Genetic analyses of the resistance of faba beans (*Vicia faba*) to the fungus *Ascochyta fabae*

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Genetic analyses of the resistance of faba beans (*Vicia faba*) to the fungus *Ascochyta fabae*

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Chapter 1. Screening of faba bean germplasm for traits related to resistance against the fungus *Ascochyta fabae* causing *Ascochyta* blight

1.1 Introduction

1.1.1 General introduction of faba beans

1.1.1. a. Origin of faba bean

Faba bean (*Vicia faba*) is a diploid (2n=2x=12) legume crop adapted to cool-season regions. The genus *Vicia* belongs to the family *Fabaceae*. Faba bean is one of the earliest domesticated crops which dates back to the early Neolithic era (Duc et al., 2015). The area and time of its origin are not fully known (Shiran et al., 2014). However, the significance of Near East as a primary centre and China as a secondary centre of origin of faba beans genetic diversity have been ascertained in the literature (Cubero et al., 1992, Zong et al., 2009). The wild progenitor of the domesticated faba bean is either still unknown or extinct (Singh et al., 2013).

1.1.1. b. Morphology of faba bean plants

Faba bean can grow 0.1 to 2m tall with indeterminate growth of stem. The leaves are up to 8cm long without tendril and are comprised of two to six leaflets (Duc et al., 2015). The flowers are grouped in inflorescences and usually have a white background and a large satin-black spot on both wing petals. Fully white and further colour variants exist. The seeds may vary in size, shape (oval, oblong, broadly oblong) and colour (yellow, green, brown, black and violet) and have a prominent hilum. Faba bean has a strong tap root system, nitrogen fixing nodules are present at tap roots and lateral root branches (Nozzolillo et al., 1989, Bond et al., 1994). Useful bacteria (*Rhizobium leguminosarum Frank*) live in structures called nodules at the roots of faba bean. These bacteria have a symbiotic association with faba bean and therefore capable of fixation of atmospheric nitrogen (Angus et al., 2015).

1.1.1. c. Winter vs. spring types of faba beans

Faba bean is an annual crop. In European regions north of Pyrenees and Alps, two different types are used, 'winter type' sown in autumn (September to November) and 'spring type' sown in spring (February to March); yet, maturity and harvest of both types is normally in early (winter types) or late (spring types) August. In Europe, faba bean is usually sown in spring time, most European winter beans are grown in UK due to the mild winters there (Sass, 2009). The winter types are not grown in Central or East Europe, because their winter hardiness is not sufficient to survive the harder winters there. In semi-arid regions such as North Africa, Australia, regions of China, faba

beans are sown as well in autumn to escape summer drought; yet, these types are not winter hardy enough for Central or East Europe.

1.1.1. d. Nutritional value of faba beans

Faba bean offers a good nutritive value (starch about 45%, protein about 30%) for humans and animals (Duc et al., 2015). Faba bean also have anti-nutritional components such as tannin, lectins, vicine, convicine and phytic acid. Tannins reduce protein digestibility and tannin-absence is controlled by either of two genes zt-1 or zt-2 (Woyengo & Nyachoti, 2012). High amount of vicine and convicine in faba bean causes hemolytic anemia, called favism, in humans. This is associated with human glucose-6-phosphate dehydrogenase (G6PD) deficiency (Khamassi et al., 2013).

1.1.1. e. Production of faba beans

In Europe, among various cultivated legumes, faba bean ranks third in production after soya bean and dry pea and before lupines, lentil and chick peas. China is main global producer of faba bean and produces about 39% of the world production. Major growing countries of faba bean are hence China with about 0.8452 mio ha, Ethiopia with about 0.4667 mio ha, Australia with about 0.230 mio ha, and UK including Northern Ireland with about 0.1370 mio ha (FAOSTAT, 2019). Almost 14% of the world area of faba bean is grown in Europe and it produces about 25% of the world production of faba beans. The average yield of faba bean is double in Europe compared to the average global yield (Kezeya et al., 2020). In Europe, the main producing countries of faba bean are UK including Northern Ireland (about 547800 tons), France (about 177380 tons), Germany (about 159500 tons) and Italy (about 132310 tons) (FAOSTAT, 2019). Faba bean cultivated area has progressively increased in Germany in the last ten years (FAOSTAT, 2019). If every flower on faba bean would produce a pod, and if each pod would produce three seeds, then the hypothetical yield of faba bean would be up to 38-43 t/ha. However, a realistic figure is about 4 t/ha (Patrick & Stoddard, 2010), although 7 tons can be achieved (Link, 2009).

