ATCTTTTCCATGCTTTCTTCA***GTTACTCTAGTGGTTGCG*** **Reverse frame 2, 170 amino acids** ATTRVTEESMEKMQYLHAALTETLRLHPALPVESKYCFSDDKWPDGFSVRKGD LVSFQPYVMGRMKFLWGEDAEKFRPERWIDENGKFQKESPFKFTAFQAGPRICL SKEFAYRQMKVFAAILLGSHSFKLADQK
EST NO: 2203 ↓ 2203- D8-3, RLRRfwd/AS8; C3, U **DETAILS:** RLRR frwd/AS8, e U_HW-070116_Plate5f02 **sequence size (bp):** 544 **BLASTX:** emb|CAO23744.1| unnamed protein product [Vitis vinifera] 161 2e-38 gb|EAZ25897.1| hypothetical protein OsJ_009380 [Oryza sativa .. 155 9e-37 gb|EAY88869.1| hypothetical protein OsI_010102 [Oryza sativa .. 155 9e-37 ref|NP_001049238.1| Os03g0192300 [Oryza sativa (japonica cult.. 155 9e-37 gb|ABF94410.1| expressed protein [Oryza sativa (japonica cultiva 155 9e-37 ref|NP_196577.1| unknown protein [Arabidopsis thaliana] >emb|.. 129 7e-29 emb|CAN64540.1| hypothetical protein [Vitis vinifera] >emb|CA.. 129 1e-28 emb|CAN67325.1| hypothetical protein [Vitis vinifera] 127 3e-28 gb|AAZ67530.1| 4D11_16 [Brassica rapa subsp. pekinensis] 125 1e-27 ref|NP_200787.1| unknown protein [Arabidopsis thaliana] >dbj|.. 124 3e-27 gb|AAL08561.1| auxin-regulated protein [Lycopersicon esculentum] 122 **CONSERVED DOMAIN:** pfam06136, DUF966, Domain of unknown function (DUF966). Family of plant proteins with .. 69644 No 1e-35 **BlastN:** AP006419.1 Lotus japonicus genomic DNA, chromosome 4, clone:LjT43O18, TM0307, complete sequence 197 4e-48 NM_121053.4 Arabidopsis thaliana unknown protein (AT5G10150) mRNA, complete cds 132 1e-28	***CGCAACACTAGAGTAAC***GAGAATGAGGCGTTGAGCGGAGCTCGTGTAACTCG TTTCCCTCATCTAATAATTCTCAGAGTATTCTTCATATTGAACCTCACTCTCCT CCTTGGGAGCATCTTCGATCATTCAATACCGCCACCTCGCTCTTTGAATTATT CTTATAAGTGCTAATAAAATTTTGTTCTGCCTGTTGTGGCTGTTTATCGCCCA AGTTTACTTGTTGAAATCTTCCGGAACATCCTTCAACTAGTTCAGATCCTTTA AGTACATACTCTGCTCCTTCTTCTGATGGATGTATTATATCCTTCAGTGCCAA GTCATGCCACACATATCCGCTCTTATAGTTTCTTTTAGTAGACCATGAATATT GAGAAGGCATGCCACTTCCTCTTAGCGCCATCAGCCGATCAAATACATCTTT CAAACGGAGAGGTTGATTAGGCAGAAGAGTGACTTCCATGAAATGTGGATG CTCCAATAAGCCATTACGAGATAGATAGTAAACAACTTGGACTTTTCTAATG ***GGCTTCCCCTTCGCCTTC*** **Reverse frame 3, 203 amino acids** *LKAKGKPIRKVQVVYYLSRNGLLEHPHFMEVTLLPNQPLRLKDVFDRLMALR GSGMPSQYSWSTKRNYKSGYVWHDLALKDIIHPSEEGAEYVLKGSELVEGCSG RFQQVNLGDKQPQQAEQNFISTYKNNSKSEVAVLNDRRCSQGGE
EST NO: 2204 ↓ 2204-D8-2; RLRRfwd/AS8; C3, U **DETAILS:** RLRRfwd/AS8 U_HW-070501-Plate5c10.b1 **sequence size (bp):** 455 **BLASTX:** gb|ABN08096.1| Galactose mutarotase-like [Medicago truncatula] 287 2e-76 ref|NP_200543.1| aldose 1-epimerase family protein [Arabidops.. 259 3e-68 **CONSERVED DOMAIN:** COG0676, COG0676, Uncharacterized enzymes related to aldose 1-epimerase [Carbohydrate .. 31020 No 1e-30	***GAAGGCGAAGGGGAAGCC***ATTTTCGTTCACCTTTGCTTATCATACTTATTTAT CAGTATCAAACATAAGTGAAGTTCGGCTTGAGGGCTTAAAGACCCTAGATTA TCTTGACAACCTGCAGAACAAGGAGCGTTTTACTGAACAGGGGGATGCTTTA ACGTTTGAATCAGAAGTGGACAAGATATATCTTAGTACTCCTACTAAGATAG CAATTATTGATCATGAAAAAAAGAGGACATTTGTTTTGCGTAAAGATGGCCT TCCTGATGCTGTGGTGTGGAATCCATGGGATAAAAAAGCAAAAGCTATGGCT GATTTCGGTGATGATGAGTATAAGCATATGCTTTGTGTAGAGGCTGCCAATA TTGAAAAGGCTATCACTTTGAAACCTGGAGAAGAATGGAAAGGAAGACTAG AGCTTTCAGCTGTTCCATCTA***GTTACTCTAGTGGTTGCG*** **Forward frame 2, 153 amino acids** RLKAKGKPFSFTFAYHTYLSVSNISEVRLEGLKTLDYLDNLQNKERFTEQGDAL TFESEVDKIYLSTPTKIAIIDHEKKRTFVLRKDGLPDAVVWNPWDKKAKAMADF GDDEYKHMLCVEAANIEKAITLKPGEEWKGRLELSAVPSSYSSGC
EST NO: D8-4 **DETAILS:** RLRRfwd/ RLRRfwd U_HW-070501-Plate5c12.b1 **sequence size (bp):** 484 **BLASTX:** gb|AAB70660.1| grr1 [Glycine max] 209 5e-53 emb|CAO44237.1| unnamed protein product [Vitis vinifera]	***CGCAACCACTAGAGTAAC***ATTAGACAAACTGGCCTTTGACCTCTCAAGCAGC GAAAGAAACCCTGCGTCTGTTATTCCCTCGAGTCCAATCAATTCAAGATGCT GAAGCAAAGGACAGAGCTTCCCAAGGACAGCAAGAGCAAAATTACCAACTC CAGGGCATTTACGGATTGATAATGACAATACTGATTTGCAAGGAGATACTGC TGGAAATTCCAAATTCAAATCTTTGATCCCATAGCATCTTATCAAAGTAAGA GCCTTCTATTTTGCTCCACAGTTAAAAAGGACACCGAAAAGCCCAAATTGGG

194 2e-48 emb|CAO65681.1| unnamed protein product [Vitis vinifera] 190 2e-47 emb|CAN83761.1| hypothetical protein [Vitis vinifera] 190 2e-47 gb|ABC24972.1| EIN3-binding F-box protein 2 [Lycopersicon escule 188 1e-46 emb|CAN82790.1| hypothetical protein [Vitis vinifera] 187 1e-46 dbj|BAF01819.1| putative glucose regulated repressor protein .. 176 3e-43 ref|NP_565597.1| EBF1 (EIN3-BINDING F BOX PROTEIN 1); ubiquit.. 176 3e-43 gb|ABB89717.1| EIN3-binding F-box protein 1 [Solanum lycopers.. 165 7e-40 ref|NP_197917.1| EBF2 (EIN3-BINDING F BOX PROTEIN 2) [Arabido.. 161 1e-38	TAACCCTGTGGCACTCTTCCAATTGTACCCTCTCAATTGATGGAGCAGCCTTG GTGAATGATACCAACCCATTGTCCGACAGAAACGCACACCTGTGAAGCTAA AAGTTTTTCACATTTGGACAACCCTTTCCTATAGCTTCAAGTCCGGTATCT***GT TACTCTAGTGGTTGCG*** **Reverse frame 2, 161 amino acids** ATTRVTDTGLEAIGKGCPNVKNF*LHRCAFLSDNGLVSFTKAAPSIERVQLEECH RVTQFGLFGVLFNCGAK*KALTLIRCYGIKDLNLEFPAVSPCKSVLSLSIRKCPGV GNFALAVLGKLCPLLQHLELIGLEGITDAGFLSLLERSKASLSNVTLVVA

ESTs in RGA-DDRT-PCR AUTORADIOGRAPH FILM-5

EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 2228 ↑ 2228- D24-4, CicerKinF/ NLRRrev; H3, I **DETAILS:** NLRRrev/NLRRrev U_HW-070116_Plate5f12 **sequence size (bp):** 396 **BLASTX:** ref|YP_001086319.1| pyruvate decarboxylase E1 component of th.. 260 2e-68 ref|YP_047975.1| pyruvate decarboxylase, E1 component of the .. 259 4e-68 ref|YP_001280299.1| 2-oxo-acid dehydrogenase E1 subunit, homo.. 237 2e-61 **CONSERVED DOMAIN:** cd02017, TPP_E1_EcPDC_like, Thiamine pyrophosphate (TPP) family, E1 of E. coli PDC-lik.. 48180 No 3e-63	***TATAAAAAGTGCCGGACT***ACTGGCAGTTCCCAACTGTATCGATGGGTCTGGG TCCGATTATGTCGATTTATCAGGCGCATATTCAGAAGTACCTGATGAACCGT GGTCTGATCAAGGAAGAAGATCGTAAGGTCTGGGCATATCTGGGCGATGGC GAGATGGATGAGCCGGAAAGCCTGGGTGCGATTTCTCTGGCTGGCCGTGAA AAGCTGGATAACCTGATTTGGGTGGTGAACTGTAACTTGCAACGTCTGGATG GTCCGGTACGTGGTAATGGCAAGATCATTCAGGAACTGGAATCCTTGTTCCG CGGTGCAGGCTGGCGTGTGATTAAAGTCGTGTGGGGCCGTCACTGGGATCCA CTGCTGGATAAAGACA***AGTCCGGCACTTTTTATA*** Forward frame 3, 131 amino acids *KVPDYWQFPTVSMGLGPIMSIYQAHIQKYLMNRGLIKEEDRKVWAYLGDGEM DEPESLGAISLAGREKLDNLIWVVNCNLQRLDGPVRGNGKIIQELESLFRGAGW RVIKVVWGRHWDPLLDKDKSGTFY

ESTs in RGA-DDRT-PCR AUTORADIOGRAPH FILM-6

EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 2296 ↓ 2296-2; NLRRfwd/NBS; C3, U **DETAILS:** NLRRfwd/NBS U_HW-Plate3f04.b1, **sequence size (bp):** 346 **BLASTX:** emb|CAN79988.1| hypothetical protein [Vitis vinifera] 181 1e-44 emb|CAO41190.1| unnamed protein product [Vitis vinifera] 179 5e-44 gb|EAZ20663.1| hypothetical protein OsJ_034872 [Oryza sativa .. 172 6e-42 gb|EAY83302.1| hypothetical protein OsI_037261 [Oryza sativa .. 172 6e-42 ref|NP_001066882.1| Os12g0514500 [Oryza sativa (japonica cult.. 172 6e-42 gb|ABG22030.1| Hsp90 protein, expressed [Oryza sativa (japoni.. 172 6e-42 ref|NP_187434.2| ATP binding [Arabidopsis thaliana] 166 6e-40 **CONSERVED DOMAIN:** pfam00183, HSP90, Hsp90 protein. 64068 No 9e-27	**T*CTAGTTGTGATGATTATTTTGC***AGGTGATAAGAATGAGGAAAGGGAAAAGG AGATGAAGCAGGAATTTAGTGGGACAATTGACTGGATTAAGAAACGTTTGG GAGATAAAGTAGCAAGTGTGCAGATTTCAAACCGACTGAGCTCTTCACCTTG TGTCCTGGTGTCAGGGAAATTTGGTTGGTCTGCAAACATGGAAAGGCTAATG AAGTCACAAACTATGGGTGATGCCAACAGCTTTGAATTCATGAGAAGCAGA AGGGTTTTTGAGATCAATCCTGACCACCATATTATCAAGAACTTGGATGCTG CATGTAAAACTAATCCTG***ACGATGCAAGAGGCCCTA*** **Forward frame 1** SSCDDYFAGDKNEEREKEMKQEFSGTIDWIKKRLGDKVASVQISNRLSSSPCVL VSGKFGWSANMERLMKSQTMGDANSFEFMRSRRVFEINPDHHIIKNLDAACKT NPDDARGP
EST NO: 2296-D19 ↓ 2296- D19-4; NLRRfwd/NBS; C3, U **DETAILS:** NLRRfwd/NBS U_HW-070116_Plate5g10 **sequence size (bp):** 348 **BLASTX:** emb|CAO16775.1| unnamed protein product [Vitis vinifera] 162 8e-39 emb|CAN70560.1| hypothetical protein [Vitis vinifera] 157 2e-37 ref|NP_192425.1| 4-coumarate--CoA ligase, putative / 4-coumar.. 151 1e-35 **CONSERVED DOMAIN:** pfam00501, AMP-binding, AMP-binding enzyme. 79614 No 8e-20	***TAGGGCCTCTTGCATCGT***ATTAGGACCGCGAACCCATATCTCCCCAAGCTGT CCAGGAGGAAGTGGCTTCAGTGTATCCACACTGACAATTTGAGCTTCCACTC CAGCAACAAGCATTCCTGCGGAGCCAGTATGCCGAACCCCCATCCTTGTATT CTCCACTGAGACAATACCAGACGTTTCAGTCATACCATAGCCCTGAGCAACG ATTGTATGAGGAAAACGCTTAGCACACTCCTCCATCAACTCTTTTCCAAGAG GAGCAGCACCAGAACCAATATACTTCAACGAAGAAAGATCATACTTACCAA CCAAAGCATGTTTA***GCAGAATAATCATCACAACTAGA*** **Reverse frame 3** SCDDYSAKHALVGKYDLSSLKYIGSGAAPLGKELMEECAKRFPHTIVAQGY GMTETSGIVSVENTRMGVRHTGSAGMLVAGVEAQIVSVDTLKPLPPGQLGEI WVRGPNTMQEAL
EST NO: 2314 2314-2, WipK-1/T5; H3, C3, I **DETAILS:** WipK-1/T5 U_HW-Plate3f07.b1, **sequence size (bp):** 608 **BLASTX:** gb|EAU83039.1| hypothetical protein CC1G_08976 [Coprinopsis c.. 33.5 8.7 **BlastN:** AP006430.1 Lotus japonicus genomic DNA, chromosome 5, clone:LjT33L13, TM0327, complete sequence 132 2e-28	***CATTATGCTGAGTGATATCTTTTTTTTTCC***TATGACATAAAATTTCAAATGTGC TTTGCTGACATGTACTTAAAAGTTAAAATATAAAATCATTCCATATAGCTGG TATTTTCTTTTATCTTTCCCTTCCCACCACAGTAAAAAACTAATAAATTTTGT CTACCTATTAATGAGTTAGTTAACCAGTGACTCCACCATCCACTTATCATCCT ACCAGTTGATAAAGTTTGTCTGTTGATTAATGAATTGGTCAATTAGTCATCCC TTTGACCTTGGTGTACATATGAGTATGCTTTGATAGTAAAGTTTATAAGGAA TCAGGGCTGTCCTGTTGCTGTTGTATGTCCAAACAAATGTATGCAGGTTTGA ATTATAAAAAATTTGTTGGCAACTGGGCGCACCATCTTATCTATTGTGGATG GGGTTATATGGATTCCTCACTGCTCTTTTATAGGGTATTGCATACTGAAGTGA CATGTTTTCAATGATTGAAATTTTTTGAGTACGGTTGGACCTTACTCTGTTTT TGGACTGCCTATCAATTGTATTTTCAAAACCTTTGATTGTAAAGATCAAATGC CTACTTGC***ATTCCATACGCACCACGACC*** **Reverse frame 1** WSWCVWNASRHLIFTIKGFENTIDRQSKNRVRSNRTQKISIIENMSLQYAIPYKR AVRNPYNPIHNR*DGAPSCQQIFYNSNLHTFVWTYNSNRTALIPYKLYYQSILCT PRSKG*

EST NO: 2325 ↓ Wipk-1/T8; C3, U **DETAILS:** Wipk-1/T8 U_HW-070116_Plate6f02 **sequence size (bp):** 368 **BLASTX:** gb|ABK78691.1| putative elongation factor 1-beta [Brassica rapa] 65.5 1e-09 emb|CAO68285.1| unnamed protein product [Vitis vinifera] 65.1 1e-09 emb|CAO67292.1| unnamed protein product [Vitis vinifera] 65.1 1e-09 gb|EAZ40980.1| hypothetical protein OsJ_024463 [Oryza sativa .. 64.7 2e-09 ref|NP_001060543.1| Os07g0662500 [Oryza sativa (japonica cult.. 64.7 2e-09 **CONSERVED DOMAIN:** pfam00736, EF1_GNE, EF-1 guanine nucleotide exchange domain. This family is the guanin.. 79691 No 6e-04	***CATTATGCTGAGTGATATCTTTTTTTTTGC***ATGGTTTGGACATCAATAACATTA ACTAACACCAAAACATATCTCTATCTCTTTTACTTCATACAATTCTTGTAACA AAACCATCACTGGAACAGACTAAACATAAAAAGATGAGATGGGAATACGGT ATCAGCAAATAACAGTCTAATCAGCCGCCGCTTGAAAATAGCTTATAAGAA ACCTTAAATTTTGTTGAAGGCCACAATGTCACAGCTCTGGACAAACTCATTA ATTGGCTCAACCGTGAGTGTTTCTTCAATAAGGTCATCCACGGACACAAGAT CATCAATGATGGTCAACATGATCTGCAGCTTCTTG***ATTCCATAAGCACCACGA CC*** **Reverse frame 1** GRGAYGIKKLQIMLTIIDDLVSVDDLIEETLTVEPINEFVQSCDIVAFNKI*GFL*AI FKRRLIRLLFADTVFPSHLFMFSLFQ*
EST NO: 2327 ↑ 2327-4, WipK-1/T9; H3, I **DETAILS:** WipK-1/T9 U_HW-Plate2c02.b1 **sequence size (bp):** 566 **BLASTX:**!Beklenmeyen Formül Sonu ref|XP_001672205.1| Hypothetical protein CBG11461 [Caenorhabd.. 34.7 3.2 **Reverse frame 1** ref|YP_001280159.1| MscS Mechanosensitive ion channel [Psychr.. 35.8 0.84 **BlastN:** AC150704.34 Medicago truncatula clone mth2-131h23, complete sequence 260 4e-67	***CTTATGCTGAGTGATATCTTTTTTTTTGG***TAAGCTTTTTTGTTCACATTTTTATT TATGAAGGCCCACACATCCTATGAGAATTCTGATTTTTGATCTGGTAATTGTG ATTGGTCTTCAACGAAATAAAAAATCCGAGGAATTCTTGATGCTGTGATGGA TACTAGTTGAACGTATTTTGACCTGTGGCTGATGCCTCATAGGAGTTGGACC TGTCATTTGTTGATTCAAACGCATATATTAAACATAAACTGTGGTTACTAAAT AAGAACCAAAATTTGCTGGTGTGTCACTTCCAATCACAGGGAAGGGGTCAAT GGTAACACATTCAAAGTTACTTTATTTAAGCCCACTGCTCAATGACTGTAGC TGGGAGGGGGTTGCTGACTCTTTTATTTGAATATGAGTTCGGGAGTTATTTA AAAAATTAACAAATTACTGTAGCAAATTGTTGCTGCCCTTTTTTGCTGGGGTC GGGTTTGCAATGATATTAGCTGTTGGTAGGTGGTGGTGATGATGGCTGTCTA GCTGGGCTGGTGGTTTCGTTG***ATTCCAAAGCACCCCTACC*** **Reverse frame 2, 194 amino acids** EINRDW*GCFGINETTSPARQPSSPPPTNS*YHCKPDPSKKGQQQFATVIC*FFK*L PNSYSNKRVSNPLPATVIEQWA*IK*L*MCYH*PLPCDWK*HTSKFWFLFSNHSL CLIYAFESTNDRSNSYEASATGQNTFN*YPSQHQEFLGFFISLKTNHNYQIKNQNS HRMCGPS*IKM*TKKLTKKKDITQHK **Reverse frame 1** RGALESTKPPAQLDSHHHHHLPTANIIANPTPAKKGSNNLLQ*FVNFLNNSRTHI QIKESATPSQLQSLSSGLK*