1.1.1. f. Biotic and abiotic stresses affecting faba beans

Faba bean production can be heavily and badly affected globally, including Europe, by abiotic and biotic stresses. Heat and drought are major abiotic stresses for faba bean in Europe. Faba bean plants are sensitive to drought at flowering, early podding and grain filling stage. Drought tolerant genotypes show the mechanism of proline accumulation (Mwanamwenge et al., 1999, Link et al.,

2007, Abid et al., 2017). Faba bean is sensitive to harsh frost; yet, even spring faba beans can bear some degree minus as juvenile plants. Winter hardiness of winter faba bean is improved by the exposure of seedlings to low non-freezing temperature before the onset of winter (Arbaoui & Link, 2008, Maqbool et al., 2010). Faba bean production is also badly effected by biotic stresses such as *Ascochyta* blight (*Ascochyta fabae*), chocolate spot (*Botrytis fabae*), downy mildew (*Peornospora viciae*), rust (*Uromyces viciae-fabae*), foot rots (*Fusarium* spp.), broomrape *(Orobanche crenata)* (Torres et al., 2006); in addition, several pests and viruses may threat the crop. *Ascochyta* blight, chocolate spot and rust are three major fungal diseases which affect the faba bean crop around the globe especially in wet weather conditions (Stoddard et al., 2010).

1.1.2 *Ascochyta* blight resistance in faba beans

Ascochyta blight being a devastating threat to faba bean attacks both spring beans and winter beans in Europe (Ahmed et al., 2016). *Ascochyta* blight is caused by the fungal pathogen *Ascochyta fabae* (telomorph; *Didemella fabae* Jellis and Punithalingam) (Omeri et al., 2012) in almost all faba bean production areas around the globe, including Europe, Australia and the Middle East (Bond & Pope, 1980; Geard, 1961; Hawtin & Stewart, 1979). It damages the aerial parts of plants and symptoms are observed on leaves, stem, and pods. Faba bean yield losses may rise to 90% for susceptible cultivars, especially in wet weather condition **(**Davidson & Kimber, 2007). *Ascochyta* spores can be present on and under the seed coat, and thus infected seeds can distribute *Ascochyta.* However, seed quarantine is a proven strategy to protect farmers from *Ascochyta* blight. Rain and wind may assist in spreading and dispersion of *Ascochyta* spores (Hanounik & Robertson, 1989). However, the application of fungicides and integrated management practices, such as late sowing, appropriate crop rotation may help in reducing *Ascochyta* incidence (Ahmed et al., 2016; Davidson & Kimber, 2007). Yet, all these approaches are limited as to their practicability and effectiveness. Out of all the practices against *Ascochyta* blight, the development of resistant cultivar is the most effective option in long run.

Ascochyta lesion numbers, lesion size, lesion area and presence versus absence of *Ascochyta* pycnidia in lesions mostly indicate the level of *Ascochyta* resistance in host plants (Maurin & Tivoli, 1992; Ondrej, 1993; Rashid et al., 1991). Several genetic sources of incomplete resistance of faba bean against *Ascochyta* blight have been reported (Bond & Pope, 1980; Hanounik & Robertson, 1989; Rubiales & Fondevilla, 2012; Siddique et al., 2013; Sillero et al., 2001). But limited and

even some contradictory information was published on the genetic basis of these resistances to *Ascochyta* blight. Both polygenic (Román et al., 2003) and major gene inheritance (Rashid et al., 1991) have been reported to cause *Ascochyta* blight resistance (Avila et al., 2004; Hanounik & Robertson, 1989; Kohpina et al., 2000; Román et al., 2003; Sillero et al., 2010; Stoddard et al., 1999). Moreover, different genetic systems in stem and leaves of faba beans have been reported for *Ascochyta* blight resistance (Avila et al., 2004; Kaur et al., 2014; Kohpina et al., 2000). The winter type inbred line 29H was reported as an excellently resistant accession of faba bean and *Ascochyta fabae* pathogen nearly fails to penetrate into the tissues of host plant (Maurin & Tivoli, 1992).

1.1.3 Molecular genetic studies on faba beans

In faba bean, DNA based marker application started in mid 1990s (Torres et al., 1993). The big genome size of faba bean (13000 Mb) is challenging for adequate genomic studies (Satovic et al., 2013). No faba bean genome sequence is open to the public but a model legume *Medicago truncatula* (*M. truncatula*) with a small genome size of 500 Mb is sequenced and data are available (Burstin et al., 2007; Djemel et al., 2005; Duc, 2004; Gnanasambandam et al., 2012). *M. truncatula* can be used for synteny based approaches to faba bean. Similar comparative studies have been reported among the legumes *M. truncatula*, *Lens culinaris* and *Lupinus albus* (Phan et al., 2007). A large number of single nucleotide polymorphism (SNP) markers and simple-sequence repeats (SSR) markers have been developed for other legumes such as *Lens culinaris* (Kaur et al., 2011; Sharpe et al., 2013), *Pisum sativum* (Kaur et al., 2012), *Cicer arietinum* (Gujaria et al., 2011; Hiremath et al., 2011; Jhanwar et al., 2012; Stephens et al., 2014). Different genetic maps of faba beans were published in different mapping populations developed from crosses Vf6 x Vf136 (Román et al., 2003, Díaz-Ruiz et al., 2009), 29H x Vf136 (Avila et al., 2004, Atienza et al., 2016) and Ascot x Icarus (Webb et al., 2016) which are enabling the transfer of genetic information from the genomic sequence of a model plant such as *M. truncatula* to faba bean linkage maps by using synteny-based approaches. Previously, different types of markers such as isozyme, RAPD, SSR and seeds protein gene markers were employed to detect and map QTL (quantitative trait loci) for *Ascochyta* resistance in faba beans (Avila et al., 2004; Cruz-Izquierdo et al., 2012; Díaz-Ruiz et al., 2009; El-Rodeny et al., 2014; Ellwood et al., 2008; Kaur et al., 2014; Román et al., 2003). Today, mainly or even only SNPs are employed for pertinent studies. QTL mapping was employed

as a tool in different studies on *Ascochyta* blight resistance in faba beans in F2 or RIL populations (Avila et al., 2004; Díaz-Ruiz et al., 2009; Román et al., 2003). A disadvantage of a QTL study is the limitation of the allelic diversity which is solely provided by the two parents of the bi-parental cross, and the limited amount of recombination limits the mapping resolution (Borevitz & Nordborg, 2003). As a supplementation and alternative to these QTL studies, genome wide association studies (GWAS) was coined as a method to detect genomic regions associated with QTLs not in bi-parental populations but in diversity panels of genotypes (Lander & Schork, 1994). The adequate choice of genotypes for such GWAS panel is the basis for a promising QTL detection. Decisive parameters include pattern of linkage among the markers, number of markers and evenness of their distribution across the genome hence genetic map resolution, entry number of the chosen panel, the ratio of variance explained by the detected QTL to the total variance, repeatability of phenotypic data, and whether there is an option of serious validation of markers and QTL. Basically, the GWAS approach involves the screening of DNA markers distributed across the genome for a statistical associations with the variation of the phenotype (the expression of the trait of interest) among the genotypes. The marker and QTL validation under different conditions is a key point in the applications of such approaches in actual breeding programs. Association mapping is dependent on the spread and genome-wide pattern of LD. To employ the findings of GWAS in applied breeding, the main interest is to find LD between QTL and closely linked marker loci. Associations between marker and QTL although they are unlinked may be considered as false positive result. In addition, variation of genetic relatedness within the set of genetic material must be considered. Inclusion of related individuals can cause biased association results (Kang et al., 2008). To partly overcome such issues, a so-called kinship matrix can be established and used in this statistical analysis (Kang et al., 2008; Vilhjálmsson & Nordborg, 2013; Zhang et al., 2010).