ESTs in RGA-DDRT-PCR AUTORADIOGRAPH FILM-7

EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: 1758 ↑ 1758- D18-4; WipK-1/T7; H3-I **DETAILS:** Primers found WIPK-1/T7 U_HW-070501-Plate5b02.b1, **sequence size (bp): 516** **BLASTX:** gb|ABD33028.1| UBA-like [Medicago truncatula] 267 2e-70 ref|NP_191233.1| ubiquitin-associated (UBA)/TS-N domain-conta.. 221 1e-56 **CONSERVED DOMAIN:** cd00194, UBA, Ubiquitin Associated domain. The UBA domain is a commonly occurring sequ.. 29156 No 1e-07	***GGTCGTGGTGCTTATGGAAT***TAGTGGGACTGTGCCTCTAGTAGGATGTTTGTT GCAACATTGACGTCTTTTCTCGCCTGCACTAGGGCTTGTCTTGCCGAATTCCT GTCAAATCCCATTGAAACAAGGGTCGTGATTGAGTCCTCGGATGGTTCTACT GCAAAATGCATTGGATATTTTCTCTCCATTTGGCGAGCTGGGTAGGAGGGTA CACTTCCCACAACATTCCTTGTTGATGTTGGCCGCGGACTCCCCATAGATGG CAAGGAAATTCGTGAAAAGAATGATGAAATAAACTCCGGGAACTTTGCTTT GCGGATATATAAAACATTCGAACGGTATACGGAACCGGCAGGGATGCCGCA CAACCCTGGTAAGGTGGATCTTTTCCATGAAAACAATAAAAGCTGAAGACC AGCTAAATATATGAATGACTTATCTGAGAAGTGGAAACCAAATAAGCGGAA CCGTGTTGAAACGGGAATG***TCAAAAAAAAAAGATATCACTCACCATAATG*** **Reverse frame 3** LW*VISFFFDIPVSTRFRLFGFHFSDKSFIYLAGLQLLLFSWKRSTLPGLCGIPAGS VYRSNVLYIRKAKFPEFISSFFSRISLPSMGSPRPTSTRNVVGSVPSYPARQMERK YPMHFAVEPSEDSITTLVSMGFDRNSARQALVQARKDVNVATNILLEAQSH*
EST NO: 1749 ↓ 1749-2-1, S2/T8; C3, U **DETAILS:** S2/? U_HW-Plate3d08, **sequence size (bp):** >431 **BLASTX:** gb|AAO69667.1| vacuolar ATPase subunit E [Phaseolus acutifolius] 117 3e-25 emb|CAO65845.1| unnamed protein product [Vitis vinifera] 115 7e-25 ref|NP_176602.1| VHA-E3 (VACUOLAR H+-ATPASE SUBUNIT E ISOFORM.. 113 4e-24 gb|ABR26124.1| vacuolar proton-translocating atpase subunit e.. 110 4e-23	***GGGGGGGTGGGGAAGACTC***CTACACTTGGTAGAGGATGTGTTGGAATCAGCT GCTGAGGAGTATGCTGAGAAGGCAAAAGTTCATCTACCAAATATTGTTGTTG ATAAGGATGTCTATCTTCCACCTGCACCCAACCATAACAATCCCCATGATCT TTATTGCTCGGGTGGCGTGGTATTGGCATCTCGAGATGGAAAGATTGTGTTT GAAAATACACTTGATGCACGTTTGGATGTAGTGTTCCGTAATAAACTCCCAC ACCTCCGCAAGGAGCTCTTTGGACAATTTGCTGTTTGAAGAATTTGGTTGTTT TGTTTCATTTCACAAAAAATATTTATAATTTACTGCAGAAATTGTCCGCGAG GGGAATATGTGTTGGGCCCTTGCTCCTGTAACTTATATTATTGCCCTTTATAA ACTTCTTTTTTTT **Forward frame 2** GGWGRLLHLVEDVLESAAEEYAEKAKVHLPNIVVDKDVYLPPAPNHNNPHDL YCSGGVVLASRDGKIVFENTLDARLDVVFRNKLPHLRKELFGQFAV*
EST NO: 1769 ↓ S2/T8; C3, U **DETAILS:** S2/T8, U_HW-Plate2b10.b1 (1769) and U_HW-070116_Plate5c05 (1880), **sequence size (bp):** 270 **BLASTX**: No significant similarity found **BLASTP** ref|NP_001061174.1| Os08g0191900 [Oryza sativa (japonica cult... 38.5 0.12 gb|EAZ05871.1| hypothetical protein OsI_027103 [Oryza sativa ... 38.5 0.13	***GGGGGGGTGGGGAAGACGAC***GACGACGATGATGACGATTACGAGGAAATGG ATGATGAAGAGTTAGATTTTGTTGCTTTCAAAGATAAGCGAGAACTAAATTT TGCGACTTGATATGTTGAAGCATTGGTGTATATTTTCTTGCTGCAAAACTTAT GATAGCTTTTCCTATGAAAAAAAGGACTTTGTGGTATTCTCGAAATGTGTCA ATACAGCTGGGCTGGATGGTGTTTTTGCCTGG***GCAAAAAAAAAGATATCACTC AGCATAATG*** ***GGGGGGATGGGGAAGACGAC***GACGACGATGATGACGATTACGAGGAAATGG ATGATGAAGAGTTAGATTTTGTTGCTTTCAAAGATAAGCGAGAACTAAATTT TGCGACTTGATATGTTGAAGCATTGGTGTATATTTTCTTGCTGCAAAACTTAT AATAGCTTTTCCTATGAAAAAAAGGACTTTGTGGTATTCTCGAAATGTGTCA ATACAGCTGGGCTGGATGGTGTTTTTGCCTGG***GCAAAAAAAAAGATATCACTC AGCATAATG*** **Reverse frame 3, 89 amino acids** GDGEDDDDDDDDYEEMDDEELDFVAFKDKRELNFAT*
EST NO: 1793 ↑ 1793-D19-1, WipK-1/T7; H3-I **DETAILS:** Primers found WipK-1/T7 U_HW-070501-Plate5b04.b1, **sequence size (bp):** 547 **BLASTX:** gb|ABD33028.1| UBA-like [Medicago truncatula] 135 9e-31 emb|CAO18203.1| unnamed protein product [Vitis vinifera] 110 4e-23 ref|NP_191233.1| ubiquitin-associated (UBA)/TS-N domain-	***GGTCGTGGTGCTTATGGAAT***TAGTGGGACTGTGCCTCTAGTAGGATGTTTGTT GCAACATTGACGTCATTTCTCGCCTGCACTAGGGCTTGTCTTGCCGAATTCCT GTCAAATCCCATTGAAACAAGGGTCGTGATTGAGTCCTCGGATGGTTCTACT GCAGAATGCATTGGATAGTTTCTCTCCATTTGGCGAGCTGGGGAGGGAGGGT ACCCTCCCCCACAACCTTTCTTTGTTGATGTTGCCCGCCCACTCCCCATAG AATGGGAAGGGAAATTCGTGAAAAGATGGATGAATAAACTCTCCGGGAACT TTTGCTTTGCGGATAATAAAAAACACATTCAAACGGTATAACGGGAAACCA GCAAGGGATGGCCGCACAAACCCTGGGGTAAGAAGTGGACCTTTTCCCTTG AAAAACAATAGAAACGCGGAAGAACCCAGCCTAGAATATATGGAATGGACT TATCTGGAAAATGGGAAACCAAATAAGCCGAACCGTGTTTAAACCGGAATG

conta.. 108 1e-22 **CONSERVED DOMAIN:** cd00194, UBA, Ubiquitin Associated domain. The UBA domain is a commonly occurring sequ.. 29156 No 4e-09 **Same band as 1758 (D18-4)** Pairwise Alignment Identities: 0,8731884	G***TCAAAAAAAAGAAT****TCC****CTCAGCATAA*** **Reverse frame 1** LC*GNSFF*PFRFKHGSAYLVSHFPDKSIPYILGWVLPRFYCFSREKGPLLTPGFV RPSLAGFPLYRLNVFFIIRKAKVPGEFIHPSFHEFPFPFYGGVGRATSTKKGCGGG YPPSPARQMERNYPMHSAVEPSEDSITTLVSMGFDRNSARQALVQARNDVNVA TNILLEAQSH*
EST NO: 1901 (or 1772) ↓ S1/AS4; C3, U **DETAILS:** S2/AS4 U_HW-070116_Plate5c07 and U_HW-Plate3e02.b1, **sequence size (bp):** 550 **BLASTX:** gb|AAD51625.1|AF169022_1 seed maturation protein PM37 [Glycine m 283 4e-75 emb|CAO66017.1| unnamed protein product [Vitis vinifera] 278 1e-73 emb|CAN82708.1| hypothetical protein [Vitis vinifera] 278 1e-73 gb|ABH06547.1| molecular chaperone [Ricinus communis] 277 3e-73 emb|CAA63965.1| DnaJ protein [Solanum tuberosum] 277 3e-73 **CONSERVED DOMAIN:** pfam00684, DnaJ_CXXCXGXG, DnaJ central domain (4 repeats). The central cysteine-rich (..64542 No 9e-08 pfam01556, DnaJ_C, DnaJ C terminal region. This family consists of the C terminal regi.. 65363 No 6e-05	***GAGGGCGAAGGGGAAGCC****ACACAGAGCTTCGGTGAGAGACAATGTGTGCTC TACAAAAAGATCTTCACTCTTTCTTCTGAACTTGGGATGTTCCTTTTGTTGTA ATACAAAGACAATATCCCCAGTGATTGTATCCGGCGCTTCATCAGCTTCACC AGGGAATGTAATCTTCTGTCCATTCTGCATTCCTTTCTCCACAATAACTTCAA GTACTTTCTTCTCTTGAACAACCTTCTCACCCTTGCACTGCCCGCATCGATCT TTGTCACTGATTGTCTCACCAGTACCCTTGCATTCATTGCAAGGATGCTGCAT TTGCTGAATCATACTAGCACCCAAATGCCTAATAGAAACCTTCATACCAGTA CCTTGACAACCAGCACATGTCATCGAAGCACCGGACTTTGAGCCTTTCCCTT TGCACTTTGAGCACAAGACATTTCGTGAAAGAGAAAGCTTCTTAGATGTTCC AAGGTAAAGATCCTCCAATGAAACCTTCAAAGGGTGAACCACATCTTCTCCA CGTCTCT***GTCGTCTTCCCCACCCCCCC*** **Reverse frame 2** GGWGRRQRRGEDVVHPLKVSLEDLYLGTSKKLSLSRNVLCSKCKGKGSKSGAS MTCAGCQGTGMKVSIRHLGASMIQQMQHPCNECKGTGETISDKDRCGQCKGE KVVQEKKVLEVIVEKGMQNGQKITFPGEADEAPDTITGDIVFVLQQKEHPKFRR KSEDLFVEHTLSLTEALCGFPFAL
EST NO: 1804 ↓ S2/T4; C3, U **DETAILS:** S2/T4 U_HW-070116_Plate6f09 , **sequence size (bp):** 470 **BLASTX:** emb|CAO64896.1| unnamed protein product [Vitis vinifera] 148 9e-35 emb|CAN78431.1| hypothetical protein [Vitis vinifera] 120 2e-26 gb|EAZ40853.1| hypothetical protein OsJ_024336 [Oryza sativa .. 102 1e-20 gb|EAZ04896.1| hypothetical protein OsI_026128 [Oryza sativa .. 102 1e-20 ref|NP_001060449.1| Os07g0644600 [Oryza sativa (japonica cult.. 102 1e-20 **CONSERVED DOMAIN:** pfam00067, p450, Cytochrome P450. Cytochrome P450s are haem-thiolate proteins involved.. 79443 No 2e-13	***CATTATGCTGAGTGATATCTTTTTTTTTCA****CTTGTCCCTTTTCACAAAAAGTAT TTGTAAATTAGGATGATGCTGAAGAAGATGATTTTTCAAGATTGGTTCTGAG TCACCAATTGAGCCTGAGTTGAGCTTTATCTCATATTTCTTGAGTAATGATGC CAACAATGTAGCAACAACTTGGATAACAAGTTTCTGTCCAACGCAGGCACGT GTACCGGATCCAAAAGGAAGAAATGCCGCGTTTTCACTTGGATCATTTAGTA CAAACGAGCTGGTTCCATAATTAAATTGTTCTTCAGAACCTGATCCTTTTGTA CTGTTTGACAAAAACCGGTATGGATTAAAATCGCTTGCATCTTTCCCCCAATT GAAGTCATCTTTGTGTACCAATTGAACAGGAACAACCAGTGCAGTTCCAGCA GGTATGGTTACACCAGTTGCAAATCTTAA***GTCGTCTTCCCCACCCCCCC*** **Reverse frame 3, 156 amino acids** GGGEDDLRFATGVTIPAGTALVVPVQLVHKDDFNWGKDASDFNPYRFLSNSTK GSGSEEQFNYGTSSFVLNDPSENAAFLPFGSGTRACVGQKLVIQVVATLLASLLK KYEIKLNSGSIGDSEPILKNHLLQHHPNLQILFVKRDK*
EST NO: 1806 ↑ 1806-2-1, S2/T4; H3, I; less C3, U **DETAILS:** S2/T4 U_HW-Plate3d12.b1 **sequence size (bp):** 292 **BLASTX:** ref|NP_001062701.1| Os09g0258600 [Oryza sativa (japonica cult.. 166 6e-40 **CONSERVED DOMAIN:** cd00336, Ribosomal_L22, Ribosomal protein L22/L17e. L22 (L17 in eukaryotes) is a core .. 48343 No 1e-19	***CATTATGCTGAGTGATATCGTTTTTTTTCA****AATAATACCAACTCTATGTGGCA TGGAGAAGACATATAAGGGTTAATTCTTCCATGGGCTCTGTATGTTCGACGT CTTTGCTTCTGTGCTTGATTAACTTGGATATGAGACACGTAAAGAGCATCAA TATCCAAACCTTTCACTTCAGCATTACTCTCTGCGTTCTTGAGCAAATCTAGA ATGAACTTGGCGGATTTGGCAGGCCACCTTCCTTGTCCATTAGAGTGTCTATT CTTGGCCTGA***TCGTCTTCCCCACCCCCCC*** **Reverse frame 1, 97 amino acids** WGGGEDDQAKNRHSNGQGRWPAKSAKFILDLLKNAESNAEVKGLDIDALYVS HIQVNQAQKQRRRTYRAHGRINPYMSSPCHIELVLFEKKRYHSA*
EST NO: 1826 ↑ 1826-1a , S2/T4; H3, I **DETAILS:** S2/T4 U_HW-070116_Plate5c06, **sequence size (bp):** 543 **BLASTX:** ref|XP_001498357.1| PREDICTED: hypothetical protein [Equus cabal 34.7 2.9 **Half is similar to 619** Optimal Global aligment Alignment score: 204 Identities: 0,4613971 **Last half:** Optimal Global aligment Alignment score: 430 Identities: 0,9733333	***CATTATGCTGAGTGATATCTTTTTTTTTCA****CCAGAATTAGGCAAGTAACCATT AGAAGAAGCCTTCATTATTATGACCTAAAATTTGATGATGACCCTTCAAAAC AAGTTAAACTATTTCTCTTTTTCCTAGCAACAAGATTTAACCTTGTTCCTCAA GTATTCATTTCAGAATAACAACACAATCCTCCAAAAATAGCAGCACCACTTC CACACGAGTAGATGAAGAATAAAAAACACTTTTTGATTCCTACCACAAAGCT ATTCAATAACATAGCAGAATCATCAACAACAATAACAAAAAGTTCACCTTTT TCACCCCAGAAAATGCCACTACTTCATTTTTTTTGGTCCAACTCCAAATTAAT AAGTTCCCAAATTCAAGAACTAGGAGAAACTCAACATAAAAAAGCCACCAC TTTCTGCAATGTTCAACTTTTCACAGCTCAAGTTTGAATGTTGAATCTTCAAC ACATAACTTCAAGTCATAACAAAAACTATCATCTTACACCATCTTCCCCACA ***GTCGTCTTCCCCACCCCCCC*** **Forward frame 3, 180 amino acids** LC*VISFFFTRIRQVTIRRSLHYYDLKFDDDPSKQVKLFLFFLATRFNLVPQVFISE *QHNPPKIAAPLPHE*MKNKKHFLIPTTKLFNNIAESSTTITKSSPFSPQKMPLLHF FWSNSKLISSQIQELGETQHKKATTFCNVQLFTAQV*MLNLQHITSSHNKNYHLT PSSPQSSSPPP
EST NO: 1868 ↑ 1868-1-1, S2/T5; H3, I **DETAILS:** S2/T5 U_HW-Plate2b11.b1, **sequence size (bp):** 474 **BLASTX:** emb|CAO49173.1| unnamed protein product [Vitis vinifera] 195 7e-49 gb|AAM65698.1| putative serine carboxypeptidase II [Arabidopsis 184 1e-45 ref|NP_186860.1| SCPL25 (serine carboxypeptidase-like 25); se.. 184 1e-45 emb|CAN79972.1| hypothetical protein [Vitis vinifera] 173 4e-42 emb|CAO66662.1| unnamed protein product [Vitis vinifera] 170 3e-41 ref|NP_194790.1| BRS1 (BRI1 SUPPRESSOR 1) [Arabidopsis thalia.. 169 5e-41 **CONSERVED DOMAIN:** pfam00450, Peptidase_S10, Serine carboxypeptidase. 79602 No 9e-35 **BlastN:** NM_111077.3 Arabidopsis thaliana SCPL25 (serine carboxypeptidase-like 25); serine carboxypeptidase (SCPL25) mRNA, complete cds 169 2e-39	***GGGGGGGTGGGGAAGACGACGC****CGGCCGGAGCTTCCAAAAATAAAAGGTTG GCCACTGTGTTCCAAGCAAACTTGTTAATGTATAGCCCAGAAGCTGTTTTGTT CAATCTAAATGGGCCAATCTCTTCGGATGCTCCATATGCCACTGATGAACAG CCTGGACCTCCATTAAGCCAAATGACAAGGGGTTTGTTGAGAGGATTTTGAG AAGCTTCAGTTAGCCAATAAAAGAGAGCTCTACCAGCAACATGATCAACAG TAACATAGCCAGAAAATTGTTGAAATAAAACCTTAGGTTGACCAGGGAGCT CAAAAATCTATCATGTTCTTCTTCTTCTACTTTTGCTTCAACATTTTATAAT TAATTTCTCTAAGTAGGAACATGACCAAGAAAATCACTACATGAACAAAGA TTTTTCTTTGCTCCATGACTTAATTGATT***GGAAAAAAAAAGATATCACTCAGCA TAATG*** **Reverse frame 2, 157 amino acids** IMLSDIFFFPIN*VMEQRKIFVHVVIFLVMFLLREINYKNVEAKVEEEEHDRIFELP GQPKVLFQQFSGYVTVDHVAGRALFYWLTEASQNPLNKPLVIWLNGGPGCSSV AYGASEEIGPFRLNKTASGLYINKFAWNTVANLLFLEAPAGVVFPTP `emb|CAO49173.1|:` Query 338 EEEEHDRIF EEEE DRI Sbjct 37 EEEEADRIT gb|AAM65698.1|: Query 431 MEQRKIFVH M + IF Sbjct 1 MAKLAIFTT