1.1.4 Review of literature on QTLs reported for the resistance against *Ascochyta* blight

Román et al. (2003) were the first to detect QTL for the resistance of faba bean against *Ascochyta* blight. They detected two QTL (*Af1, Af2*) in a study under controlled conditions with artificial *Ascochyta fabae* inoculation. The F_2 population was derived from a cross of *Ascochyta* resistant line Vf6 with susceptible line Vf136. Vf6 comes from the collection of genetic variants at the E.T.S.I.A.M in Córdoba. Vf136 is susceptible to *Ascochyta* blight; it was obtained at CIDA-Córdoba from a cross Vf1071x Alameda (Cubero et al. 1992). QTL *Af1* showed additive gene action

and was located on chromosome III. The second QTL *Af2* was located on chromosome II and displayed a dominant gene action for *Ascochyta* blight resistance. *Af1* explained 25.2% and *Af2* as 21% of the phenotypic variation in that study. In both of the QTLs, the resistance-associated alleles were contributed by the resistant parent Vf6.

Avila et al. (2004) continued the work of Román et al. (2003) by studying the *Ascochyta* blight resistance in stem and leaves of faba bean by using two pathogenically distinct isolates. They suggested the presence of six QTLs (*Af3, Af4, Af5, Af6-Af7, Af8*) in a genetic map developed from an F_2 population, which was derived from a cross of the *Ascochyta* resistant line 29H with the susceptible lineVf136. The genotype 29H was provided by Berthelem and Le Guen (INRA-Rennes, France). 29H is a small-seeded, i.e., minor type of winter faba bean and several authors have broadly revealed its resistance (Bond et al., 1994; Maurin & Tivoli, 1992; Sillero et al., 2001; Sillero et al., 2010; Tivoli et al., 2006; Tivoli et al., 1987). The resistance trial was conducted under controlled conditions. The QTL *Af3, Af4, Af5* and *Af7* were described for *Ascochyta* resistance in both stem and leaf. QTL *Af6* was reported only in leaf and *Af8* in stem resistance. *Af3* was located on chromosome III, just as QTL (*Af1*) published by Román et al. (2003). However, the homology of the chromosomal location of previously published *Af1* and *Af3* was unclear because of the absence of useful common markers.

Díaz-Ruiz et al. (2009) confirmed and validated two QTLs (*Af1, Af2*) in a recombinant inbred line (RIL) population derived from the same cross Vf6 x Vf136 as used by Román et al. (2003) for an F2 population phenotyped under controlled conditions. Both QTLs together explained 24% of the phenotypic variation for disease severity on leaves and 16% of the phenotypic variation for disease severity on stem. *Af1* seemed to be located in the same chromosomal region as *Af3* reported by Avila et al. (2004) because of a common marker linked (<10cM) to flanking markers in both mapping populations. The further QTLs reported by (Avila et al., 2004) were incomparable due to a lack of common markers.

Atienza et al. (2016) recently identified two QTLs (*Af1, Af3*) in a RIL population developed from a cross 29H x Vf136 and tested in both greenhouse and field conditions. *Af2* being present on chromosome II is considered the same as reported in the previous studies (Díaz-Ruiz et al., 2009; Kaur et al., 2014; Román et al., 2003).

Kaur et al. (2014) employed SNP markers and reported four QTLs (QTL-1, QTL-2, QTL-3, QTL-4) in a RIL population derived from the cross of Icarus (susceptible cultivar) x Ascot (resistant cultivar). Both of the cultivars belong to Australian-bred germplasm, hence different from the genotypes used in previous studies. The 95 RILs were tested under controlled conditions for resistance to *Ascochyta* blight and a QTL analyses was performed which revealed four QTL. Within these, QTL-3 was discussed to be identical to the prior reported QTL Af2 (Díaz-Ruiz et al., 2009; Román et al., 2003) because both were located on chromosome II; however, actual homology was not confirmed because of the lack of common markers. QTL-1, QTL-2 and QTL-4 were considered as different from QTL presented by (Avila et al., 2004; Díaz-Ruiz et al., 2009; Román et al., 2003).

Objectives

In the present study, a panel of 224 faba bean genotypes was tested for *Ascochyta* blight resistance under controlled conditions and with artificial inoculation. This is aimed at:

- Assessment and analyses of genetic variation and repeatability of phenotypic data for sub traits of *Ascochyta* blight resistance in these 224 faba bean lines including A-set lines (see below). These data was further used for genome wide association study (Chapter 2).

- To search for useful (for breeding purpose) correlations among the assessed sub traits of faba bean *Ascochyta* blight resistance.

1.2 Material and methods

1.2.1 Plant material

The plant material consisted of N= 224 homozygous lines including 11 so-called founder lines, moreover 188 A-set lines, five spring faba bean lines, nine lines from a cross (Hiverna/2 x 29H), three Australian lines, twice *Ascochyta* resistant line (29H) and six additional lines from 'Göttingen Winter Bean Population'(GWBP). Following are further details about these groups of lines.

1- Founder lines (N= 11). These are highly inbred winter bean lines, originated in different regions of Europe: Germany (Webo/1, Wibo/1, Hiverna/1, L79/79, L977/88/S1wn, and L979/S1/1/sn), France (Côte d' Or/1 and Arrisot), UK (Banner/1, Bourdon/1 and Bulldog/1).

2- A-set (N= 188) homozygous lines, named as association set (A-set) because these were used for the genome-wide association analysis (*cf*. Chapter 2). These A-set lines were bred via single seed descent from the Göttingen Winter Bean Population (GWBP) to generation F>9. The GWBP was created in 1989 by combining the so called 11 founder lines through open pollination and natural selection into a population for up to 8 generations (Gasim, 2003). Afterwards, 400 SSD (single seed descent) lines were drawn from that recombined population and inbred up to F>9 generation. The A-set was randomly taken from these 400 inbred lines for association study.

3- Spring bean lines (N=5) were Limbo-7, Melodie-7, Hedin/2, Minica-5 and ILB938/2-2. These were added as outgroup types to oversee their level and variation for *Ascochyta* resistance.

4- Winter bean lines (N=9) derived from a cross between these two lines: 29H (*Ascochyta* resistant) and Hiverna/2 (German winter hardy line).

5- Australian lines (N=3) were available as check entries; Icarus-1, Manafest-1, Manafest-2.

6- 29H is reported as *Ascochyta* resistant line in literature (Atienza et al., 2016). 29H is employed twice in the current plant material, as double-check.

6- Additional winter bean lines (N=6) from research activities at Göttingen: (Côte d' Or/1-1 X BPL4628/1521.1)-18, Côte d'Or/1-1 X BPL4628/1521.1)-95.4, Côte d' Or/1-1, Hiverna/2-

5_EP1, Hiverna/1-1-2_EP3-2-4, and Webo/1-1-1 EP_10-1-1-2. These six lines do not or not directly belong to the Göttingen Winter Bean Population. Therefore these lines were not genotyped nor used for the GWAS (Chapter 2).

1.2.2 Green house experiments

1.2.2.1 Sowing of plants

The experiments were conducted in green houses at the divisions of 'Plant Breeding Methodology' and 'Plant Pathology and Plant Protection' at Georg-August-University Göttingen (Germany) through two successive seasons, 2017 and 2018. The plants were evaluated in six experiments, each one with the same genotypes and treatments. Each experiment was designed as an alpha-lattice experiment with two replicates (blocks) and the above-mentioned 224 entries (16 partial blocks x 14 genotypes per partial block) in each replicate; so across the six experiments, we count a total R=12 replicates. The seeds were stored in dry paper envelops and arranged in a tray before sowing (see Fig. 1.1 (a)). The seeds were sown in pots (size 13cm x13cm). The pots were more than half-filled (with 900g of soil mixture; local compost soil, ready-mixed soil (Fruhstorfer Erde by Co. 'Hawita') and sand as 3:1:1, respectively). Seeds were sown as one seed in one pot (Fig. 1.1 (b)). After sowing, the pots were filled up to 1600g of soil mixture; therefore, seed gaining 5cm of sowing depth (Fig.1.1 (c)). The pots were put on movable wagons, which were rotated every third day to randomize for any slight light intensity and temperature inequality for each plant in the greenhouse. When the juvenile plants reached the four expanded leaf stage (within about 40 days after sowing), pots were transferred from the greenhouse at the 'Division of Plant Breeding Methodology' to the greenhouse at the Division of 'Plant Pathology and Plant Protection' for inoculation with fungal spores and symptom scoring. Pots were arranged at the tables in that greenhouse according to the randomization plans, with 14 pots grouped together to make one partial block of pots, and then the 16 partial blocks arranged together to make one replicate.