EST NO: 1881 ↑ 1881-2, S2/T7; H3.I **DETAILS:** S2/T7 U_HW-Plate4g01.b1, **sequence size (bp):** 206 **BLASTX:** emb|CAO17868.1| unnamed protein product [Vitis vinifera] 85.9 7e-16 ref|NP_568355.1| methyltransferase [Arabidopsis thaliana] >gb.. 82.4 8e-15	***GGGGGGGTGGGGAAGACGAC***AAAAGAGGGTCAAGCGTGCTGAGGTATGGGT AAAAGACCCGGCTCGGGATAACATACTTTTCTTGTTTGCAAATGCCCCAATT TCTTTCAAGCTGTTGGTAGAATCATATCCTGGGCCTTTGCAATTAGTTTCAAT TTTGTGTCCAGACCCTCATT***TCAAAAAAAAAGATATCACTCAGCATAATG*** **Forward frame 2, 68 amino acids** GGWGRRQKRVKRAEVWVKDPARDNILFLFANAPISFKLLVESYPGPLQLVSILC PDPHFKKKDITQHN
ESTs of GSP-RT-PCR	
EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: ACTIN Act-F/Act-R **DETAILS:** Act-F/Act-R, U_HW-Plate2d12.b1, **sequence size (bp):** 465 **BLASTP:** i|544861|gb|AAC60565.1| actin [Striga asiatica] 160 5e-38 gi|3860317|emb|CAA10126.1| actin [Cicer arietinum] 160 5e-38	***GTAACATTGTCTTGAGGGGGGG***TTCTACTATGTTCCCCGGAATTGCTGATAGA ATGAGCAAAGAGATTACAGCATTGGCACCCAGCAGCATGAAAATCAAGGTT GTAGCACCACCAGAGAGGAAATACAGTGTCTGGATTGGAGGCTCCATTTTGG CATCTCTCAGCACTTTCCAACAGATGTGGATTGCAAAGGCAGAATATGATGA ATCTGGCCCATCAATAGTACACAGGAAATGCTTCTAAGTTCAAATCATGGAG CACTGAGAGCTGAACCAAGGAAATTACAATATATACTTTGTACTACATAAAT ACCATCTAGTGGTTGAGGAACTTCCATTTCCTATTCTTTTACCATTTCTTTTAT CACGTTTCTCTGTGTTTTCCTTTCTTGATATGTTGGGATAAGAGCATGAAGGC TAGCAAGATATGAAAGATTCAGTTTT***CCCCTCATTTTGTTGTAAAG*** **Forward frame 1, 261 amino acids** CNIVLRGGSTMFPGIADRMSKEITALAPSSMKIKVVAPPERKYSVWIGGSILASLS TFQQMWIAKAEYDESGPSIVHRKCF*
EST NO: Fdh-3 Fdh-3, FDH-fwd/FDH-rev **DETAILS:** FDH-fwd/FDH-rev, U_HW-Plate3g12.b1, **sequence size (bp):** 404 **BLASTX:** mb|CAE12168.2| formate dehydrogenase [Quercus robur] 249 3e-65 gb|EAZ37051.1| hypothetical protein OsJ_020534 [Oryza sativa .. 247 1e-64 gb|EAZ00985.1| hypothetical protein OsI_022217 [Oryza sativa .. 247 1e-64 **CONSERVED DOMAIN:** pfam02826, 2-Hacid_dh_C, D-isomer specific 2-hydroxyacid dehydrogenase, NAD binding do.. 79959 No 6e-35	***ACAGAGCTTATGATCTTGAAGG***AAAGACGATAGGAACTGTTGGTGCTGGACG AATCGGGAAGCTTTTACTTCAAAGACTGAAACCCTTTAACTGTAACCTTTTGT ATCATGATAGACTTAAGATAGCACCTGAATTGGAGAAAGAAATTGGAGCTA AGTTTGAGGAGGATCTTGATGCTATGCTTCCGAAGTGCGATGTAATTGTTAT CAACCCCCCCCTCACCGATAAGGCAAGAGGATTGTTTGACAAAAATAGAAT CGCAAAGTTGAAGAAGGGTGTCTTGATTGTTAACAATGCTCGCGGGGCGACT ATGGACACGCAAGCAGTTGCTGATGCTTGTTCTAGTGGCCACATTGCAGGTT ATAGTGGTGATGTTTGGTT***CCCACACCCAGCTCCTAAGGAC*** **Forward frame 3, 134 amino acids** RAYDLEGKTIGTVGAGRIGKLLLQRLKPFNCNLLYHDRLKIAPELEKEIGAKFEE DLDAMLPKCDVIVINPPLTDKARGLFDKNRIAKLKKGVLIVNNARGATMDTQA VADACSSGHIAGYSGDVWFPHPAPKD
EST NO: FPIP Fpip-3; FPIP-fwd/FPIP-rev **DETAILS:** FPIP-fwd/FPIP-rev, U_HW-Plate3h02.b1, **sequence size (bp):** 400 **BLASTX:** dbj|BAA77395.1| SLL2-S9-protein [Brassica rapa] 187 1e-46 emb|CAO17056.1| unnamed protein product [Vitis vinifera] 187 2e-46	***AGGGCATGTACTAGAACCAC***CAAACATGAACGTCGGATATGTGCGGACATGA CTAACATATCGAAATAGGGGATCTCTGCATGATTCATCATCTGGGGCAGTAG CACTTCTTCTTTGGTTGAAACTTTCCACTTCTTGCACTAACTCATCCTTTGTAC TGTTACATGATGTGATAACAAGTAATCCACCAGAAGCAACCAGCCTTGAAAC AGAATCCCAATACATCATTCTCTTGACAGGACCATCGGGATGTAATCCAATA GCATCAAGTGTCCCTTTATCCATGACAAGTTGAAACACTTGTTCCAACTTGT TTCAAGGACATCATCAACCAAAAATTTGATGTGGGGAAACCCATCACGGTTA GCAAGGCTTTG***GGCGAGGCTTATTTTCGACTTG*** **Reverse frame 2, 133 amino acids** KSKISLAQSLANRDGFPHIKFLVDDVLETKLEQVFQLVMDKGTLDAIGLHPDGP VKRMMYWDSVSRLVASGGLLVITSCNSTKDELVQEVESFNQRRSATAPDDESC RDPLFRYVSHVRTYPTFMFGGSSTCP
EST NO: Pe-3 PE-3; PE-fwd/PE-rev **DETAILS:** PE-fwd/PE-rev, eU_HW-070116_Plate5e09, **sequence size (bp):** 422 **BLASTX:** emb|CAN77092.1| hypothetical protein [Vitis vinifera] 169 7e-41 emb|CAO42327.1| unnamed protein product [Vitis vinifera] 167 3e-40 ref|NP_173733.1| pectinesterase family protein [Arabidopsis t.. 156 3e-37 **CONSERVED DOMAIN:** pfam01095, Pectinesterase, Pectinesterase. 64931 No 2e-47	***GTGGCAAAAGATGGCAGTG***GAAACTACAAAACAATATCGGAGGGTGTTGCT GCAGCTGCAAAACTAAAAGGAAAAGGAAGAGTTGTTGTTTATGTGAAAAGA GGTGTTTATAAAAGAGAATGTTGATGTTAAAAAAACAGTGAAGAATTTAATG ATTGTTGGAGATGGAATGGATTCAACAATTATTACTAGTAATCACAATGCTC AACATGGCTATACTACTTTTCGTTCAGCTACTTTTGGTATTATGAGTGATGGT TTCATAGCAAAGGACATGACTTTTGAAAACACAGCCGGACCACAAAATCAT CAAGCAGTGGCTCTACGTTCTGGCGCCGATTACTCTGTATTTTATCGTTGCGC CTTCAAAGGTTATCAAGACACATTATATGTCTACGCTCAA***CGCCAATTCTACC GTGACTG*** **Forward frame 1, 143 amino acids** SIVAKDGSGNYKTISEGVAAAAKLKGKGRVVVYVKRGVYKENVDVKKTVKNL MIVGDGMDSTIITSNHNAQHGYTTFRSATFGIMSDGFIAKDMTFENTAGPQNHQ AVALRSGADYSVFYRCAFKGYQDTLYVYAQRQFYRD*
EST NO: Eds1-1 EDS-1, EDS-fwd/EDS-rev **DETAILS:** EDS-frw/EDS-fwd, U_HW-070501-Plate6a01.b1, **sequence size (bp):** 791 **BLASTX:** emb|CAO42468.1| unnamed protein product [Vitis vinifera] 366 1e-111 ref|NP_172777.1| DIS1 (Distorted Trichomes 1); structural con.. 362 1e-107 **CONSERVED DOMAIN:** cd00012, ACTIN, Actin; An ubiquitous protein involved in the formation of filaments th.. 28896 Yes 5e-51	***CCTCGGATGATGCTTGCTC***TGGCTGCTGGTTACACAACGTCTAAGTGTGAGA TGACAGGAGTTGTATTTGATATTGGAGACGGGGCTACACATGTTGTACCAGT TGCGGATGGTTATGTTATTGGAAGTAGCATCAAATCGGTTCCTATTGCTGGA AAGGATGTTACTCTTTTTGTCCAGCAGCTAATGCGGGAAAGAGGGGAGAAT GTACCCCCAAAAGACTCTTTTGAAGTGGCTCGGAAAGTGAAAGAAATGCATT GCTACCCCTGCTCTGACATAGTGAAGGAATATAATAAGCATGACAAAGAAC CAGCCAAGTATATCAAGCAATGGCGAGGTGTTAAACCAAAGAGTGGGGCTC CATATTCTTGTGATATTGGCTATGAACGCTTTCTTGGCCCCGAGGTTTTCTTT AATCCTGAAATATATAGCAGCGACTTTTCCACTCCTTTGCCAGTTGTAATAG ACAAGTGCATTCAGTCTGCACCAATTGACACAAGGAGATCACTATATAAAA ATATAGTGCTGTCAGGAGGATCAACAATGTTCAAGGATTTTCACAGGAGATT GCAACGTGATCTAAAAGAAAATCGTGGATGCTCGTGTCCTTTGCAGCTGACG CGCGCCTAAATGGGGAGATAAATCACATCCAGTGAAGGTCAATGTAGTCAG CCATCCTATCCAAAAATTTGCCGTTTGGTTTAGGAGGATCAGTGCTTGCATC AACACCTGAATTTTTACGGCTTGTCATACAAGGGCAGATATGAAGAATAT***GA GCAGCATATCCGAGG*** **Forward frame 1, 263 amino acids** PRMMLALAAGYTTSKCEMTGVVFDIGDGATHVVPVADGYVIGSSIKSVPIAGK DVTLFVQQLMRERGENVPPKDSFEVARKVKEMHCYPCSDIVKEYNKHDKEPAK YIKQWRGVKPKSGAPYSCDIGYERFLGPEVFFNPEIYSSDFSTPLPVVIDKCIQSA PIDTRRSLYKNIVLSGGSTMFKDFHRRLQRDLKENRGCSCPLQLTRA
EST NO: Eds1-4 EDS-4, EDS-fwd/EDS-rev **DETAILS:** EDS-frw/ EDS-rev, U_HW-070116_Plate6g11, **sequence size (bp):** 586 **BLASTX:** emb|CAO38897.1| unnamed protein product [Vitis vinifera] 303 4e-81 ref|NP_200333.2| trigger factor type chaperone family protein..	***CCTCGGATGATGCTAGCTC***CACCTCTTGGAATCTCTCTAATAGTAAGTCTTTG ACCTGCTCCACTGTGGTGCATCCAGGGATAAGCTTATCAGCAATAGAATCAT CCAGCTCTGGTAAATCTCTGTAAAAAAGTTCTTTGCACTCAACAGTAAACTG AGCATGAACACCACGGAGATTTTCCTGTTTCCACGTTTCTGGGAAAAAAAGG TGAAATGACTTTGCTTCGCCTCGACGAATTCCAATTATAGAATCAAGGAAAC CTGGTACTAATATTTCACCATCCTCTGTATCAAAATTAAATCCTTTACTTTCA GCAGAAGGAATATTTCTAATATTTGATTCATCTTGATCAATGGTGGTTGCTG AGATGTCAATAACAACAACATCACCAACCTGAAGTCCTCTATCAGTAACAAC

265 2e-69 **CONSERVED DOMAIN:** PRK01490, tig, trigger factor. 74013 Yes 4e-21	TTTCAGTGCGCCTGTAGACTTGTAACGCCTTCTAAATTCTTTTTCAGATGCTA TGTGAGCATCTATATCACTGTCTATCTCAACAACAACCTTCAAATTCTTGTAT GCGTTATCAGAAATCCATTTGATTTCTGGTGCAACATCAAC***AATGACTTCAAT GCTAACGTC*** **Reverse frame 1, 195 amino acids** DVSIEVIVDVAPEIKWISDNAYKNLKVVVEIDSDIDAHIASEKEFRRRYKSTGAL KVVTDRGLQVGDVVVIDISATTIDQDESNIRNIPSAESKGFNFDTEDGEILVPGFL DSIIGIRRGEAKSFHLFFPETWKQENLRGVHAQFTVECKELFYRDLPELDDSIAD KLIPGCTTVEQVKDLLLERFQEVELASSE
EST NO: HRP HRP-fwd/HRP-rev **DETAILS:** HRP-fwd/HRP-rev, U_HW-Plate3d04.b1, **sequence size (bp):** 283 **BLASTX:** emb|CAO65595.1| unnamed protein product [Vitis vinifera] 52.4 9e-06 gb|AAK92807.1| putative receptor protein kinase [Arabidopsis tha 45.1 0.001 ref|NP_180241.1| leucine-rich repeat transmembrane protein ki.. 45.1 0.001	***AGATGTTCTTGAGCCTGGTTGC***CGCCGACGTCGTTTACGCAGGCAGAGTAAC AGAATTAGAAGTAGTAACGCTATGAAAAGAACGGAACCAACTACGATTGCG ACAATGGCGCCGGTCGAGAGCTTCTTTGACTTCTTCCCTGGTTTAATCACCGG TGGAATGGAAGGTGACGGCGCCGGCGCCGGGAAGAAAGGCGTGCAAGGAT CCAACGGTGATCCACAGAGATCTGAATTTCCAATGAATGAAGATTTCGGGAA TTT***CGACAGTGTTTTGGGTTTCGCT*** **Reverse frame 2, 94 amino acids** AKPKTLSKFPKSSFIGNSDLCGSPLDPCTPFFPAPAPSPSIPPVIKPGKKSKKLSTGA IVAIVVGSVLFIALLLLILLLCLRKRRRRQPGSRTS
EST NO: Rar1-1 Rar-1 **DETAILS:** /?/? U_HW-070116_Plate6e09, **sequence size (bp):** 501 **BLASTX:** gb|AAY82249.1| mitochrondrial voltage-dependent anion-selecti.. 290 3e-77 gb|AAQ87019.1| VDAC1.1 [Lotus corniculatus var. japonicus] 286 3e-76 sp|P42054|VDAC_PEA Outer plastidial membrane protein porin (V.. 284 2e-75 **CONSERVED DOMAIN:** pfam01459, Porin_3, Eukaryotic porin. 65270 No 2e-36	TTCCGTCTCCGCCGACATCGGCAAGAAAGCCAGAGATCTGTTGTTCAAGGAC TATCACAGTGATCAGAAATTCAGCATCACCACTTACTCACCAACTGGAGTTG CTATCACTTCATCAGGTACAAAGAAAGGTGAACTGTTTCTGGCTGATGTTAA TACCCAGTTGAAGAACAAAAACGTCACCACTGATATCAAAGTTGATACAGA ATCTAACCTTTTCACAACTATCACTGTCAACGAACCTGCTCCTGGTGTGAAG GCTATTTTAAGCTTCAAAGTCCCTGATCAGACTTCTGGGAAGGTGGAACTAC AATACTTGCACGACTATGCTGGATTGAGCGGCAGCTTTGGGTTGAAAGCAAA CCCAATTGTTAACTTATCTAGTGTTGTTGGAACTAATGTTCTTGCTATTGGTG CTGATCTTTCTTTTGATACCAAACTTGGGGAGTTGACAAAATCTAATGCTGG ACTGAGCTACGCCAAAGATGACTTGATTGCCTA **Forward frame 2, 166 amino acids** SVSADIGKKARDLLFKDYHSDQKFSITTYSPTGVAITSSGTKKGELFLADVNTQL KNKNVTTDIKVDTESNLFTTITVNEPAPGVKAILSFKVPDQTSGKVELQYLHDYA GLSGSFGLKANPIVNLSSVVGTNVLAIGADLSFDTKLGELTKSNAGLSYAKDDLI A
EST NO: Rar1-4 Rar-4 **DETAILS:** /?/ U_HW-070501-Plate5f01.b1, **sequence size (bp):** 613 **BLASTX:** emb|CAO71127.1| unnamed protein product [Vitis vinifera] 236 9e-61 emb|CAN75984.1| hypothetical protein [Vitis vinifera] 235 2e-60 ref|NP_187252.1| homeotic gene regulator, putative [Arabidops.. 189 1e-46	CCGAACAGATTCGACAGCCCAGGACAGAAAAGAGATGTTGGAGGTGATTAT GCGTCGAGGTTCAAGCTCACTTGGGGCAGATGTACCTAGTGAGAGAGAAAT TAACCGCCTTGCTGCTCGATCAAATGAGGAATTTTGGCTGTTTGAGAAAATG GATGAAGAGAGAAGACAAAAGGAGAATTACAGATCACGTCTCATGGAAGA GCATGAATTGCCAGATTGGGTTTATGCTCTGATTAAAAAGGATGACAAGGCT AAAAGTTTCAACAGTGGTGTTACTGGAAAGCGAAAAAAAAAAAGACGTGGTA TATGCTGATACATTAAGTGAGCTACAGTGGATGAAGGCCATGGAGAAATGGG GAAGACATGTCAAAGCTTTCAGCTAAAGGGAAAAGAAGAGAGTCAAGAGAT TACCTCTCATCTGACAGCATAGCACAAGCCAGTGATAATACAGGGGCAGAT GAAAGCTTATTAGAGTCGAGGACTAAAATTGTCCCCGTGGCGAGTGAGAGA ACAAGTGAAGATAGTTTCCATGTCACTCCTTCCTCAAAGAGATTCAAGCCTG AAGGAACAATTTTCCAGAAACATGCTTATGAGGATGTCGGCGGAGACGGA **Forward frame 2, 204 amino acids** RTDSTAQDRKEMLEVIMRRGSSSLGADVPSEREINRLAARSNEEFWLFEKMDEE RRQKENYRSRLMEEHELPDWVYALIKKDDKAKSFNSGVTGKRKKKDVVYADT LSELQWMKAMENGEDMSKLSAKGKRRESRDYLSSDSIAQASDNTGADESLLES RTKIVPVASERTSEDSFHVTPSSKRFKPEGTIFQKHAYEDVGGDG
Undetermined RGA and DD ESTs	
EST INFORMATION **EST NO:** EST no/Clone no(if different)/ original primer/ plant sample **DETAILS:** primer found/96-well plate no/sequence size, (?) primer not complete or not obvious	**SEQUENCE** **DNA sequence of clone 5' to 3'** GGAATTCGAT*ins*ATCACTAGTG ***Bold : primers*** **Translated sequence related to BlastX**
EST NO: B20 **DETAILS:** Ptokin-1/ Ptokin-2 U_HW-070116_Plate5g08 **sequence size (bp):** 463 **BLASTX:** dbj|BAF74756.1| CBL-interacting protein kinase [Vigna unguiculat 210 1e-66 dbj|BAD95889.1| Ser/Thr protein kinase [Lotus japonicus] 210 5e-66 emb|CAO67343.1| unnamed protein product [Vitis vinifera] 207 1e-65 emb|CAN70422.1| hypothetical protein [Vitis vinifera] 207 1e-65 gb|ABJ91211.1| CBL-interacting protein kinase 3 [Populus trichoc 206 2e-65 gb|ABJ91212.1| CBL-interacting protein kinase 4 [Populus trichoc 199 3e-63 ref|NP_850095.2| CIPK3 (CBL-INTERACTING PROTEIN KINASE 3); ki.. 199 5e-63 **CONSERVED DOMAIN:** cd00180, S_TKc, Serine/Threonine protein kinases, catalytic domain. Phosphotransferase.. 29142 Yes 1e-30	***GCATTGGAACAAGGTGAA***ATTTGCAAGGAACTCTGAGACAAATGAGGCGGTG GCTCTCAAAATTCTTGACAAAGAGAAAGTTCTCAAGCATAAGATGGCTGAGC AAATCAAGCGGGAAATAGCTACAATGAAGTTAATCAAGCATCTGAATGTTG TTCAACTATATGAGGTCATGGGAAGCCGGACAAAAATATATATTGTTTTGGA GTTTGTGACCGGCGGCGAGCTTTTTGACAAAATTGTAAACCATGGACGAATG AGCGAAAGTGAAGCGCGTAGGTATTTCCAGCAGCTTATAAATGTTGTTGATT ATTGTCATAGCAGGGGTGTCTATCACAGAGACTTAAAGAAAATTTGCTATTA GATGATAAAGGTAACCTTAAAGTCTCCGATTTCGGGTTGAGTGCACTCTCCC AGCAAGTTAGGGACGACGGACTTCTCCATA***CTACGTGGTGGTCCCCCT*** **Forward frame 2, 154 amino acids** HWNKVKFARNSETNEAVALKILDKEKVLKHKMAEQIKREIATMKLIKHLNVVQ LYEVMGSRTKIYIVLEFVTGGELFDKIVNHGRMSESEARRYFQQLINVVDYCHS RGVYHRDLKKICY*
EST NO: B20-2 **DETAILS:** Ptokin-1/ Ptokin-2 U_HW-070501-Plate5b06.b1 **sequence size (bp):** 400 **BLASTX:** gb|ABH08754.1| ubiquitin [Arabidopsis thaliana] 241 1e-62 gb|ABH08753.1| ubiquitin [Arabidopsis thaliana] 241 1e-62 gb|ABH08755.1| ubiquitin [Arabidopsis thaliana] 241 1e-62 gb|ABR25718.1| polyubiquitin containing 7 ubiquitin monomers .. 241 1e-62 **CONSERVED DOMAIN:** cd01803, Ubiquitin, Ubiquitin (includes Ubq/RPL40e and Ubq/RPS27a fusions as well as h.. 29205 No 3e-34 cd01803, Ubiquitin, Ubiquitin (includes Ubq/RPL40e and Ubq/RPS27a fusions as well as h.. 29205 No 1e-20	***GCATTGGAACAAGGTGAA***GGCAAAAATTCAAGACAAGGAGGGTATTCCTCCA AACCAGCAACGATTGATCTTCGCCGGAAAACAATCAAAAGACGGAAGGACA CTGGCGGATTACAATATCCAAAAGGAATCAACGCTTCATTTGGTGCTTCGAC TTCGCGGCGGGATGCATATTTTCGTCAAAACCCTAACCGGAAAAACCATCAC CTTGGAGGTTGAGAGTTCTGATACTATTGATAACGTGAAAGCGAAGATTCAG GATAAAGAGGGAATTCCACCGGATCAACAGCGTTTGATATTTGCTGGTAAGC AGCTTGAGGATGGAAAAACTCTTGCTGATTATAACATTCAAAAGGAATCTAC ACTTCACCTTGTTCTCAGG***CTACGTGGTGGTCCCCCT*** **Forward frame 2, 133 amino acids** HWNKVKAKIQDKEGIPPNQQRLIFAGKQSKDGRTLADYNIQKESTLHLVLRLRG GMHIFVKTLTGKTITLEVESSDTIDNVKAKIQDKEGIPPDQQRLIFAGKQLEDGKT LADYNIQKESTLHLVLRLRGGPP
EST NO: 350 S2/T2; B1, B2	***CATTGGAACAAGGTGAA***GTGTAGATTCCTTCTGAATGTTATAATCAGCAAGA GTTCTTCCATCCTCAAGCTGCTTACCAGCAAATATCAAACGCTGTTGATCCG