(a) (b) (c)

Fig. 1.1 (a) Seed envelops arranged in a tray (b) Seed sowing in half filled pots (900 g soil mixture) (c) Refilling of pots after sowing (1600 g soil mixture)

1.2.2.2 Inoculation of plants

1.2.2.2. a. Collection and purification of fungal isolates

Fungal material was collected and purified by a former researcher (Remer, 2016). During the growing season 2015, faba bean leaves infected with *Ascochyta fabae* had been collected at the experimental fields of the private breeding company 'Norddeutsche Pflanzenzucht', located in Hohenlieth. These leaves were air-dried and stored in paper bags for spore isolation. The infected leaflets were first placed separately on tap water-moistened filter paper in Petri dishes. Subsequently, these Petri dishes were labelled with numbers as given to the isolates and incubated for two days at 20 °C near UV light as a stress factor to stimulate the formation of pycnidia. An adequate level of humidity facilitated pycnidia in opening and escaping of spore mucus. Pycnidia with emerging spore mucus on a lesion of a field bean leaf infected with *Ascochyta fabae* can be observed in Fig. 1.2. Under sterile conditions, spore slime was picked with a needle and streaked onto the 'Synthetic Nutrient-Poor Agar' (SNA) medium in Petri dishes, which additionally contained 200 ppm streptomycin. The purpose of adding streptomycin was to inhibit the possible growth of existing bacteria. These Petri dishes were incubated at 20°C for at least one week near UV light until fungal mycelium grew and pycnidia were formed again, from which spore mucus

emerged. Afterward, spore mucus was transferred to streptomycin-enriched V8A (V8 Agar) medium in Petri dishes. These plates were stored for two to five weeks at 20°C near UV light for pycnidia or spore mucus formation again. After pycnidia formation on V8A medium, the preparation of the spore suspensions was carried out under sterile conditions. At first, some sterile tap water was added to the respective Petri dish. A slide served to scrape the spores off the medium. The water including the spores was filtered through a gauze cloth into a Falcon tube to retain mycelial debris and impurities onto the gauze cloth.

These isolates were labelled with given names and dates to store at -20°C as parent spore suspension stock (initial suspension to prepare further spore suspensions). Two isolates named as 50 and 51 (Chapter 3, Table 3.1) were taken from this stock and propagated for this current study (Remer, 2016).

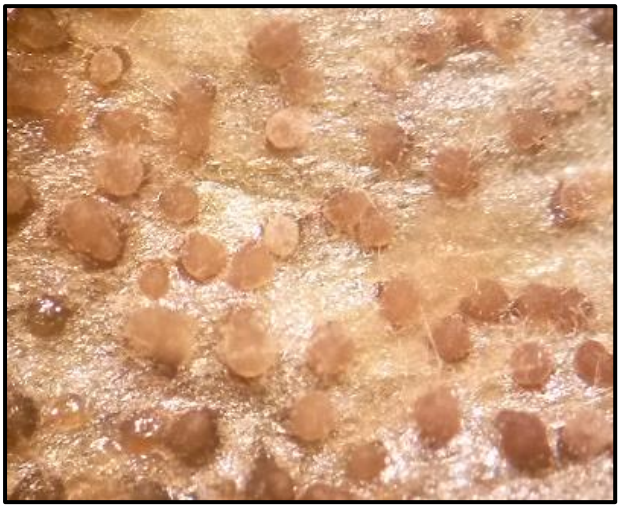


Fig. 1.2 Pycnidia with emerging spore mucus on a lesion of a field bean leaf infected with *Ascochyta fabae* (Remer 2016).

1.2.2.2. b. Preparation of V8A (V8 Agar) media

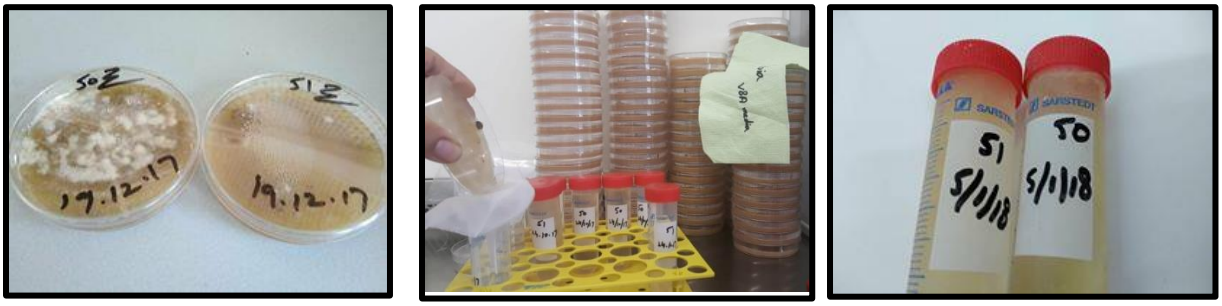
V8A (V8 Agar) media was prepared in 1000 ml flask by mixing vegetable juice ('Tomaten-Gemüse-Saft' as offered by the ALDI trade brand 'GutBio'), agar-agar, $CaCO_3$ and H_2O (Chapter 3, Table 3.2). Prepared V8A media flasks can be seen in Fig. 1.3. The mixture was autoclaved at 121 °C for 3 hours. After that, under the laminar airflow chamber, streptomycin (200 mg/l) was added and mixed in V8A media to avoid any bacterial contamination. The media was poured in such a way that it covered the surface of each Petri dish. The media plates were labelled with the

name of media (V8A) and date to store for 24 hours for the solidification of media. Next day half of the media plates were inoculated with isolate 50 and half plates with isolate 51. The prepared plates were closed and stored in a UV incubator at 20°C for 20 days for the growth of fungus.

Fig. 1.3 Prepared V8A media in flasks

1.2.2.2. c. Preparation of spore suspension

After 20 days, the fungal isolates grown on Petri plates can be seen (Fig. 1.4 (a)). Each Petri plate was filled with 10ml of autoclaved normal tap water under the laminar airflow chamber. After five minutes, the surface of each plate was scraped with the help of a glass slide to scratch mycelial network grown on plates and release fungal spores in water. The suspension from each plate was filtered through a gauze cloth into a labelled Falcon tube separately for each of the two isolates (50 and 51) to get spore suspension into Falcon tube and to retain mycelial debris onto gauze cloth (see Fig. 1.4 (b),(c)). The spore concentration of spore suspension was measured by 'Fuchs Rosenthal' under a microscope. Isolate 51 was producing roughly twice the number of spores in comparison to isolate 50. The concentration of each spore suspension was further diluted by adding sterile tap water to maintain the spore concentration to $1x10^6$ conidia spores per milliliters (ml). Spore suspensions from isolate 50 and 51 were mixed in such a way to achieve a ratio of 1:1. As a common observation, 2ml spore suspension was required for the inoculation of one plant by spray method, so almost 448 milliliters of spore's suspension was prepared for the inoculation of 224 plants. A fresh spore suspension was prepared to inoculate each replicate for assured viability of fungal spores.