DETAILS: Ptokin-1/T6 U_HW-070116_Plate5b08, **sequence size (bp):** 494 **BLASTX:** gb|EAY84584.1| hypothetical protein OsI_005817 [Oryza sativa .. 282 5e-75 gb|ABU40645.1| polyubiquitin [Triticum aestivum] 282 6e-75 gb|ABH08754.1| ubiquitin [Arabidopsis thaliana] 282 **CONSERVED DOMAIN:** cd01803, Ubiquitin, Ubiquitin (includes Ubq/RPL40e and Ubq/RPS27a fusions as well as h.. 29205 No 3e-34 cd01803, Ubiquitin, Ubiquitin (includes Ubq/RPL40e and Ubq/RPS27a fusions as well as h.. 29205 No 2e-31 **BlastN:** AY057500.1 Arabidopsis thaliana AT4g05320/C17L7_240 mRNA, complete cds 432 9e-119 U77939.1 Phaseolus vulgaris ubiquitin-like protein mRNA, complete cds 421 2e-115 NM_116771.2 Arabidopsis thaliana UBQ10 (POLYUBIQUITIN 10); protein binding (UBQ10) mRNA, complete cds 419 6e-115	GTGGAATTCCCTCTTTATCCTGAATCTTCGCTTTCACGTTATCAATAGTATCA GAACTCTCAACCTCCAAGGTGATGGTTTTTCCGGTTAGGGTTTTGACGAAAA TCTGCATCCCACCGCGAAGTCGAAGCACCAAATGAAGCGTTGATTCCTTTTG GATATTGTAATCCGCCGGTGTCCTTCCGTCTTCTAATTGTTTTCCGGCGAAGA TCAATCGTTGCTGGTCTGGAGGAATACCCTCCTTGTCTTGAATTTTTGCCTTC ACGTTGTCGATTGTATCGGAACTCTCTACCTCTAGGGTAATGGTTTTGCCGGT TAGGGTTTTCACGAAGATCTGCATGATGAATTTGTAGTTTTGTG***CGAAAAAAA*** ***AAGATATCACTCAGCATAATG*** Query 447 IMQIFVK Sbjct 1 MMQIFVK **Reverse frame 3, 164 amino acids** LC*VISFFFAQNYKFIMQIFVKTLTGKTITLEVESSDTIDNVKAKIQDKEGIPPDQQ RLIFAGKQLEDGRTPADYNIQKESTLHLVLRLRGGMQIFVKTLTGKTITLEVESS DTIDNVKAKIQDKEGIPPDQQRLIFAGKQLEDGRTLADYNIQKESTLHLVPM **Longer sequence of B20-2** Pairwise Alignment Optimal Global aligment Alignment score: 505 Identities: 0,6300676
EST NO: 453 NLLRfwd/T8 **DETAILS:** WipK-1/T5, U_HW-070116_Plate6e04, **sequence size (bp):** 211 **BLASTX:** emb|CAO42125.1| unnamed protein product [Vitis vinifera] 55.1 1e-06 emb|CAN78632.1| hypothetical protein [Vitis vinifera] 55.1 1e-06	***CATTATGCTGAGTGATATCTTTTTTTTTCC***AGCTTCATTGAAAGTTGACCATGA TGACTGCAAATACCCAGGAGATCCATCTATTTTCAGTTCCTCGGTCGTGCCAT CTTTAACTAACATGGTTATGTGTAGGTGACTCCATGTTGTTTTCTATAGAATG GTGTCTACTTTTCGGGTTATCTAGTTTTTTC***ATTCCATAAGCACCACGACC*** **Forward frame 2, 70 amino acids** IMLSDIFFFPASLKVDHDDCKYPGDPSIFSSSVVPSLTNMVMCR*
EST NO: B46 1992 (PtofeS X T7), 1994(PtofeS X T7), 1998 (PtofeS X T3); all H3,C3,U,I **DETAILS:** NLRR fwd/T2, U_HW-070501-Plate6g01.b1 **sequence size (bp):** 203 **BLASTX:** ref|YP_304164.1| phycocyanin alpha phycocyanobilin lyase rela.. 37.7 0.24	***TAGGGC7TCTTGC7CGT***GGCTTGAAAAAAGTTCCCATTCTTGTGGATACTGGT ATGACAACCAAAAGACAATGCCCCCCATCGACTGAATCTTATTATCCATGTT TGTAAAACCTGAAAAGTAAACTAGGCTTAATATTTTCAACTTGTAAAGAAAA AAAATGATCTGTTTTATTGT***GTAAAAAAAAGATATCCTCACATATG*** **Reverse frame 3, 67 amino acids** YVRISFFYTIKQIIFFLYKLKILSLVYFSGFTNMDNKIQSMGGIVFWLSYQYPQEW ELFSSHEQEAL
EST NO: 1507a P9/T7 **DETAILS:** P4/T4, U_HW-070501-Plate6h05 **sequence size (bp):** 357 **BLASTX:** ref|XP_001507622.1| PREDICTED: similar to RasGEF domain famil.. 33.1 6.0 **BlastN:** AC126007.16 Medicago truncatula clone mth2-11o4, complete sequence 306 5e-80 AF056621.1 Medicago sativa putative Cu/Zn superoxide dismutase precursor, mRNA, nuclear gene encoding chloroplast protein, complete cds 293 3e-76 J04087.1 Pea chloroplastic copper/zinc-superoxide dismutase mRNA, complete cds 183 4e-43	***ATTAACCCTCACTAAATGCTGGTA***AAAAATTAGCTTGGGGTGTGGTTGGCTTG ACTCCAATATAAATGCTACGAATGCTTTTGTACCAGCAGTGTTATATTATCTG GTATTTGATTTCTTCAGTTACCCTTGTTTTATGAAGCTTGATGGCTTACAATT ATTTGCTTTCTTCGCTGTCTTCTGATAGCAATGTAAACTATGAAGCTTAATGT CCTAGATGAGTGTGTAAACGGTATGTTATTCTTTTACCAAAAAGATTCATCA TGCAGTCGGGGTGCTTACTCCCCAATAAAATTTAATTTCTGATGTAGTTCTTGT TGCCTGCTTG***TGAAAAAAAAAGATATCACTCAGCATAATG*** **Reverse frame 2, 118 amino acids** IMLSDIFFFHKQATRTTSEIKFYWGVSTRLHDESFW*KNNIPFTHSSRTLSFIVYIAI RRQRRKQIIVSHQAS

APPENDIX E

PREDICTION OF MOLECULAR FUNCTION, BIOLOGICAL PROCESS AND CELLULAR COMPONENTS OF SELECTED ESTs

Prediction of molecular function, biological process and cellular components of selected ESTs which have similarity score above 40 bits are shown in the below Table. Functions were predicted from the information in the BLASTX hits mainly. These hits were shown in Appendix K. Final classification of function-process-component was stated in bold.

Table E.1 Prediction of molecular function, biological process and cellular components of selected ESTs which have similarity score above 40 bits.

INFORMATION AND BLASTX HIT OF EST EST no / Sequence size: bp **BLASTX** (Hit / Score(Bits) - E Value) **CONSERVED DOMAIN** (Title /E-value)	**MOLECULAR FUNCTION**	**BIOLOGICAL PROCESS**	**CELULAR COMPO-NENT**
	RGA-DDRT-PCR-Trials		
NO: R13 263 bp **BLASTX** gb|AAY85658.1| plastid glucose-6-phosphate/phosphate transloc.. 80.1 4e-14	TPT (PF03151) IPR004853 UAA (PF08449) **transport**	Transporters-sugars **Cellular Transport..** **(MIPS 20)**	**integral membrane**
NO: R21 306 bp **BLASTX:** gb|ABK23952.1| unknown [Picea sitchensis] 58.2 2e-07 ref|NP_565854.1| zinc finger (C2H2 type) family protein [Arab.. 57.4 3e-07	nucleic acid binding **(nucleic acid binding)** zinc ion binding **(ion binding)**	**UNKNOWN**	GO:0005634* **nucleus**
NO: R46 261 bp **BLASTX** emb|CAO62495.1| unnamed protein product [Vitis vinifera] 127 3e-28 emb|CAN66563.1| hypothetical protein [Vitis vinifera] 127 3e-28 **CONSERVED DOMAIN** cd00180, S_TKc, Serine/Threonine protein kinases, catalytic domain. Phosphotransferase.. 5e-12	SM00220 S_TKc protein kinase activity **(catalytic activity)** ATP binding **(nucleotide binding)**	SM00220 S_TKc protein amino acid phosphorylation (protein modification) Protein destination and storage **Protein Fate..** **(MIPS 14)**	GO:0016021* **integral to membrane**
NO: R44 473 bp **BLASTX:** emb|CAO40090.1| unnamed protein product [Vitis vinifera] 70.9 3e-11	**UNKNOWN**	**UNKNOWN**	**unknown**

NO: R48 394 bp **BLASTX** dbj|BAA10929.1| cytochrome P450 like_TBP [Nicotiana tabacum] 121 1e-26 pir||T02955 probable cytochrome P450 monooxygenase - maize (frag 101 2e-20	unspecific monooxygenase activity **(catalytic activity)**	**UNKNOWN** probably **Cell Rescue** **(MIPS 32)**	**unknown**
NO: R50 177 bp **BLASTX** emb|CAC44142.1| putative polyprotein [Cicer arietinum] 89.0 9e-17	IPR001584 IPR012337 DNA binding **(nucleic acid binding)**	IPR001584 IPR012337 DNA integration (DNA metabolic process) Retroelement **Transposable Elements..** **(MIPS 38)**	**nucleus**
RGA-RT-PCR			
NO: 31 541 bp **BLASTX** ref|NP_568011.1| RNA recognition (RRM)-containing prote.. 258 9e-68 **CONSERVED DOMAIN** smart00360, RRM, RNA recognition motif 1e-12	SM00360 IPR000504 IPR012677 RNA binding **(nucleic acid binding)**	Unknown Probable: Ribonucleo proteins, regulation of alternative splicing, regulation of RNA stability and translation **Transcription** **(MIPS 11)**	**Unknown**
NO: 104 368 bp **BLASTX** emb|CAC86495.1| RGA-F protein [Cicer arietinum] 194 1e-48 **CONSERVED DOMAIN** pfam00931, NB-ARC, NB-ARC domain. 9e-10	IPR002182 **ATP binding** **(nucleotide binding)**	IPR000767 IPR002182 defense response (response to stimulus) apoptosis (cell death) **Cell Fate** **(MIPS 40)**	**Unknown**
NO: 19 356 bp **BLASTX** ref|NP_568958.1| unknown protein [Arabidopsis thaliana] >gb|A.. 183 3e-45	Tic22 (PF04278) IPR007378 Unknown Probable: **acyltransferase activity** **(catalytic activity)**	Tic22 (PF04278) IPR007378 Translocationof proteins through the inner membrane of chloroplast (transport) **Cellular Transport**. **(MIPS 20)**	**inner envelope membrane of chloroplasts**
NO: 2 557 bp **BLASTX** emb|CAA57721.1| protein kinase [Medicago sativa] 291 2e-77 gb|AAF73236.1|AF153061_1 MAP kinase 3 [Pisum sativum] 290 4e-77 **CONSERVED DOMAIN** cd00180, S_TKc, Serine/Threonine protein kinases, catalytic domain. 1e-33	IPR000719 IPR011009 IPR002290 IPR008271 IPR003527 IPR008351 MAP kinase activity (protein serine/threonine kinase activity) **(catalytic activity)** ATP binding **(nucleotide binding)**	IPR000719 IPR011009 IPR002290 IPR008271 IPR003527 IPR008351 response to stimulus signal transduction **Cellular Communication**.. **(MIPS 30)**	**Unknown**
NO: 116 (eU_HW-070116_Plate5e01), 376 bp **BLASTX** gb|AAZ94162.1| enzymatic resistance protein [Glycine max] 87.4 3e-16 gb|AAQ56193.1| aminotransferase 2 [Cucumis melo] 83.6 5e-15 **CONSERVED DOMAIN** COG0075, COG0075, Serine-pyruvate aminotransferase/archaeal aspartate aminotransferase.. 0.006	COG0075 transferase activity serine-glyoxylate transaminase activity **(catalytic activity)**	Amino Acid Metabolism Enzymatic Resistance **Metabolism** **(MIPS 01)**	**Unknown**

NO: 38 737 bp emb|CAN77792.1| hypothetical protein [Vitis vinifera] 166 2e-39 emb|CAO70481.1| unnamed protein product [Vitis vinifera] 150 9e-35 ref|NP_001078319.1| EIF4G (EUKARYOTIC TRANSLATION INITIATION .. 138 3e-33 **CONSERVED DOMAIN:** pfam02847, MA3, MA3 domain. Domain in DAP-5, eIF4G, MA-3 and other proteins. 2e-07	IPR003891 MA3 (PF02847) IPR003890 **protein binding (protein binding) translation factor activity, nucleic acid binding (nucleic acid binding)**	IPR003891 MA3 (PF02847) IPR003890 response to virus regulation of translational initiation, RNA metabolic process (nucleobase, nucleoside, nucleotide and nucleic acid metabolic process) **Protein Synthesis (MIPS 12)** also **Cell Fate (MIPS 40)**	**Cytoplasm**
NO:179 336 bp **BLASTX** gb|ABU98947.1| dynein light chain [Lupinus albus] 74.3 2e-12 emb|CAN81003.1| hypothetical protein [Vitis vinifera] 74.3 2e-12 **CONSERVED DOMAIN** pfam01221, Dynein_light, Dynein light chain type 1. 2e-11	**motor activity**	microtubule-based process (cytoskeleton organization and biogenesis) Cell structure:Cytoskeleton **Biogenesis of Cell. Comp. (MIPS 42)**	microtubule associated complex **(cytoskeleton)**
NO: 253 571 bp **BLASTX** sp|P14584|CB21_RAPSA Chlorophyll a-b binding of LHCII type 1 .. 149 1e-53 emb|CAA10284.1| chlorophyll a/b binding protein [Cicer arietinum 162 1e-38	**binding**	IPR001344 photosynthesis, light harvesting **Energy (MIPS 02)**	GO:0016020* **membrane**
NO: 262 487 bp **BLASTX** emb|CAO71692.1| unnamed protein product [Vitis vinifera] 211 1e-53 ref|NP_179336.1| leucine-rich repeat family protein [Arabidop.. 174 2e-42 **CONSERVED DOMAINS** COG4886, COG4886, Leucine-rich repeat (LRR) protein [Function unknown]. 3e-06	IPR001611 IPR003591 **protein binding**	Unknown Probable: involved in a variety of biological processes **UNKNOWN**	GO:0005575* **Unknown**
NO: 154 366 bp**,** Similar to 417 and 405. **BLASTX** emb|CAN64195.1| hypothetical protein [Vitis vinifera] 107 3e-22 gb|AAK25760.1|AF334840_1 ribosomal protein L33 [Castanea sativa] 106 4e-22	Ribosomal_L35Ae **structural constituent of ribosome**	Translation **Protein Fate.. (MIPS 14)**	GO:0005840* **ribosome**
NO: 353 426 bp **BLASTX:** emb|CAO63363.1| unnamed protein product [Vitis vinifera] 59.7 6e-08	Probably: RNA binding **(nucleic acid binding)**	**UNKNOWN**	**unknown**
NO: 489 374 bp **BLASTX:** emb|CAO64530.1| unnamed protein product [Vitis vinifera] 46.2 7e-04 emb|CAN77176.1| hypothetical protein [Vitis vinifera] 46.2 7e-04	**UNKNOWN**	**UNKNOWN**	**unknown**
NO: 402 459 bp **BLASTX** gb|ABO61516.1| GAI1 [Glycine max] 131 2e-29	IPR005202 transcription factor activity **(nucleic acid binding)**	signal transduction (gibberellic acid mediated signaling) response to stimulus (ethylene, salt, abscisic acid) developmental process, regulation of nitrogen utilization **Cellular Communication.. (MIPS 30)**	GO:0005634* **nucleus**

NO: 384 628 bp **BLASTX** emb|CAO65845.1| unnamed protein product [Vitis vinifera] 125 2e-27 gb|AAO69667.1| vacuolar ATPase subunit E [Phaseolus acutifolius] 124 3e-27 **CONSERVED DOMAIN** PRK02292, PRK02292, V-type ATP synthase subunit E. 2e-04 pfam01991, vATP-synt_E, ATP synthase (E/31 kDa) subunit. 2e-14 **Similar to 1749**	PRK02292 vATP-synt_E (PF01991) IPR002842 hydrogen ion transporting ATP synthase activity, rotational mechanism hydrogen ion transporting ATPase activity, rotational mechanism **(ion transmembrane transporter activity)** **Transport ATPases**	IPR002842 ATP synthesis coupled proton transport (cellular metabolic process) organelle organization and biogenesis, development response to stimulus **Cellular Transport..(MIPS 20)** also **Interaction With Cell. Env.** **(MIPS 34)**	GO:0016469 Proton transporting two-sector ATPase complex **(vacuolar membrane)**
EST NO: 505 317 bp **BLASTX:** ref|NP_564517.1| unknown protein [Arabidopsis thaliana] >gb|A.. 51.2 2e-05	**UNKNOWN**	**UNKNOWN**	**unknown**
NO: 372 519 bp **BLASTX** dbj|BAE71197.1| hypothetical protein [Trifolium pratense] 238 1e-61 dbj|BAF01924.1| hypothetical protein [Arabidopsis thaliana] 129	Probable: **nucleotide binding**	Unknown Probable: variety of functions ranging from signal transduction and transcription regulation to cell cycle control and apoptosis **UNCLEAR**	Probable: GO:0009507* **chloroplast**
NO: 417 531 bp, Similar to 405 and 154 **BLASTX** gb|ABK93583.1| unknown [Populus trichocarpa] >gb|ABK94271.1| .. 220 4e-56 gb|ABK93065.1| unknown [Populus trichocarpa] 219 8e-56 gb|AAK25760.1|AF334840_1 ribosomal protein L33 [Castanea sativa] 218 1e-55 **CONSERVED DOMAIN** pfam01247, Ribosomal_L35Ae, Ribosomal protein L35Ae. 1e-28	Ribosomal_L35Ae (PF01247) IPR001780 structural constituent of ribosome **(structural molecule activity)**	Ribosomal_L35Ae (PF01247) IPR001780 translation **Protein Fate..** **(MIPS 14)**	GO:0005840* **ribosome**
NO: 405 >537 bp Similar to 417 and 154 **BLASTX** gb|ABK93583.1| unknown [Populus trichocarpa] >gb|ABK94271.1| .. 191 2e-47 gb|ABK93065.1| unknown [Populus trichocarpa] 190 4e-47 emb|CAN64195.1| hypothetical protein [Vitis vinifera 190 4e-47 pfam01247, Ribosomal_L35Ae, Ribosomal protein L35Ae. 2e-21	Ribosomal_L35Ae (PF01247) IPR001780 structural constituent of ribosome **(structural molecule activity)**	Ribosomal_L35Ae (PF01247) IPR001780 translation **Protein Fate..** **(MIPS 14)**	GO:0005840* **ribosome**
NO: 427 585 bp **BLASTX** ref|NP_190930.1| inorganic pyrophosphatase, putative (soluble.. 293 5e-78 **CONSERVED DOMAIN:** cd00412, pyrophosphatase, Inorganic pyrophosphatase. 3e-52	IPR008162 magnesium ion binding **(ion binding)** inorganic diphosphatase activity **(catalytic activity)**	IPR008162 phosphate metabolic process **Metabolism** **(MIPS 01)**	GO:0005737* **cytoplasm**
EST NO: 937 224 bp **BLASTX:** mb|CAN77440.1| hypothetical protein [Vitis vinifera] >emb|CA.. 53.9 3e-06 gb|AAN65067.1| Similar to CGI-126 protein [Arabidopsis thaliana] 53.9 3e-06 ref|NP_564289.1| unknown protein [Arabidopsis thaliana] >gb|A.. 53.9 3e-06 **CONSERVED DOMAIN:** pfam08694, UFC1, Ubiquitin-fold modifier-conjugating enzyme 1. Ubiquitin-like (UBL) po.. 87601 No 1e-04	UFC1 (PF08694) ubiquitin-like **protein binding**	UFC1 (PF08694) post-translational modifier **Protein Fate..** **(MIPS 14)**	**Unknown**

NO: 645 288 bp Same hit as 2132 and 1934	IPR000626 Ubiquitin Probable: IPR002906 **structural constituent of ribosome**	IPR000626 protein modification **Protein Fate..** **(MIPS 14)** also **Protein Synthesis** **(MIPS 12)**	Probable GO:0005840* **ribosome**
NO: 863 >520 bp Similar to 2 **BLASTX** emb|CAA57721.1| protein kinase [Medicago sativa] 68.6 1e-15 gb|ABF82263.1| MAP kinase [Cicer arietinum] 48.9 2e-15 **CONSERVED DOMAIN** cd00180, S_TKc, Serine/Threonine protein kinases, catalytic domain. 4e-07	IPR000719 IPR011009 IPR002290 IPR008271 IPR003527 IPR008351 MAP kinase activity (protein serine/threonine kinase activity) **(catalytic activity)** ATP binding **(nucleotide binding)**	IPR000719 IPR011009 IPR002290 IPR008271 IPR003527 IPR008351 response to stimulus signal transduction **Cellular Communication..** **(MIPS 30)**	**Unknown**
NO: 860 564 bp **BLASTX** emb|CAA08906.1| cysteine proteinase [Cicer arietinum] 188 1e-46	IPR000169 IPR000668 IPR013128 cysteine-type peptidase activity **(catalytic activity)**	Proteolysis (metabolism) programmed cell death **Cell Fate (MIPS 40)** also **Protein Fate..** **(MIPS 14)**	GO:0012505* **endomembrane system**
NO: 853 328 bp **BLASTX** ref|NP_180560.1| NDA2 (ALTERNATIVE NAD(P)H DEHYDROGENASE 2); .. 147 2e-34 emb|CAB52796.1| putative internal rotenone-insensitive NADH d.. 147 3e-34 **CONSERVED DOMAIN** COG1252, Ndh, NADH dehydrogenase, FAD-containing subunit [Energy production and conver.. 2e-12	NADH dehydrogenase activity **(electron carrier activity)** **(catalytic activity)**	electron transport **Energy (MIPS 02)**	GO:0031304* **intrinsic to mitochondrial inner membrane**
NO: 892 669 bp **BLASTX** gb|AAM65279.1| unknown [Arabidopsis thaliana] 130 8e-29 ref|NP_176940.1| PSBY (photosystem II BY) [Arabidopsis thalia.. 130 8e-29	manganese-binding polypeptide L-arginine metabolizing enzyme activity **(catalytic activity)**	photosynthesis **Energy (MIPS 02)**	GO:0009535* **chloroplast thylakoid membrane**
NO: 882 373 bp **BLASTX:** gb|ABE11607.1| COV1-like protein [Solanum chacoense] 207 2e-52	**UNKNOWN**	**UNKNOWN**	**integral membrane**
NO: 818 801 bp **BLASTX** emb|CAO38916.1| unnamed protein product [Vitis vinifera] 382 1e-104 ref|NP_567760.1| alanine racemase family protein [Arabidopsis th 369 9e-101 **CONSERVED DOMAIN** cd00635, YBL036c_PLPDEIII, PLP dependent enzymes class III (PLPDE_III). 7e-73	alanine racemase activity **(catalytic activity)** pyridoxal phosphate binding **(cofactor binding)**	biosynthesis of amino acids , amino acid-derived metabolites, amino sugars, catabolism of neurotransmitters **Metabolism** **(MIPS 01)**	**unknown**
NO: 997 376 bp **BLASTX** gb|ABD32881.1| Nascent polypeptide-associated complex NAC; UB.. 219 4e-56 **CONSERVED DOMAIN** pfam01849, NAC, NAC domain. 1e-11	NAC (PF01849) EGD2 IPR009060 **binding**	NAC (PF01849) EGD2 IPR009060 Targeting **Protein Fate..** **(MIPS 14)**	GO:0005854* nascent polypeptide-associated complex **(cytoplasm)**
NO: 955 353 bp **BLASTX** emb|CAD27943.1| PsbY-like protein precursor [Oryza sativa] 72.8 7e-12	manganese-binding polypeptide **(binding)** L-arginine metabolizing enzyme activity **(catalytic activity)**	photosynthesis **Energy (MIPS 02)**	GO:0009535** **chloroplast thylakoid membrane**

NO: 1072 504 bp **BLASTX** gb|ABB85180.1| NBS-LRR type disease resistance protein [Vicia fa 269 4e-72 gb|ABB85195.1| NBS-LRR type disease resistance protein [Cicer.. 266 2e-71 emb|CAC86496.1| RGA-G protein [Cicer arietinum] 266 2e-71 **CONSERVED DOMAIN** pfam00931, NB-ARC, NB-ARC domain. 1e-06	IPR002182 NB-ARC (PF00931) Q8VXR6_CICAR ATP binding **(nucleotide binding)**	IPR002182 NB-ARC (PF00931) Q8VXR6_CICAR apoptosis (cell death) **Cell Fate (MIPS 40)**	**unknown**
NO: 1158 382 bp; similar to 860 **BLASTX** emb|CAA08906.1| cysteine proteinase [Cicer arietinum] 121 2e-26	IPR000169 IPR000668 IPR013128 cysteine-type peptidase activity **(catalytic activity)**	Proteolysis (metabolism) programmed cell death **Cell Fate** **(MIPS 40)** also **Protein Fate..** **(MIPS 14)**	GO:0012505* **endomembrane system**
NO: 1422a 501 bp **BLASTX** gb|AAK64167.1| putative methionine synthase [Arabidopsis thalian 262 5e-69 ref|NP_187028.1| AtMS2 (Arabidopsis thaliana methionine synth.. 262 5e-69 cd03311, CIMS_C_terminal_like, CIMS - Cobalamine-independent methonine synthase, or Me…1e-43	IPR011060 IPR011254 IPR013215 IPR002629 IPR006276 5-methyltetrahydropteroyltriglutamate-homocysteine S-methyltransferase activity methionine synthase activity **(catalytic activity)**	IPR011060 IPR011254 IPR013215 IPR002629 IPR006276 methionine biosynthetic process (amino acid metabolism) **Metabolism** **(MIPS 01)**	GO:0005829* **cytosol**
NO: 241 251 bp emb|CAO61326.1| unnamed protein product [Vitis vinifera] 81.3 2e-14 emb|CAN78410.1| hypothetical protein [Vitis vinifera] 81.3 2e-14 ref|NP_195062.1| terpene cyclase/mutase-related [Arabidopsis .. 79.0 9e-14	IPR001509 3Beta_HSD (PF01073) dihydrokaempferol 4-reductase activity **(catalytic activity)** coenzyme binding **(cofactor binding)**	IPR001509 3Beta_HSD (PF01073) terpenoid metabolic process (cellular lipid metabolic process) **Metabolism** **(MIPS 01)**	GO:0005575* **unknown**
NO: 826 458 bp **BLASTX** emb|CAN78606.1| hypothetical protein [Vitis vinifera] 90.5 3e-17 ref|NP_200269.1| RNA recognition motif (RRM)-containing prote.. 90.5 3e-17 **CONSERVED DOMAIN** smart00360, RRM, RNA recognition motif; . 3e-05	IPR000504 IPR012677 RNA binding **(nucleic acid binding)**	Unknown Probable: Ribonucleo proteins, regulation of alternative splicing, regulation of RNA stability and translation **Transcription** **(MIPS 11)**	**Unknown**
NO: 447 > 392 bp **BLASTX** ref|NP_200901.2| chromosome-associated kinesin, putative [Arabid 47.4 3e-04	Probable: microtubule motor activity **(motor activity)** ATP binding **(nucleotide binding)**	Probable: microtubule-based movement (cellular component organization and biogenesis) **Biogenesis of Cell. Comp.** **(MIPS 42)**	Probable: GO:0005875* microtubule associated complex **(cytoskeleton)**
NO: B20 463 bp dbj|BAF74756.1| CBL-interacting protein kinase [Vigna unguiculat 210 1e-66 **CONSERVED DOMAIN** cd00180, S_TKc, Serine/Threonine protein kinases, catalytic domain. 1e-30	S_TKc IPR002290 SMART SM00220 protein serine/threonine kinase activity **(catalytic activity)** ATP binding **(nucleotide binding)**	S_TKc IPR002290 SMART SM00220 post-translational protein modification (protein modification process) **Cellular Communication..** **(MIPS 30)** also **Protein Fate..** **(MIPS 14)**	**Unknown**

NO: B20-2 400 bp Similar to 350 **BLASTX** gb|ABH08754.1| ubiquitin [Arabidopsis thaliana] 241 1e-62 **CONSERVED DOMAIN** cd01803, Ubiquitin, Ubiquitin (includes Ubq/RPL40e and Ubq/RPS27a… 3e-34 cd01803, Ubiquitin, Ubiquitin (includes Ubq/RPL40e and Ubq/RPS27a… 1e-20	cd01803 Ubiquitin IPR000626 **protein binding**	cd01803 Ubiquitin IPR000626 protein modification process, aging (developmental process) response to salicylic acid stimulus (response to stimulus) **Protein Fate..** **(MIPS 14)** also **Interaction with Env.** **(MIPS 36)**	**Unknown**
NO: 350 494 bp, Similar to B20-2 **BLASTX** gb|EAY84584.1| hypothetical protein OsI_005817 [Oryza sativa .. 282 5e-75 gb|ABU40645.1| polyubiquitin [Triticum aestivum] 282 6e-75 gb|ABH08754.1| ubiquitin [Arabidopsis thaliana] 282 6e-75 **CONSERVED DOMAIN** cd01803, Ubiquitin, Ubiquitin (includes Ubq/RPL40e and Ubq/RPS27a… 3e-34 cd01803, Ubiquitin, Ubiquitin (includes Ubq/RPL40e and Ubq/RPS27a… 2e-31	cd01803 Ubiquitin IPR000626 **protein binding**	cd01803 Ubiquitin IPR000626 protein modification process, aging (developmental process) response to salicylic acid stimulus (response to stimulus) **Protein Fate..** **(MIPS 14)** also **Interaction with Env.** **(MIPS 36)**	**Unknown**
NO: 453 211 bp **BLASTX:** emb|CAO42125.1| unnamed protein product [Vitis vinifera] 55.1 1e-06	**UNKNOWN**	Probably: Agenet pfam05641 **UNKNOWN**	**Unknown**
	DDRT-PCR ESTs		
NO: 1465a **> 427 bp** **BLASTX:** emb|CAO62021.1| unnamed protein product [Vitis vinifera] 57.4 3e-07 ref|NP_565129.1| unknown protein [Arabidopsis thaliana] >gb|A.. 57.4 3e-07 **CONSERVED DOMAIN:** pfam03138, DUF246, Plant protein family. The function of this family of plant proteins.. 66790 No 2e-08	DUF246 **Unknown**	**UNKNOWN**	GO:0005739* **mitochondrion**
NO:**1490** 329 bp **BLASTX** emb|CAC44123.1| N3 like protein [Medicago truncatula] 77.8 2e-13	IPR004316 **Unknown**	Ripening (Development) **Development..** **(MIPS 41)**	IPR004316 **Transmembrane**
NO:**1468** 483 bp **BLASTX** ref|NP_001046190.1| Os02g0196000 [Oryza sativa (japonica cult.. 132 5e-30 **CONSERVED DOMAIN** PRK04201, PRK04201, zinc transporter ZupT. 6e-06	IPR003689 GO:0046873 metal ion transport **(Ion Transmembrane Transporter Activity)**	IPR003689 GO:0030001 Ion Transport **Cellular Transport..** **(MIPS 20)**	IPR003689 GO:0016020* **Membrane**
NO: **1480** 443 bp **BLASTX** emb|CAO65523.1| unnamed protein product [Vitis vinifera] 59.7 6e-08	nucleotide binding oxidoreductase activity **(catalytic activity)**	metabolic process resulting in cell growth metabolic process **Metabolism** **(MIPS 01)**	**unknown**
NO: **1934** 294 bp **BLASTX** emb|CAA80334.1| ubiquitin extension protein [Lupinus albus] 72.0 1e-11 SAME HIT AS 2132 and 645 **CONSERVED DOMAIN** pfam01599, Ribosomal_S27, Ribosomal protein S27a. 2e-06	PF01599 IPR0002906 structural constituent of ribosome **(structural molecule activity)**	PF01599 IPR0002906 translation **Protein Fate..** **(MIPS 14)** also **Protein Synthesis** **(MIPS 12)**	GO:0005840* **Ribosome**

NO: **1937** 386 bp **BLASTX** emb|CAO67101.1| unnamed protein product [Vitis vinifera] 65.1 1e-09 **CONSERVED DOMAIN** pfam05602, CLPTM1, Cleft lip and palate transmembrane protein 1 (CLPTM1) 3e-10	CLPTM1 (PF05602) IPR008429 **UNKNOWN**	CLPTM1 (PF05602) IPR008429 Unknown Probable: Apoptosis **Cell Fate (MIPS 40)**	GO:0016020* **Membrane**
NO: **1479** 501 bp **BLASTX** gb|ABN08405.1| Peptidase aspartic, active site [Medicago truncat 65.5 1e-09	pfam08284: RVP_2 cd01647: RT_LTR Reverse transcriptase Retroviral aspartyl protease **(catalytic activity)**	Retroelement Cell growth/division-DNA synth/replication **Transposable Elements..** **(MIPS 38)**	**nucleus**
EST NO: 1476a U_HW-070116_Plate6b02 300 bp **BLASTX:** ref|YP_173415.1| hypothetical protein NitaMp073 [Nicotiana ta.. 136 9e-36 emb|CAO46934.1| unnamed protein product [Vitis vinifera] 123 3e-27 gb|ABR26094.1| retrotransposon protein [Oryza sativa (indica cul 110 4e-23	GTP-binding **(nucleotide binding)** or retrotransposase activity	**UNCLEAR**	**unclear**
NO: 1931 336 bp **BLASTX** gb|ABB29467.1| salt-tolerance protein [Glycine max] 37.4 0.29	Probable: SM00336 IPR000315 **zinc ion binding** **(ion binding)**	Probable: IPR000315 Salt tolarence **Cell Rescue..** **(MIPS 32)**	**Probable:** GO:0005622* **intracellular**
EST NO: 1477a-a (P7*P7) 298 **BLASTX** emb|CAO21131.1| unnamed protein product [Vitis vinifera] 48.5 1e-04 ref|NP_568392.1| unknown protein [Arabidopsis thaliana] >gb|A.. 46.2 7e-04	**UNKNOWN**	**UNKNOWN**	**chloroplast envelope membrane protein**
NO: **1943-D5** 473 bp **BLASTX** ref|NP_563986.1| unknown protein [Arabidopsis thaliana] >gb|A.. 112 5e-24 gb|EAZ42142.1| hypothetical protein OsJ_025625 [Oryza sativa .. 102 1e-20	NCBI CDD COG0859 transferase activity **(catalytic activity)**	Metabolism **Metabolism** **(MIPS 01)**	GO:0009507* **chloroplast**
NO: 1943 (P9) 416 bp **BLASTX:** emb|CAO49820.1| unnamed protein product [Vitis vinifera] 210 3e-53 ref|NP_566924.1| unknown protein [Arabidopsis thaliana] >ref|.. 196 3e-49	methyltransferase activity **(catalytic activity)**	**UNKNOWN**	**chloroplast thylakoid membrane**
NO: 1940-2 398 bp **BLASTX** dbj|BAA02117.1| GTP-binding protein [Pisum sativum] >prf||200.. 75.5 1e-12	IPR001806 IPR002078 IPR005225 IPR003579 IPR013753 ATP binding- GTP binding **(nucleotide binding)** transcription factor binding **(protein binding)**	IPR001806 IPR003579 IPR002078 IPR005225 small GTPase mediated signal transduction (signal transduction) regulation of transcription, DNA-dependent (transcription regulation) protein transport **Cellular Communication..** **(MIPS 30)**	GO:0005622* **intracellular**
NO: 1560 410 bp **BLASTX** emb|CAO45205.1| unnamed protein product [Vitis vinifera] 140 2e-32 ref|NP_001031934.1| AAP7 (amino acid permease 7) [Arabidopsis th 140 2e-32 **CONSERVED DOMAIN** pfam01490, Aa_trans, Transmembrane amino acid transporter protein. 1e-16	Aa_trans (PF01490) IPR013057 Amino acid transmembrane **transporter activity**	Aa_trans (PF01490) IPR013057 Amino acid Transport Stres response **Cellular Transport..** **(MIPS 20)**	GO:0016020 **membrane**

NO: 1528 508 bp **BLASTX** emb|CAO65716.1| unnamed protein product [Vitis vinifera] 227 2e-58	hATC (PF05699) IPR008906 protein dimerization activity **(protein binding)**	hATC (PF05699) IPR008906 Transposable element **Transposable Elements..** **(MIPS 38)**	**unknown**
NO: 1562 352 bp **BLASTX** emb|CAO16829.1| unnamed protein product [Vitis vinifera] 89.0 2e-20 gb|AAB95218.1| putative serine-glyoxylate aminotransferase [F.. 89.7 4e-20 **CONSERVED DOMAIN** COG0075, COG0075, Serine-pyruvate aminotransferase/archaeal aspartate aminotransferase 1e-06	COG0075 transaminase activity serine-glyoxylate transaminase activity **(catalytic activity)**	Amino Acid Metabolism probable Enzymatic Resistance **Metabolism** **(MIPS 01)**	**unknown**
NO: 1538 313 bp **BLASTX** pdb|1J93|A Chain A, Crystal Structure And Substrate Binding M.. 69.7 5e-11 **CONSERVED DOMAIN** cd00717, URO-D, Uroporphyrinogen decarboxylase (URO-D) 7e-07	IPR006361 IPR000257 uroporphyrinogen decarboxylase activity **(catalytic activity)**	IPR006361 IPR000257 porphyrin biosynthetic process (cofactor biosynthetic process) **Metabolism** **(MIPS 01)**	GO:0009507* **chloroplast**
NO: 1555 226 bp **BLASTX** emb|CAO50143.1| unnamed protein product [Vitis vinifera] 47.0 4e-04 ref|NP_195760.1| ESP4 (ENHANCED SILENCING PHENOTYPE 4); bindi.. 34.7 2.0	Probable: **binding**	**Probable:** RNA-mediated posttranscriptional gene silencing (regulation of gene expression) RNA processing (nucleobase, nucleoside, nucleotide and nucleic acid metabolic process) **Transcription** **(MIPS 11)**	Probable: GO:0005847* (**nucleus)**
NO: 1642 245 bp **BLASTX** emb|CAE00491.2| 1-deoxy-D-xylulose-5-phosphate reductoisomera.. 52.0 1e-05	IPR003821 **catalytic activity**	IPR003821 isoprenoid metabolic process (cellular lipid metabolic proces) Lipid Metabolism **Metabolism** **(MIPS 01)**	GO:0009507* **chloroplast**
NO: 1536 447 bp **BLASTX** gb|ABD32724.1| Helicase, C-terminal; Argonaute and Dicer prot.. 229 3e-59	IPR000999 IPR003100 IPR001159 double-stranded RNA binding **(nucleic acid binding)** ribonuclease III activity **(catalytic activity)** probable: IPR011545 ATP-dependent helicase activity **(catalytic activity)** ATP binding **(nucleotide binding)**	IPR000999 IPR003100 IPR001159 probable: IPR011545 posttranscriptional gene silencing developmental process DNA modification RNA processing (RNA metabolic process) **Transcription** **(MIPS 11)**	GO:0005634* **nucleus**
NO: 1571 ~400 bp **BLASTX** emb|CAA04767.1| ripening-induced protein [Fragaria vesca] 54.3 6e-06	IPR002085 IPR002364 IPR011032 IPR013149 PF00107 IPR013154 zinc ion binding **(ion binding)** oxidoreductase activity **(catalytic activity)**	Electron-transport Energy **Detoxification MIPS 32.07** also **Energy** **(MIPS 02)**	**unknown**
NO: 1633 445 bp **BLASTX** gb|AAO33590.1|AF479308_1 putative caffeic acid methyl transferas 80.9 2e-14	IPR001077 O-methyltransferase activity **(catalytic activity)**	lignin biosynthesis **Metabolism** **(MIPS 01)**	**unknown**

NO: 1606 337 bp **BLASTX** sp|Q41001|BCP_PEA Blue copper protein precursor >emb|CAA80963.. 107 2e-22 **CONSERVED DOMAIN** pfam02298, Cu_bind_like, Plastocyanin-like domain. 3e-14	PF02298 IPR003245 IPR008972 copper ion binding **(ion binding)** electron carrier activity **(catalytic activity)**	IPR003245 IPR000923 electron transport (generation of precursor metabolites and energy) **Detoxification MIPS 32.07** also **Energy (MIPS 02)**	GO:0031225* **anchored to membrane**
1595 611 bp **BLASTX** dbj|BAE71297.1| hypothetical protein [Trifolium pratense] 308 2e-82 emb|CAA66481.1| transcription factor [Vicia faba var. minor] 285 1e-75 **CONSERVED DOMAIN** pfam04774, HABP4_PAI-RBP1, Hyaluronan / mRNA binding family. 1e-09	IPR006861 RNA binding **(nucleic acid binding)**	Probable: transcription factor **Transcription** **(MIPS 11)**	GO:0005634* **nucleus**
1609 292 bp **BLASTX** gb|**EAZ25827.1**| hypothetical protein OsJ_009310 [Oryza sativa .. 48.1 2e-04	Probable: ATP binding **(nucleotide binding)** **protein transporter activity**	Probable: intracellular protein transport **UNCLEAR**	Probable: GO:0030126* COPI vesicle coat **(cytoplasm)**
1619 363 bp **BLASTX** emb|CAO41131.1| unnamed protein product [Vitis vinifera] 110 4e-23 **CONSERVED DOMAIN:** COG1530, CafA, Ribonucleases G and E [Translation, ribosomal structure and biogenesis]. 0.009	IPR004659 ribonuclease activity **(catalytic activity)**	IPR004659 RNA processing (RNA metabolic process) Transcription **Transcription** **(MIPS 11)**	GO:0005737* **cytoplasm**
NO: 1612 198 bp **BLASTX** ref|NP_192536.1| ATCSLC12 (Cellulose synthase-like C12); tran.. 111 1e-23	Probable: pfam00535 IPR000312 transferase activity, transferring glycosyl groups **(catalytic activity)**	Probable: Metabolism **Metabolism** **(MIPS 01)** also **Biogenesis of Cell. Comp.** **(MIPS 42)**	**Unknown**
NO: 1601 > 400 bp **BLASTX** gb|AAN32497.1| ATP synthase beta subunit [Cypripedium passerinum 207 2e-52 **CONSERVED DOMAIN:** cd01133, F1-ATPase_beta, F1 ATP synthase beta subunit, nucleotide-binding domain 2e-51	IPR000194 IPR005722 nucleotide binding hydrogen ion transporting ATPase activity **(transporter activity)**	ATP synthesis coupled proton transport Energy Respiration **Energy** **(MIPS 02)**	GO: 0016469* proton-transporting two-sector ATPase complex **membrane** also GO:0009579* **thylakoid membrane**
NO: 1626 394 bp **BLASTX** gb|ABD33010.1| Fumarylacetoacetase [Medicago truncatula] 225 8e-58 **CONSERVED DOMAIN** pfam01557, FAA_hydrolase, Fumarylacetoacetate (FAA) hydrolase family. 2e-07	fumarylacetoacetase activity **(catalytic activity)**	amino acid metabolic process **Metabolism** **(MIPS 01)**	GO:0005575* **Unknown**
NO: 1623 384 bp emb|CAO47145.1| unnamed protein product [Vitis vinifera] 180 3e-44 ref|NP_051110.1| photosystem I subunit VII [Arabidopsis thali.. 178 1e-43 **CONSERVED DOMAIN** cd01916, ACS_1, Acetyl-CoA synthase (ACS), also known as acetyl-CoA decarbonylase 6e-07 CHL00065, psaC, photosystem I subunit VII. 3e-37	electron carrier activity **(catalytic activity)**	electron transport (generation of precursor metabolites and energy) photosynthesis **Energy** **(MIPS 02)**	**Chloroplast**

EST NO: 1611 >370 bp **BLASTX:** gb|AAK37555.1|AF349572_1 SHOOT1 protein [Glycine max] 157 4e-37 emb|CAN75787.1| hypothetical protein [Vitis vinifera] 131 2e-29 BlastN: AC153124.20 Medicago truncatula clone mth2-21e10, complete sequence 336 6e-90 ..... NM_104423.3 Arabidopsis thaliana binding / protein binding (AT1G55480) mRNA, complete cds 136 1e-29	RNA binding **(nucleic acid binding)** **(protein binding)**	various processes, such transcriptional control, signaling, protein transport, protein folding **Protein Fate..** **(MIPS 14)**	Probably: **chloroplast thylakoid membrane**
EST NO: 1597a 304 bp BLASTX: gb|EAZ37628.1| hypothetical protein OsJ_021111 [Oryza sativa .. 75.9 8e-13	DUF1680 Putative glycosyl hydrolase **(catalytic activity)**	**UNKNOWN**	**UNKNOWN**
NO: 1508 >549 bp **BLASTX** emb|CAO15460.1| unnamed protein product [Vitis vinifera] 97.4 4e-19 gb|ABF94173.1| ReMembR-H2 protein JR702, putative, expressed .. 89.0 1e-16 ref|NP_001049082.1| Os03g0167500 [Oryza sativa (japonica cult.. 89.0 1e-16 **CONSERVED DOMAIN** cd02123, PA_C_RZF_like, PA_C-RZF_ like: Protease-associated (PA) domain C_RZF-like. 1e-04	peptidase activity **(catalytic activity)** **protein binding** zinc ion binding **(ion binding)**	Proteolysis (cellular protein metabolic process) **Protein Fate..** **(MIPS 14)**	GO:0012505* endomembrane system **(membrane)**
	RGA-DDRT-PCR		
NO: 1990 459 bp **BLASTX** dbj|BAE99990.1| peptidylprolyl isomerase [Arabidopsis thaliana] 195 7e-49 ref|NP_199668.1| peptidyl-prolyl cis-trans isomerase, putativ.. 195 7e-49 **CONSERVED DOMAINS** pfam00254, FKBP_C, FKBP-type peptidyl-prolyl cis-trans isomerase. 3e-14	IPR001440 IPR011990 IPR001179 FKBP_C (PF00254) IPR013026 calmodulin binding **(protein binding)** peptidyl-prolyl cis-trans isomerase activity **(catalytic activity)**	IPR001440 IPR011990 IPR001179 FKBP_C (PF00254) IPR013026 protein folding Folding and stability Protein destination and storage **Protein Fate..** **(MIPS 14)**	GO:0005575* **unknown**
NO: 2132 287 bp SIMILAR CLONES: 645, 1934 **BLASTX** ref|NP_001054720.1| Os05g0160200 [Oryza sativa (japonica cult.. 140 3e-32 gb|ABK42077.1| ubiquitin extension protein [Capsicum annuum] 138 1e-31 **CONSERVED DOMAIN** cd01803, Ubiquitin, Ubiquitin (includes Ubq/RPL40e and Ubq/RPS27a fusions as well as h.. 2e-32	IPR000626 Ubiquitin Probable: IPR002906 **structural constituent of ribosome**	IPR000626 protein modification Folding and stability Protein destination and storage Probable: IPR002906 Translation **Protein Fate..** **(MIPS 14)** also **Protein Synthesis (MIPS 12)**	Probable: GO:0005840 **ribosome**
NO: 2150 364 bp **BLASTX** gb|ABQ41357.1| mitochondrial dihydroorotase [Vicia faba] 127 3e-28 **CONSERVED DOMAIN** cd01294, DHOase, Dihydroorotase (DHOase)… 4e-19	IPR004721 IPR002195 dihydroorotase activity **(catalytic activity)**	IPR004721 IPR002195 'de novo' pyrimidine base biosynthetic process (nucleobase metabolic process) **Metabolism** **(MIPS 01)**	Probable: **mitochondria**
NO: 2166(B28-2161) 240 bp gb|AAL47004.1| unknown [Davidia involucrata] 134 2e-30 gb|ABV89652.1| early-responsive to dehydration 4 [Brassica rapa] 128 1e-28 ref|NP_564354.1| ERD4 (EARLY-RESPONSIVE TO DEHYDRATION 4) [Ar.. 126 4e-28 **CONSERVED DOMAIN** pfam02714, DUF221, Domain of unknown function DUF221… 6e-09	DUF221 (PF02714) IPR003864 **unknown**	response to water deprivation (response to stimulus) **Interaction with Env.** **(MIPS 36)**	GO:0016020* **membrane**

NO: 2191 511 bp **BLASTX** gb|ABC59094.1| cytochrome P450 monooxygenase CYP704G9 [Medicago 250 4e-66 **CONSERVED DOMAIN** pfam00067, p450, Cytochrome P450… 4e-23	p450 (PF00067) IPR001128 iron ion binding **(ion binding)** heme binding **(tetrapyrrole binding)** monooxygenase activity **(catalytic activity)**	p450 (PF00067) IPR001128 electron transport (generation of precursor metabolites and energy) Energy Electron-transport **Cell Rescue..** **(MIPS 32)** also **Energy (MIPS 02)**	**unknown**
NO: 2204 455 bp **BLASTX** gb|ABN08096.1| Galactose mutarotase-like [Medicago truncatula] 287 2e-76 ref|NP_200543.1| aldose 1-epimerase family protein [Arabidops.. 259 3e-68 **CONSERVED DOMAIN** COG0676, COG0676, Uncharacterized enzymes related to aldose 1-epimerase. 1e-30	COG0676 IPR011013 IPR008183 aldose 1-epimerase activity **(catalytic activity)** **carbohydrate binding**	COG0676 IPR011013 IPR008183 galactose metabolic process (carbohydrate metabolic process) **Metabolism** **(MIPS 01)**	GO:0005575* **unknown**
NO: 2203 544 bp **BLASTX** emb|CAO23744.1| unnamed protein product [Vitis vinifera] 161 2e-38 **CONSERVED DOMAIN** pfam06136, DUF966, Domain of unknown function (DUF966). 1e-35	DUF966 pfam06136 IPR010369 **unknown**	**UNKNOWN**	**unknown**
NO: D8-4 484 bp **BLASTX** gb|AAB70660.1| grr1 [Glycine max] 209 5e-53 … gb|ABC24972.1| EIN3-binding F-box protein 2 [Lycopersicon escule 188 1e-46	SM00367 LRR_CC147IPR006553 **protein binding** **Probable:** pfam00646: F-box ubiquitin-protein ligase activity **(catalytic activity)**	SM00367 LRR_CC147IPR006553 Probable: ubiquitin-dependent protein catabolic process Protein destination and storage-Proteolysis **Protein Fate..** **(MIPS 14)** also **Interaction with Env.** **(MIPS 36)**	**Probable:** GO:0005634* **nucleus**
NO: 2296-D19 348 bp **BLASTX** emb|CAO16775.1| unnamed protein product [Vitis vinifera] 162 8e-39 emb|CAN70560.1| hypothetical protein [Vitis vinifera] 157 2e-37 ref|NP_192425.1| 4-coumarate--CoA ligase, putative / 4-coumar.. 151 1e-35 **CONSERVED DOMAIN** pfam00501, AMP-binding, AMP-binding enzyme 8e-20	(PF00501)pfam00501 IPR000873 4-coumarate-CoA ligase activity **(catalytic activity)**	(PF00501)pfam00501 IPR000873 phenylpropanoid metabolic process (amino acid and derivative metabolic process) auxin metabolic process jasmonic acid biosynthetic process (hormone biosynthetic process**)** **Metabolism (MIPS 01)**	GO:0005777* **peroxisome**
NO: 2296 346bp **BLASTX** emb|CAN79988.1| hypothetical protein [Vitis vinifera] 181 1e-44 **CONSERVED DOMAIN** pfam00183, HSP90, Hsp90 protein. 9e-27	HSP90 (PF00183) IPR001404 **nucleotide binding** unfolded protein binding **(protein binding)**	HSP90 (PF00183) IPR001404 response to unfolded protein (response to biotic stimulus) protein folding Protein destination and storage- Folding and stability **Cell Rescue..** **(MIPS 32)** also **Protein Fate..** **(MIPS 14)**	GO:0005739* **mitochondrion**
NO: 2325 368 bp **BLASTX** gb|ABK78691.1| putative elongation factor 1-beta [Brassica rapa] 65.5 1e-09 **CONSERVED DOMAIN** pfam00736, EF1_GNE, EF-1 guanine nucleotide exchange domain. 6e-04	pfam00736 EF1_GNE (PF00736) IPR014038 translation elongation factor activity **(nucleic acid binding)**	pfam00736 EF1_GNE (PF00736) IPR014038 translational elongation (translation) **Protein Synthesis** **(MIPS 12)**	GO:0005853* eukaryotic translation elongation factor 1 **(cytoplasm)**

NO: 1749 431 bp **BLASTX** gb|AAO69667.1| vacuolar ATPase subunit E [Phaseolus acutifolius] 117 3e-25 **CONSERVED DOMAIN** COG1390, NtpE, Archaeal/vacuolar-type H+-ATPase subunit E… 3e-08 pfam01991, vATP-synt_E, ATP synthase (E/31 kDa) subunit.. 8e-13	PRK02292 vATP-synt_E (PF01991) IPR002842 hydrogen ion transporting ATP synthase activity, rotational mechanism hydrogen ion transporting ATPase activity, rotational mechanism **(ion transmembrane transporter activity)** Transport ATPases	IPR002842 ATP synthesis coupled proton transport (cellular metabolic process) organelle organization and biogenesis development response to stimulus **Cellular Transport..(MIPS 20)** also **Interaction With Cell. Env. (MIPS 34)**	GO:0016469* Proton transporting two-sector ATPase complex **(vacuolar membrane)**
NO: 1901 **(or 1772),** 550 bp **BLASTX** gb|AAD51625.1|AF169022_1 seed maturation protein PM37 [Glycine m 283 4e-75 **CONSERVED DOMAIN** pfam00684, DnaJ_CXXCXGXG, DnaJ central domain (4 repeats). 9e-08 pfam01556, DnaJ_C, DnaJ C terminal region. 6e-05	DnaJ_CXXCXGXG (PF00684) DnaJ_C (PF01556) IPR002939 DnaJ (PF00226) IPR001623 unfolded protein binding heat shock protein binding **(protein binding)**	DnaJ_CXXCXGXG (PF00684) DnaJ_C (PF01556) IPR002939 DnaJ (PF00226) IPR001623 protein folding Protein destination and storage- Folding and stability **Protein Fate.. (MIPS 14)**	**unknown**
NO: 1868 474 bp **BLASTX** emb|CAO49173.1| unnamed protein product [Vitis vinifera] 195 7e-49 **CONSERVED DOMAIN** pfam00450, Peptidase_S10, Serine carboxypeptidase. 9e-35	IPR001563 Peptidase_S10 (PF00450) serine carboxypeptidase activity **(catalytic activity)**	IPR001563 Peptidase_S10 (PF00450) proteolysis (cellular macromolecule metabolic process) **Protein Fate..** **(MIPS 14)** also **Cellular Communication..** **(MIPS 30)**	GO:0012505* **endomembrane system**
NO: 1804 470 bp **BLASTX** emb|CAO64896.1| unnamed protein product [Vitis vinifera] 148 9e-35	CypX COG2124 p450 (PF00067) IPR001128 iron ion binding **(ion binding)** heme binding **(tetrapyrrole binding)** monooxygenase activity **(catalytic activity)**	p450 (PF00067) IPR001128 electron transport (generation of precursor metabolites and energy) Energy Electron-transport **Cell Rescue..** **(MIPS 32)** also **Energy (MIPS 02)**	**unknown**
NO: 1806 292 bp **BLASTX** ref|NP_001062701.1| Os09g0258600 [Oryza sativa (japonica cult.. 166 6e-40 **CONSERVED DOMAIN** cd00336, Ribosomal_L22, Ribosomal protein L22/L17e. L22 (L17 in eukaryotes) is a core .. 1e-19	IPR001063 IPR005721 Ribosomal_L22 PF00237 **structural constituent of ribosome**	IPR001063 IPR005721 Ribosomal_L22 PF00237 translation **Protein Synthesis** **(MIPS 12)**	GO:0015934* large ribosomal subunit **(ribosome)**
NO: 1881 206 bp **BLASTX** emb|CAO17868.1| unnamed protein product [Vitis vinifera] 85.9 7e-16 ref|NP_568355.1| methyltransferase [Arabidopsis thaliana] >gb.. 82.4 8e-15	IPR004395 IPR003358 methyltransferase activity **(catalytic activity)**	**Metabolism** **(MIPS 01)**	GO:0009507* **chloroplast**
NO: 1998 > 473bp Primers found: PtoFenS*? Similar to 262 **BLASTX** emb|CAO71692.1| unnamed protein product [Vitis vinifera] 187 2e-46 ref|NP_179336.1| leucine-rich repeat family protein [Arabidop.. 155 8e-37 **CONSERVED DOMAIN** COG4886, COG4886, Leucine-rich repeat (LRR) protein [Function unknown]. 1e-04	IPR001611 IPR003591 **protein binding**	Probable: involved in a variety of biological processes **UNKNOWN**	GO:0005575* **Unknown**

NO: 1793 (D19-1) similar to 1758 (D18-4) 547 bp **BLASTX** gb|ABD33028.1| UBA-like [Medicago truncatula] 135 9e-31 emb|CAO18203.1| unnamed protein product [Vitis vinifera] 110 4e-23 ref|NP_191233.1| ubiquitin-associated (UBA)/TS-N domain-conta.. 108 1e-22 **CONSERVED DOMAIN** cd00194, UBA, Ubiquitin Associated domain…4e-09	DER1 (PF04511) IPR009060 IPR000449 **Unknown**	DER1 (PF04511) IPR009060 IPR000449 Unknown Probable: ubiquitin/proteasome pathway, DNA excision-repair, and cell signalling, translation **Protein Fate..** **(MIPS 14)**	GO:0012505* endomembrane system **(membrane)**
NO: 1758 (D18-4) 516 bp **BLASTX** gb|ABD33028.1| UBA-like [Medicago truncatula] 267 2e-70 ref|NP_191233.1| ubiquitin-associated (UBA)/TS-N domain-conta.. 221 1e-56 **CONSERVED DOMAIN** cd00194, UBA, Ubiquitin Associated domain… 1e-07	IPR009060 IPR000449 **Unknown**	IPR009060 IPR000449 Unknown Probable: ubiquitin/proteasome pathway, DNA excision-repair, and cell signalling, translation **Protein Fate..** **(MIPS 14)**	GO:0012505* endomembrane system **(membrane)**
EST NO: 1465 >427 bp **BLASTX:** emb|CAO62021.1| unnamed protein product [Vitis vinifera] 57.4 3e-07 ref|NP_565129.1| unknown protein [Arabidopsis thaliana] >gb|A.. 57.4 3e-07 **CONSERVED DOMAIN:** pfam03138, DUF246, Plant protein family. The function of this family of plant proteins.. 66790 No 2e-08	DUF246 IPR004348 **Unknown**	DUF246 IPR004348 Probable: auxin-independent growth regulation **UNKNOWN**	GO:0005739* **mitochondrion**
GSP-RT-PCR			
NO: Fdh-3 (FDH) 404 bp **BLASTX** mb|CAE12168.2| formate dehydrogenase [Quercus robur] 249 3e-65 gb|EAZ37051.1| hypothetical protein OsJ_020534 [Oryza sativa .. 247 1e-64	IPR006139 2-Hacid_dh_C (PF02826) IPR006140 NAD binding **(cofactor binding)** oxidoreductase activity, acting on the CH-OH group of donors, NAD or NADP as acceptor **(catalytic activity)**	IPR006139 2-Hacid_dh_C (PF02826) IPR006140 Energy production and conversion /Coenzyme metabolism (metabolism) **Cell Rescue..** **(MIPS 32)** also **Metabolism** **(MIPS 01)**	GO:0009507* **chloroplast** GO:0005739* **mitochondrion**
NO: FPIP 400 bp **BLASTX** dbj|BAA77395.1| SLL2-S9-protein [Brassica rapa] 187 1e-46 emb|CAO17056.1| unnamed protein product [Vitis vinifera] 187 2e-46	pfam05175 MTS (PF05175) IPR007848 IPR013216 methyltransferase activity **(catalytic activity)**	pfam05175 MTS (PF05175) IPR007848 IPR013216 **Metabolism** **(MIPS 01)**	**Unknown**
NO: Pe-3 (PE), 422 bp **BLASTX** emb|CAN77092.1| hypothetical protein [Vitis vinifera] 169 7e-41 emb|CAO42327.1| unnamed protein product [Vitis vinifera] 167 3e-40 ref|NP_173733.1| pectinesterase family protein [Arabidopsis t.. 156 3e-37 **CONSERVED DOMAIN** pfam01095, Pectinesterase, Pectinesterase. 2e-47	Pectinesterase (PF01095) IPR000070 pectinesterase activity aspartyl esterase activity **(catalytic activity)**	Pectinesterase (PF01095) IPR000070 cell wall modification (cellular component organization and biogenesis) **Biogenesis of Cell. Comp.** **(MIPS 42)**	GO:0009505* cellulose and pectin-containing cell wall **(cell wall)**

NO: Eds1-1 791 bp **BLASTX** emb|CAO42468.1| unnamed protein product [Vitis vinifera] 366 1e-111 ref|NP_172777.1| DIS1 (Distorted Trichomes 1); structural con.. 362 1e-107 **CONSERVED DOMAIN** cd00012, ACTIN, Actin; An ubiquitous protein involved in the formation of filaments th.. 5e-51	ACTIN SM00268 IPR004000 **protein binding structural constituent of cytoskeleton**	ACTIN SM00268 IPR004000 actin filament organization-cell morphogenesis trichome -morphogenesis (cellular component organization and biogenesis) multidimensional cell growth (regulation of cell size) **Biogenesis of Cell. Comp. (MIPS 42)**	GO:0005885* Arp2/3 protein complex **(cytoskeleton)**
NO: Eds1-4 586 bp **BLASTX** emb|CAO38897.1| unnamed protein product [Vitis vinifera] 303 4e-81 ref|NP_200333.2| trigger factor type chaperone family protein.. 265 2e-69 **CONSERVED DOMAIN** PRK01490, tig, trigger factor. 4e-21	IPR008881 IPR008880 IPR001179 PRK01490 Trigger_N pfam05697 FKBP_C pfam00254 Trigger_C pfam05698 peptidyl-prolyl cis-trans isomerase activity **(catalytic activity)**	IPR008881 IPR008880 IPR001179 PRK01490 Trigger_N pfam05697 FKBP_C pfam00254 Trigger_C pfam05698 protein folding protein transport Protein destination and storage: Folding and stability-targeting **Protein Fate.. (MIPS 14)**	**Unknown**
NO: Hrp-1 (HRP) 283 bp **BLASTX** emb|CAO65595.1| unnamed protein product [Vitis vinifera] 52.4 9e-06 gb|AAK92807.1| putative receptor protein kinase [Arabidopsis tha 45.1 0.001	Probable: protein serine/threonine kinase activity **(catalytic activity)** ATP binding **(nucleotide binding)**	Probable: protein amino acid phosphorylation (protein modification) transmembrane receptor protein tyrosine kinase signaling pathway (signal transduction) **Cellular Communication.. (MIPS 30)**	Probable: endomembrane system **(membrane)**
NO: Rar1-1 501 bp **BLASTX** gb|AAY82249.1| mitochrondrial voltage-dependent anion-selecti.. 290 3e-77 gb|AAQ87019.1| VDAC1.1 [Lotus corniculatus var. japonicus] 286 3e-76 **CONSERVED DOMAIN** pfam01459, Porin_3, Eukaryotic porin. 2e-36	Porin_3 (PF01459) IPR001925 voltage-gated ion-selective channel activity **(ion transmembrane transporter activity)**	Porin_3 (PF01459) IPR001925 anion transport (ion transport) **Cellular Transport..(MIPS 20)**	GO:0005741* mitochondrial outer membrane **(mitochondria)**
NO: Rar1-4 613 bp **BLASTX** emb|CAO71127.1| unnamed protein product [Vitis vinifera] 236 9e-61 emb|CAN75984.1| hypothetical protein [Vitis vinifera] 235 2e-60	SrmB COG0513 helicase activity **(catalytic activity)** **nucleic acid binding**	SrmB COG0513 DNA replication, recombination, and repair / Transcription /Translation, ribosomal structure and biogenesis Probable: Transcriptional regulator **Transcription (MIPS 11)** also **cell cycle and DNA processing (MIPS 10)**	Probable: GO:0035060* brahma complex **(nucleus)**

* The Gene Ontology (AmiGO) accession dates are as follows: GO:0016423 28.03.2008; GO:0008152 19.10.2007; GO:0005634 14.10.2007; GO:0016021 19.10.2007; GO:0016020 19.10.2007; GO:0005575 13.10.2007; GO:0005840 19.10.2007; GO:0005634 14.10.2007; GO:0016469 23.10.2007; GO:0009507 14.10.2007; GO:0005840 19.10.2007; GO:0005737 17.10.2007; GO:0012505 02.10.2007; GO:0031304 23.10.2007; GO:0009535 14.10.2007; GO:0005854 11.01.2008; GO:0009535 14.10.2007; GO:0012505 02.10.2007; GO:0005829 14.10.2007; GO:0005575 13.10.2007; GO:0005875 04.12.2007; GO:0005739 23.10.2007; GO:0016020 19.10.2007; GO:0005840 19.10.2007; GO:0016020 19.10.2007; GO:0005622 19.10.2007; GO:0009507 14.10.2007; GO:0005622 19.10.2007; GO:0005847 15.10.2007; GO:0005634 14.10.2007; GO:0031225 07.12.2007; GO:0030126 10.01.2008; GO:0005737 17.10.2007; GO:0016469 23.10.2007; GO:0009579 10.01.2008; GO:0005575 13.10.2007; GO:0012505 02.10.2007; GO:0005840 19.10.2007; GO:0005739 23.10.2007; GO:0005853 23.10.2007; GO:0016469 23.10.2007; GO:0015934 16.11.2007; GO:0009507 14.10.2007; GO:0009505 25.10.2007; GO:0005885 25.10.2007; GO:0005741 19.12.2007; GO:0035060 11.01.2008

Table E.2 ESTs having no significant similarity and ESTs which have similarity score below 40 bits

Experiment	EST no (experimental sample)	Predicted Functional Role
RGA-DDRT-PCR trials	R14 (Uninf)	no significant similarity
	R15 (Uninf)	no significant similarity
	R17 (inf)	no significant similarity
	R23 (Uninf)	no significant similarity
RGA-RT-PCR	619 (B1, B3)	no significant similarity
	928 (B1, B2, B3)	no significant similarity
	896 (B1, B3)	no significant similarity
	843 (B1, B3)	no significant similarity
	845-2a (B1, B3)	no significant similarity
DD-RT PCR	1550 (H1, H2),	no significant similarity
	1528-D6-4 (H2, H3)	no significant similarity
	1544 (H1, H3)	no significant similarity
RGADD RT-PCR	2180-D22-1 (H3, I)	no significant similarity
	2163-D23-1 (C3, U)	no significant similarity
GSP-RT PCR	none	
RGA-DD-RT-PCR trials	R18 (inf)	possible cytoplasmic protein having oxidoreductase activity probably involved in energy metabolism
	R22 (Uninf)	possible protein having metal binding activity
	R49-2 (Uninf)	possible protein no known function
RGA-RT-PCR	283 (B2, B3)	a membrane protein of unknown function
	304 (B1, B3)	hypothetical protein assigned as kinesin related motor activity
	542 (B1, B3)	a protein having probably protein kinase activity
	879 (B1, B2, B3)	a protein belonging to proton-transporting two-sector ATPase complex in membrane
	1034 (B1, B3)	a protein of unknown function
	1130 (B1, B2, B3)	similarity to proteins having Ser/Thr kinase activity especially a special type of kinase 3-phosphoinositide-dependent protein kinase-1, Pdk1
DDRT-PCR	1644a (H1, H2, H3)	hypothetical protein of unknown function
	1486-4 (C1,C2)	a possible membrane protein having receptor activity involved in cell-cell recognition process
	1501 (C1, C2, C3)	a membrane protein having potassium ion transmembrane transporter activity
	1507a (source undetermined)	similar to RasGEF domain having protein
	1517-1a (H1, H2, H3)	possible protein no known function
	1531 (C1, C2)	a protein with protein transporter activity involved in protein import into nucleus
	1558-1-1 (H1, H2, H3)	a cytoplasmic protein having acyl-CoA synthase activity
	1540-4 (H1, H2)	a protein having subtilase activity and identical protein binding activity involved in regulation of catalytic activity
	1565 -D3-3-1 (C1,C2,C3)	a protein having protein serine/threonine kinase activity involved in protein modification process
	1568 (C1, C2, C3)	hypothetical protein assigned as probable helicase activity
	1597-2a (C1, C2)	a membrane protein probably having transporter or channel activity
	1598 (C3, less H3)	predicted protein and hypothetical proteins having many functions
	1631-2a (H1, H2)	a hypothetical protein having ubiquitin conjugating enzyme activity
	1931-3 (more in C3, less in H1, H2)	a possible zinc ion binding protein probably involved in salt tolerance
RGA-PCR	1209 (B1, B2, B3)	a probable mitochondrial or chloroplast protein having RNA binding activity involved in RNA/DNA metabolism
	1151-1-2 (B1, B2, B3)	a ribonucleoprotein in ribonucleoprotein complex involved in translation
	1450 (B1, B3)	a protein of unknown function
	391-1a (source undetermined)	a outer membrane protein having hydrolase activity peptidoglycan turnover- carbohydrate metabolic process
	737 (B1, B2, B3)	a photosystem I protein involved in photosynthesis
	617 (B1)	a possible protein having phosphotransferase activity involved in amine and carbohydrate metabolic process
	824 (B1, B2, B3)	DnaJ protein
	1107 (B1, B3)	a protein having Leucine-rich repeats (LRRs) domain with possible involment in a variety of biological processes
	B46 (source undetermined)	a probable protein having lyase activity
	925 (source undetermined)	a probable protein integral to membrane with no known function
RGA-DDRT-PCR	2007 (C3, U)	a protein having ribonuclease III activity involved in transcription
	2327 (H3, I)	a lipid binding protein transporter protein involved in lipid transport mechanism
	2314 (H3, C3, I)	a hypothetical protein having like transposable element
	1826-1a (H3, I)	a protein probably having FAD binding activity involved in electron transport
	1769 (C3, U)	a probable mitochondrial or chloroplast protein having RNA binding activity involved in RNA/DNA metabolism
	2010 (H3, I)	p450 monooxygenase protein involved in electron transport and energy metabolism
GSP-RT-PCR	none	

APPENDIX F

AUTORADIOGRAPH PICTURES OF DIFFERENTIALLY EXPRESSED ESTS

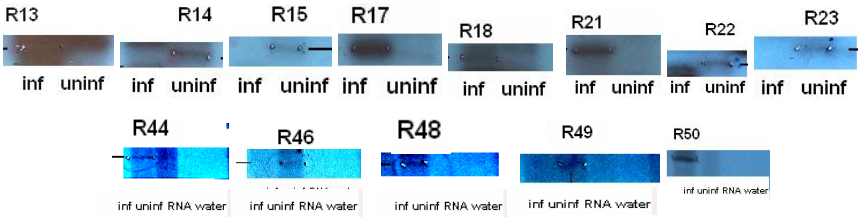


Figure F.1 Band pictures RGA-DDRT-PCR Trials

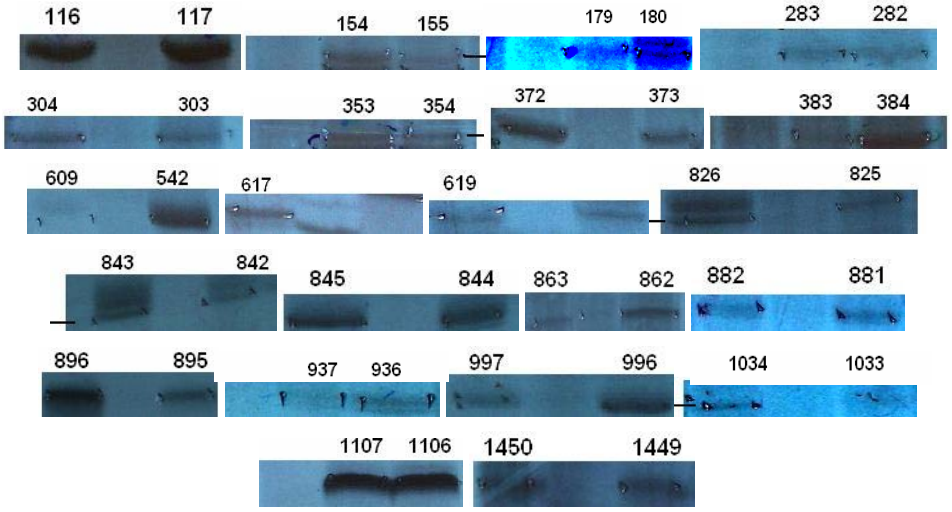


Figure F.2 Differentially expressed RGA-RT-PCR bands (order of bands: B1/B2/B3)

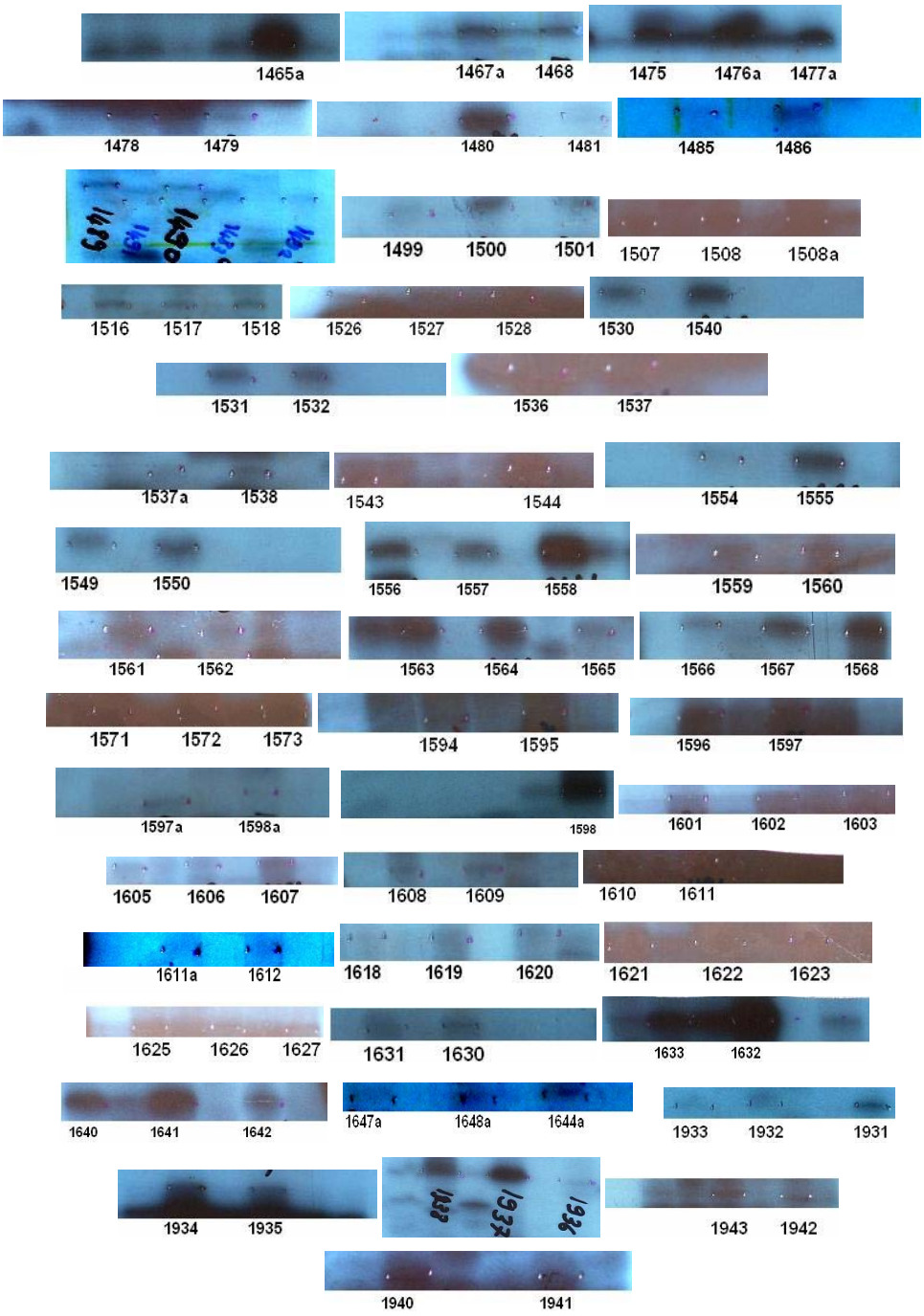


Figure F.3 DDRT-PCR band pictures (order of bands: H1/C1/H2/C2/H3/C3)

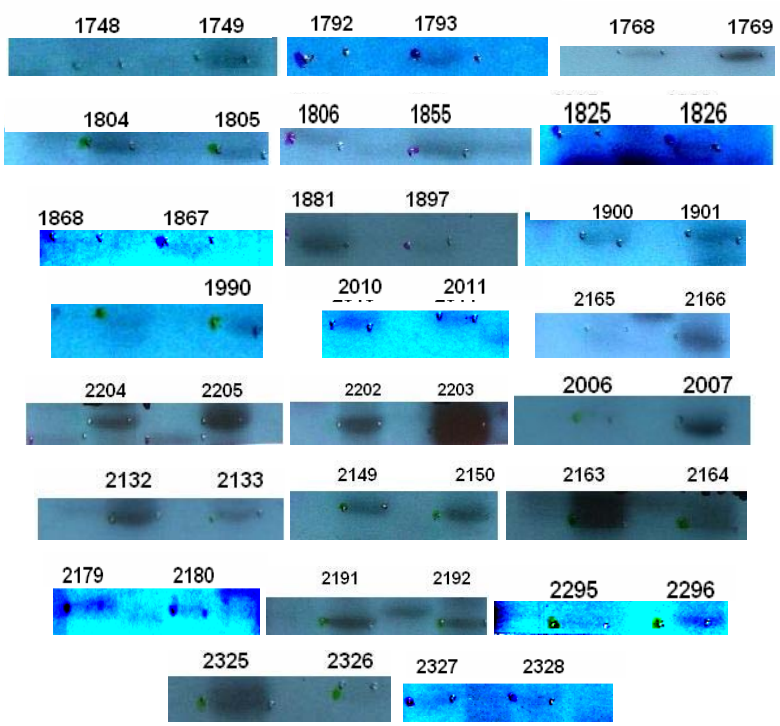


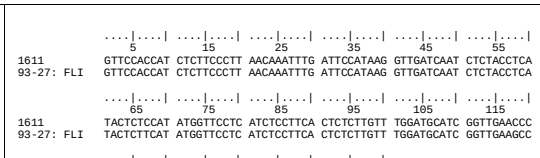
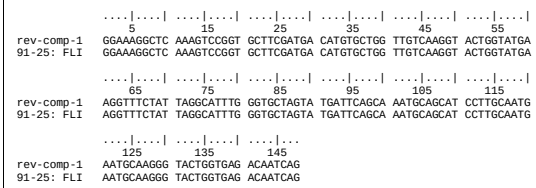
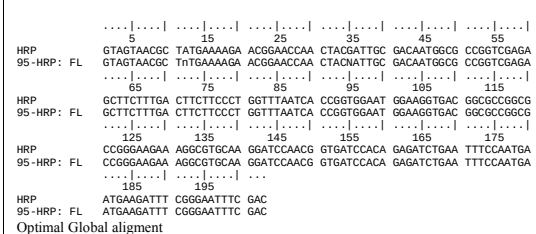
Figure F.4 RGA-DDRT-PCR band pictures (order of bands: H3/C3/I/u)

APPENDIX G

SEQUENCE DATA AND ALIGNMENTS FOR SNP ANALYSIS

Table G.1 Sequence data and alingments for SNP analysis. BLASTX done in June 2007, Alignments done with the corresponding EST sequence of original clone sequence of ILC195

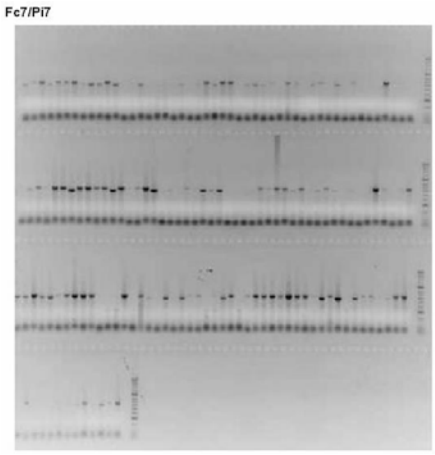
Marker	Sequence Data of PCR amplified DNA fragment from FLIP84-32C(3) and PI 599072 and BLASTX done in June 2007 (BLASTX : Score (Bits) E –Value)	Alignment of FLIP84-32C(3) / PI 599072 with EST data of ILC195
R46	**96-7: FLIP84-32C(3)** NNNNNNNNNNNNNNNCAGNNNNNNNCAGAGANGT GAGGCTCCNTGTTTTCATCAAGAAGGATGTTGCTAGA TTTTATGTCGCGATGCACAATTTTCGGGCTGCATTCA TGGTGCAAGTATGCCAAACCCC**GAG**CAGANCCAAGG GTTATCTTTAGGGGATCACTCCAA **96-8: PI 599072** NNNNNNNNNNCNNNGCAGANCAAAATCAGAGANGT GAGGCTCCATGTTTTCATCAAGAAGGANGTTGCTAG ATTTTATGTCGCGATGCACAATTTTCGGGCTGCATTC ATGGTGCAAGTATGCCAAACCCC**GGG**CAGAACCAAG GGTTATCTTTAGGGGATCACTCCAA **BLASTX** emb|CAN66563.1| hypothetical protein [Vitis vinifera] 84.7 2e-15 dbj|BAB10839.1| receptor-like protein kinase [Arabidopsis thalia 78.2 1e-13 ref|NP_201077.2| leucine-rich repeat family protein / protein... 78.2 1e-13 gb|AAP69763.1| ERECTA-like kinase 1 [Arabidopsis thaliana] 77.0 3e-13	rev-comp-R46 GAGATGTGAG GCTCCATGTT TTCATCAAGA AGGATGTTGC TAGATTTTAT GTCGCGATGC 96-7: FLIP GAGANGTGAG GCTCCNTGTT TTCATCAAGA AGGATGTTGC TAGATTTTAT GTCGCGATGC rev-comp-R46 ACAATTTTCG GGCTGCATTC ATGGTGCAGG TATGCCAAAC CCC**GAG**CAGA ACCAAGGGTT 96-7: FLIP ACAATTTTCG GGCTGCATTC ATGGTGCAAG TATGCCAAAC CCC**GAG**CAGA NCCAAGGGTT rev-comp-R46 ATCTTTAGGG GATCACTCCA A 96-7: FLIP ATCTTTAGGG GATCACTCCA A Optimal Global aligment: Alignment score: 279; Identities: 0,9716312 rev-comp-R46 GAGATGTGAG GCTCCATGTT TTCATCAAGA AGGATGTTGC TAGATTTTAT GTCGCGATGC 96-8: PI59 GAGANGTGAG GCTCCATGTT TTCATCAAGA AGGANGTTGC TAGATTTTAT GTCGCGATGC rev-comp-R46 ACAATTTTCG GGCTGCATTC ATGGTGCAGG TATGCCAAAC CCC**GAG**CAGA ACCAAGGGTT 96-8: PI59 ACAATTTTCG GGCTGCATTC ATGGTGCAAG TATGCCAAAC CCC**GGG**CAGA ACCAAGGGTT rev-comp-R46 ATCTTTAGGG GATCACTCCA A 96-8: PI59 ATCTTTAGGG GATCACTCCA A Optimal Global aligment: Alignment score: 276; Identities: 0,9716312
1998	**59-1998: FLIP84-32C(3)** CGAGTCGATTGGCTACCTGATTCGATAGGAAAGTTA NNNNNNTTGGTCACCCTTGATTTATCAGAGAATAGG ATTGTTGCTTTACCTTCCACAATTGGTGGCCTTTCCTC ACTGACCAAATTGGACTTGCATTCCAATAG **60-1998: PI 599072** GTCGAnTGGCTACCTGATTCGATAGGAAAGTTATCNA GTTTGGTCACCCTTGATTTATCAGAGAATAGGATTGT TGCTTTACCTTCCACAATTGGTGGCCTTTCCTCACTG ACCAAATTGGACTTGCATTCCAATAGGA ref|NP_195272.1| leucine-rich repeat family protein [Arabidop... 75.5 9e-13 ref|NP_179336.1| leucine-rich repeat family protein [Arabidop... 70.5 3e-11	1998 CGAGTCGATT GGCTACCTGA TTCGATAGGA AAGTTATCTA GTTTGGTCAC CCTTGATTTA 59-1998: F CGAGTCGATT GGCTACCTGA TTCGATAGGA AAGTTANNNN NNTTGGTCAC CCTTGATTTA 1998 TCAAAGAATA GGATTGTTGC TTTACCTTCC ACAATTGGTG GCCTTTCCTC ACTGACCAAA 59-1998: F TCAGAGAATA GGATTGTTGC TTTACCTTCC ACAATTGGTG GCCTTTCCTC ACTGACCAAA 1998 TTGGACTTGC ATTCCAATAG 59-1998: F TTGGACTTGC ATTCCAATAG Optimal Global aligment: Alignment score: 277; Identities: 0,9500000
FDH	**95-29: FLIP84-32C(3)** NNNNNNNNNNNNNNNNGNNTNNANCCTTTACTGTA ACCTTTTGTATCATGATAGACTTAAGATAGCACCTGA ATTGGAGAAAGAAANTGGAGCTAAGTTTGAGGAGGA TCTTGATGCTATGCTTCCGAAGTGCGATGTAATTGTT ATCAN **96-30: PI 599072** NNNNNNNNNNNNNNNNGNNTNNNNCCTTTACTGTAA CCTTTTGTATCATGATAGACTTAAGATAGCACCTGAA TTGGAGAAAGAAATTGGAGCTAAGTTTGAGGAGGAT CTTGATGCTATGCTTCCGAAGTGCGATGTAATTGTTA TCAACNNNA **BLASTX** gb|EAZ37051.1| hypothetical protein OsJ_020534 [Oryza sativa ... 77.8 2e-13 gb|EAZ00985.1| hypothetical protein OsI_022217 [Oryza sativa ... 77.8 2e-13 ref|NP_001057666.1| Os06g0486800 [Oryza sativa (japonica cult... 77.8 2e-13 dbj|BAA77337.1| Nad-dependent formate dehydrogenase [Oryza sativ 77.8 2e-13 ..... emb|CAE12168.2| formate dehydrogenase [Quercus robur] 72.4 8e-12	FDH CCTTTAACTG TAACCTTTTG TATCATGATA GACTTAAGAT AGCACCTGAA TTGGAGAAAG 95-29: FLI CCTTTA-CTG TAACCTTTTG TATCATGATA GACTTAAGAT AGCACCTGAA TTGGAGAAAG FDH AAATTGGAGC TAAGTTTGAG GAGGATCTTG ATGCTATGCT TCCGAAGTGC GATGTAATTG 95-29: FLI AAANTGGAGC TAAGTTTGAG GAGGATCTTG ATGCTATGCT TCCGAAGTGC GATGTAATTG FDH TTATCA 95-29: FLI TTATCA Optimal Global aligment Alignment score: 247 Identities: 0,9841270

1611	**93-27: FLIP84-32C3)** NNNNNNNNNCNTNNTGNNGTTCCACCATCTCTTCCCT TAACAAATTTGATTCCATAAGGTTGATCAATCTCTAC CTCATACTCTTCATATGGTTCCTCATCTCCTTCACTCT CTTGTTTGGATGCATCGGTTGAAGCCTTAACCACAAA AAGGAAGGGCCTTGACAAAAGGGTGTTAGANAAN **94-28: PI 599072** NNNNNANNNNNNNNGNNNGTTCCNCCATCTCTTCC CTTAACAAATTTGATTCCATAAGGTTGATCAATCTCT ACCTCATACTCTTCATATGGTTCCTCATCTCCTTCACT CTCTTGTTTGGATGCATCGGTTGAAGCCTTAACCACA AAAAGGAAGGGCCTTGACAAAAGGGTGTTAGAGAA **BLASTX** gb|ABE87465.1| PDZ/DHR/GLGF; Tetratricopeptide-like helical [... 59.3 7e-08 gb|AAK37555.1|AF349572_1 SHOOT1 protein [Glycine max] 50.8 2e-05	 Optimal Global aligment Alignment score: 316 Identities: 0,9875776
1901	**91-25: FLIP84-32C(3)** NNNNNNNNNNGNNGGNCNANTANCATGTTGGANGA NCCTTTGTAATGACTTTGGTTTCTGACATTGTTGGTTG GTTTGATTACAGGAAAGGCTCAAAGTCCGGTGCTTC GATGACATGTGCTGGTTGTCAAGGTACTGGTATGAA GGTTTCTATTAGGCATTTGGGTGCTAGTATGATTCAG CAAATGCAGCATCCTTGCAATGAATGCAAGGGTACT GGTGAGACAATCAGNGACTANNANNNGNTTGANAG GNTGCTTTCACTTTNGCTAGCCTNTCCNNTACNTACN AGANCTAGCCNTATTCNCCTTTTTCTTTTGACCNTTTG ACTCTNACTTNTGNANGCTTTGNTNATNCATGNN **92-26: PI 599072** NNNNNNNNNNNGNNNGNCNNNTANNNTGNTGGNNG ANCCTTTGTAATGACTTTGGTTTCTGACATTGTTGGTT GGTTTGATTACAGGAAAGGCTCAAAGTCCGGTGCTT CGATGACATGTGCTGGTTGTCAAGGTACTGGTATGA AGGTTTCTATTAGGCATTTGGGTGCTAGTATGATTCA GCAAATGCAGCATCCTTGCAATGAATGCAAGGGTAC TGGTGAGACAATCANNGACTA **BLASTX** gb|ABO78476.1| Heat shock protein DnaJ [Medicago truncatula] ... 102 7e-21 gb|ABE78148.1| Heat shock protein DnaJ [Medicago truncatula] ... 102 7e-21	 Optimal Global aligment Alignment score: 296 Identities: 1,0000000
FPIP	**89-21: FLIP84-32C(3)** NNNNNNNNNNNNNNNNANCNCTTCTTCTTTGGTTGAA CTTTCCNCTTCTTGCACTAACTCATCCTTTGTACTGTT ACATGATGTGATAACCTTACATAACACAAGGGGAAG TCAGAAAGGACAAACATCATAAAGTAGAAGAGCTGC AACAATGTCATGAGCACAACAATGGAGAAAGAAAG CAAGTGAATAAGCATCAATTAACAGCAGAAAATAAA ATCAAATAATTGCGATTTATTCTGTAACGAATAAAGG AATCCCACTTTGTAATAGGCAAAATTCATATCACGGG CCTAA**ACT**ACTTGGCAGAAAATTATAACACGGACCT AAATCTAACATTTTTAATGTTACGGAATCTAACATGA ACATCGAACAAGTTTAAAAGTGCTTACAAGTAATCC ACCAGAAGCAACCAGCCTTGAAACAGA **90-22: PI 599072** NNNNNNNNNNNNNNNNNCNNTTCTTCTTTGGTTGAAC TTTCCACTTCTTGCACTAACTCATCCTTTGTACTGTTA CATGATGTGATAACCTTACATAACACAAGGGGAAGT CAGAAAGGACAAACATCATAAAGTAGAAGAGCTGC AACAATGTCATGAGCACAACAATGGAGAAAGAAAG CAAGTGAATAAGCATCAATTAACAGCAGAAAATAAA ATCAAATAATTGCGATTTATTCTGTAACGAATAAAGG AATCCCACTTTGTAATAGGCAAAATTCATATCACGGG CCTAA**ATT**ACTTGGCAGAAAATTATAACACGGACCT AAATCTAACATTTTTAATGTTACGGAATCTAACATGA ACATCGGACAAGTTTAAAAGTGCTTACAAGTAATCC ACCAGAAGCAACNNNN **BLASTX** gb|ABE93662.1| SAM (and some other nucleotide) binding motif ... 36.2 0.63 gb|EAZ13128.1| hypothetical protein OsJ_002953 [Oryza sativa ... 34.3 2.4	Blast ResultBlast 2 Sequences (NCBI) Sequence 1: EST-FPIP Length = 400 (1 .. 400) Sequence 2: 90-22: PI 599072 Length = 392 (1 .. 392) Score = 119 bits (62), Expect = 7e-24 Identities = 69/70 (98%), Gaps = 1/70 (1%) Strand=Plus/Plus **Query 108 TTCTTCTTTGGTTGAAACTTTCCACTTCTTGCACTAACTCATCCTTTGTACTGTTACATG 167** **||||||||||||||| ||||||||||||||||||||||||||||||||||||||||** **Sbjct 1 TTCTTCTTTGGTTGAA-CTTTCCACTTCTTGCACTAACTCATCCTTTGTACTGTTACATG 59** **Query 168 ATGTGATAAC 177** **||||||||||** **Sbjct 60 ATGTGATAAC 69** Score = 44.9 bits (23), Expect = 0.28 Identities = 23/23 (100%), Gaps = 0/23 (0%) Strand=Plus/Plus **Query 176 ACAAGTAATCCACCAGAAGCAAC 198** **|||||||||||||||||||||||** **Sbjct 370 ACAAGTAATCCACCAGAAGCAAC 392**
HRP	**95-HRP: FLIP84-32C(3)** GTAGTAACGCTnTGAAAAGAACGGAACCAACTACNA TTGCGACAATGGCGCCGGTCGAGAGCTTCTTTGACTT CTTCCCTGGTTTAATCACCGGTGGAATGGAAGGTGAC GGCGCCGGCGCCGGGAAGAAAGGCGTGCAAGGATC CAACGGTGATCCACAGAGATCTGAATTTCCAATGAA TGAAGATTTCGGGAATTTCGAC **96-HRP: PI 599072** GTAGTAACGCTATGNnAAGAACGGAACCAACTACnA TTGCGACAATGGCGCCGGTCGAGAGCTTCTTTGACTT CTTCCCTGGTTTAATCACCGGTGGAATGGAAGGTGAC GGCGCCGGCGCCGGGAAGAAAGGCGTGCAAGGATC CAACGGTGATCCACAGAGATCTGAATTTCCAATGAA TGAAGATTTCGGGAATTTCGACA **BLASTX** gb|AAK92807.1| putative receptor protein kinase [Arabidopsis tha 40.4 0.034 ref|NP_180241.1| leucine-rich repeat transmembrane protein ki... 40.4 0.034 gb|AAM60932.1| putative disease resistance protein [Arabidopsis 39.7 0.057	 Optimal Global aligment Alignment score: 403 Identities: 0,9901478

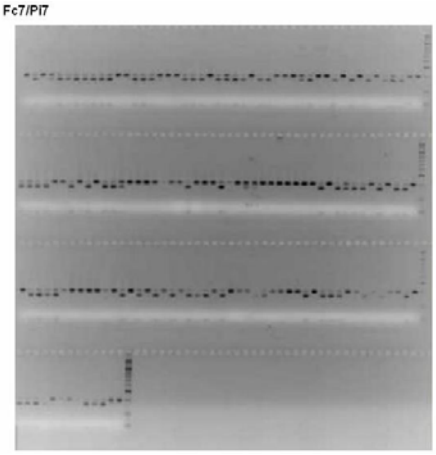
1468	**87-9: FLIP84-32C(3)** NNNNNNNNNNNNNNNNNNNNNNNNNCCATAACTC CNCCGACTGTAATAAACATAGAACCAATTTGCATAA GATTATTAATGCTGAAAATCCCATTGTAACAACAAG AAGCTGTGTCATTGCAAACCTGATCCAAGTAGACCTT CTAGAATCTCAGGATTTAAGCTGCTAGGGAATAGAT AGGCTACAGAGAAAGAAAAAGAAATCATCAAATTA AGTTCAACTTAGTAGCTCGCGAATATCT **88-10: PI 599072** NNNNNNTNNNNNNNNGNTANNNNGCCATAACTCCA CCGACTGTAATAAACATAGAACCAATTTGCATAAGA TTATTAATGCTGAAAATCCCATTGTAACAACAAGAA GCTGTGTCATTGCAAACCTGATCCAAGTAGACCTTCT AGAATCTCAGGATTTAAGCTGCTAGGGAATAGATAG GCTACAGAGAAAGAAAAAGAAATCATCAAATTAAGT TCAACTTAGTAGCTCGCGAATATCTNANATACCCCCG AGNNN **BLASTX** gb|EAZ22087.1| hypothetical protein OsJ_005570 [Oryza sativa ... 42.7 0.007 gb|EAY84865.1| hypothetical protein OsI_006098 [Oryza sativa ... 42.7 0.007 ref|NP_001046190.1| Os02g0196000 [Oryza sativa (japonica cult... 42.7 0.007 gb|AAZ20768.1| branchy [Setaria italica] 42.4 0.009 ….. protein OsI_026496 [Oryza sativa ... 40.0 0.044 dbj|BAD03545.1| putative Zinc transporter zupT [Oryza sativa ... 40.0 0.044	....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| 5 15 25 35 45 55 rev-comp-1468 CTGATCCAAG TAGACCTTCT AGAATCTCAG GATTTAAGCT GCTAGGGAAT AGATAGGCTA 87-9: FLIP CTGATCCAAG TAGACCTTCT AGAATCTCAG GATTTAAGCT GCTAGGGAAT AGATAGGCTA ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|.. 65 75 85 95 105 115 rev-comp-1463 CAGAGAAAGA AAAAGAAATC ATCAAATTAA GTTCAACTTA GTAGCTCGCG AATATCT 87-9: FLIP CAGAGAAAGA AAAAGAAATC ATCAAATTAA GTTCAACTTA GTAGCTCGCG AATATCT Optimal Global aligment: Alignment score: 234; Identities: 1,0000000
447	**55-447: FLIP84-32C(3)** CTTTCNGNCTGATCTTAGGCCCTGACTCACCCTCCGT GGACGAACCTTGCGGAGGAACCCTTnNGTTTTCGGGG CATTGGATTCTCACCAATGTTTGCGTTACTCAnGCCG ACnTTCTCACTTCCGCTTCGTCCAACCCAGCTCGCGC GGGTCCTTCCTTCTAAGGCGGAACGCTCCCCTACCGA TTCATTTTTACATCCCACAGCTTCGGCAGATCGCTTA GCCCCGTTCATCTTCGGCGCAAGAGCGCTCGATCAGT GAGCTATTACGCACTCTTTGAAGGGTGGCTGCTTCTA GGCAAACCTCCTGGCTGTCTCTGCACTCCTACCTCCT TTATCACTTAGCGATCATTTAGGGGCCTTAGCTGGTG ATCTGGGCTGTTTCCCTCTCGACGATGAAGCTTATCC CCCATCGTCTCACTGGCCGACCTTGACCCTTCTTATTT TGGGGTCATATCTAGTATTCAGAGTTTGCCTCGATTT GGTACCGCTCTCGCGGCCCGCGCCGAAnCAGTGCTTT ACCCCTAGATGTCCAGTCAACTGCTGCGCCTCAACGC ATTTCGGGGAGAACCAGCTAGCTCTGGGTTCGAGTG GCATTTCACCGCTAACCnCAACTCATCCGCTGANTTT TCAACATCAGTCNnTTCGnACCTCCACTTAGTTTCNCC TAAGCTTCATCCTGGnCATGGNNA **56-447: PI 599072** CTGATCTTAGGCCCTGACTCACCCTCCGTGGACGAAC CTTGCGGAGGAACCCTTANGTTTTCGGGGCATTGGAT TCTCACCAATGTTTGCGTTACTCAnGCCGACATTCTC ACTTCCGCTTCGTCCAACCCAGCTCGCGCGGGTCCTT CCTTCTAAGGCGGAACGCTCCCCTACCGATTCATTTT TACATCCCACAGCTTCGGCAGATCGCTTAGCCCCGTT CATCTTCGGCGCAAGAGCGCTCGATCAGTGAGCTATT ACGCACTCTTTGAAGGGTGGCTGCTTCTAGGCAAACC TCCTGGCTGTCTCTGCACTCCTACCTCCTTTATCACTT AGCGATCATTTAGGGGCCTTAGCTGGTGATCTGGGCT GTTTCCCTCTCGACGATGAAGCTTATCCCCCATCGTC TCACTGGCCGACCTTGACCCTTCTTATTTTGGGGTCA TATCTAGTATTCAGAGTTTGCCTCGATTTGGTACCGC TCTCGCGGCCCGCGCCGAAACAGTGCTTTACCCCTAG ATGTCCAGTCAACTGCTGCGCCTCAACGCATTTCGGG GAGAACCAGCTAGCTCTGGGTTCGAGTGGCATTTCA CCGCTNAnCACNACTCATCCGCTGATTTTTCAACATC AGTCGG **BLASTX** gb|ABE87179.1| hypothetical protein MtrDRAFT_AC151668g5v1 [Me... 102 9e-45 ref|YP_173415.1| hypothetical protein NitaMp073 [Nicotiana ta... 131 2e-31 gb|ABO82236.1| hypothetical protein MtrDRAFT_AC147496g40v1 [M... 126 1e-27	**No significant similarity was found**
1619	**57-1619: FLIP84-32C(3)** GTACCnTGCnNGAANTGCAGTACTGGAGGANGACAA ATTGGTTGAGTTACTATTGGAACCAGTTAAGACTAAT GTGCAATGTGATAGTGTGTATGTTGGGGTAATCACA AAGCTTCTTCCTTCTATGGGAGGAG **58-1619: PI 599072** NGAANTGCAGTACTGGAGGANGACAAATTGGTTGAG TTnCNNTTGGAACCAGTTAAGACTAATGTGCAATGTG ATAGTGTGTATGTTGGGGTAATCACAAAGCTTCTTCC TTCNATGGGAG **BLASTX** gb|AAD27911.1| putative ribonuclease E [Arabidopsis thaliana] 44.7 0.002 ref|NP_178508.2| glycoside hydrolase starch-binding domain-co... 44.7 0.002	....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| 5 15 25 35 45 55 1619 TACCATGCAA AGAATTGCAG TACTGGAGGA TGACAAATTG GTTGAGTTAC TATTGGAACC 57-1619: F TACCnTGCnN -GAANTGCAG TACTGGAGGA NGACAAATTG GTTGAGTTAC TATTGGAACC ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| ....|....| 65 75 85 95 105 115 1619 AGTTAAAACT AATGTGCAAT GTGATAGTGT GTATGTTGGG GTAATCACAA AGCTTCTTCC 57-1619: F AGTTAAGACT AATGTGCAAT GTGATAGTGT GTATGTTGGG GTAATCACAA AGCTTCTTCC ....|....| .... 125 1619 TTCTATGGGA GGAG 57-1619: F TTCTATGGGA GGAG Optimal Global aligment : Alignment score: 254; Identities: 0,9477612

APPENDIX H

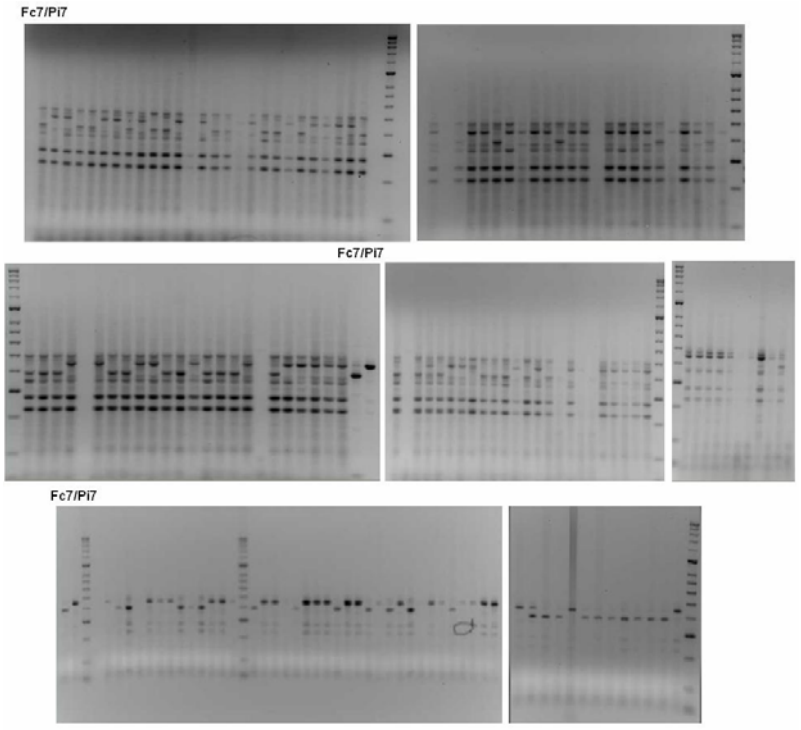
SCREENING OF POLYMORPHIC EST MARKERS ON CRIL7



EST Marker 1934



EST Marker R46 digested with SmaI



EST Marker FPIP digested with *Taq*I

Fc7/Pi7: parents of CRIL-7 (FLIP 84-92C(3), resistant and *C. reticulatum* (PI 599072), susceptible)

CURRICULUM VITAE

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WORK EXPERIENCE

Year Place Enrollment

2001 - 2003 Patent Examiner -Turkish Patent Institute
1997 - 2001 Assistant Patent Examiner -Turkish Patent Institute

FOREIGN LANGUAGES

Advanced English, Intermediate German

PUBLICATIONS

Avcioglu, B. Eyupoglu, B. Bakir, U. 2005. Production and characterization of xylanases of a *Bacillus* strain isolated from soil. World Journal of Microbiology and Biotechnology. 21:65–68.

Avcıoğlu, B. Biyoteknolojik Buluşlar ve Genetik Kaynaklar (Biotechnological Inventions and Genetic Resources). Avrupa Birliği ve Türkiye'de Sinai Haklarda Son Gelişmeler Kongresi (Congress of Recent Developments in Industrial Rights in European Union and Turkey). 10 Ekim (October) 2003. Fikri ve Sınai Haklar Araştırma ve Uygulama Merkezi (FISAUM) (The Research Centre of Intellectual and Industrial Property). Ankara. Published in 2005. p:107-124.

MEETINGS

Rajesh, P.N. Avcioglu, B., Nayak, S., Winter, P., Varshney, R., McPhee, K., Zhang, H., Muehlbauer, F., Chen, W. Integration of Additional Molecular Markers and Genetic Analysis of Ascochyta Blight Resistance In Chickpea. Poster no: P386. Plant & Animal Genomes XVI Conference. January 12-16, 2008. Town & Country Convention Center, San Diego, CA

Avcıoğlu-Dündar, B. and Akkaya, M.S. *Ascochyta rabiei* Yanıklığı Hastalığına Karşı Dirençlik Mekanizmasında Yer Alan Olası Nohut Genlerinin Analizi (Analysis of putative genes involved in resistance mechanism of chickpea against *Ascochyta rabiei* blight disease). Poster no: P-BHB8, 15. Ulusal Biyoteknoloji Kongresi (15th National Biotechnology Congress), Biyoteknoloji Derneği. 28-31 Ekim (October) 2007. Antalya. p.17

Avcıoğlu-Dündar, B., Bayraktar, H., Dolar, S., Akkaya, M.S. Nohut (*Cicer arietinum* L.) *Ascochyta rabiei* yanıklığı hastalığına karşı dayanıklılık mekanizmasinda rol alan genlerin saptanması (Determination of genes involved in resistance mechanism of chickpea (*Cicer arietinum* L.) to *Ascochyta rabiei* blight disease). 14. Biyoteknoloji Kongresi (14th National Biotechnology Congress), Biyoteknoloji Derneği, 31 Ağustos (August) - 2 Eylül (September) 2005. Eskişehir.

ACADEMIC ACHIEVEMENTS

Scholarship number 2214 from The Scientific and Technological Research Council of Turkey (TUBITAK); March-June 2007.