(a) (b) (c)

Fig. 1.4 (a) Fungal isolates grown on Petri plates (b) Filtering of spore suspension (c) Filtered spore suspension

1.2.2.2. d. Inoculation of plants by spray method

The greenhouse tables were covered with a black porous polythene sheet, including a soak-able sheet underneath to sustain leached water during the irrigation of plant pots on tables. A tunnel made of polythene sheet was prepared onto plants and it was left open from one side to allow inoculation. The plants were inoculated by spray method at their approximate four-leaf stage (see Fig. 1.5). An electric compressor was attached to an atomizer assisting in up-taking of spore suspension from container and spraying. All plants were sprayed one by one with spore suspension until the level of run-off. The tunnel was closed for three days. Plants were watered from outside of tunnel only onto sheet beneath plant pots to maintain 100% humidity inside the tunnel and to offer favourable infection conditions. After 72 hours, the tunnel was removed. The plants were scored the first time after seven days of inoculation.

Fig. 1.5 Inoculation of plants by spray method

1.2.2.3 Scoring of plants for the traits related to resistance against *Ascochyta* blight

The faba beans plants were scored eight times for each replication, first scoring was performed after seven days of inoculation and later with alternating three and four days of interval, so for a total period of 30 days for each replication. A total of eight traits were assessed, aimed at *Ascochyta fabae* symptoms scoring:

1- No. of lesions per leaflet (LNL) were visually scored by counting the number of lesions from a leaflet per plant which was at present the most infected leaflet. This was executed in this way at each scoring-visit of a plant.

2- Size of biggest lesion per leaflet (LSL) was visually measured in millimetres (mm) from the biggest lesion of the leaf which was scored for LNL as most infected leaflet of the plant during each visit for scoring. Lesion size was measured in mm between the two most-distant points of the lesion by using a handover ruler.

3- Area covered by lesions per leaflet (LAL) was anticipated in percentage (based on well-trained experience) from this same most infected leaflet of the plant identified for scoring at each visit. For the training to score area covered by the lesion per leaflet, a 47 days old leaflet of faba bean was detached from the plant and the shape transferred on paper. By using a colour marker,

the area of whole leaflet was split into 100 equal parts and the area under one part was considered as 1%, area, similarly area under 5 parts was considered as 5%, area under 10 parts as 10% area of the leaflet. Such training was patiently employed to allow adequate scoring of the percentage of area covered by the lesions per leaflet (LAL).

4- Presence or absence of pycnidia per leaflet (LPL) was visually recorded as '1' for presence of pycnidia and '0' for the absence of pycnidia at lesions on this most infected leaflet of plant. Pycnidia are visible to a naked eye; however, a hand-held magnifying glass was used for clear observation.

5- No. of lesions per stem (LSL) were visually scored by counting the number of lesions at the inoculated segment of stem; there was never any lesion observed other than the inoculated segment of the stem during all the experiments.

6- Size of biggest lesion per stem (LSS) was visually measured in millimetres by using a hando-ver ruler and by measuring the two most-distant points of the lesion from the biggest lesion at stem.

7- Area covered by lesions at stem was anticipated in percentage (by estimating the infected area out of total area of inoculated segment of stem) from inoculated part of stem.

8- Presence or absence of pycnidia at stem (LPS) was visually recorded as '1' for presence of pycnidia and '0' for absence of pycnidia at lesions of stem.

All observations on leaves were recorded from the most infected leaflet per plant found during each visit for scoring. Hence, there is possibility that a different leaflet was picked to make all leaflet observation during this and the next scoring visit. Only the main stem of plant was inoculated and scored while any offshoots were removed with sterilized scissors occasionally.

After scoring plants eight times on the consecutive dates for each replication, the eight data points were summed up into one summative value for each trait. Datasheets, including summative values for each replication, were used as final score sheets for further analysis.

(a) (b)

Fig. 1.6 (a) *Ascochyta* blight lesions at leaflet (b) *Ascochyta* blight lesions at stem

1.2.2.2.4 Method validation test

It is assumed that with high heritability, such as h^2=0.7, the data is reliable enough to motivate further analysis such as genome wide association study. Therefore the expected, ultimate heritability values were predicted early in the project, based on the ANOVA of first experiment only (initial two replicates). Such predictions are so-called prospective predictions. These prospective predictions helped in judging the future required number of replicates to achieve nearly 70% of heritability. Such heritability predictions were estimated by the algebra explained below, based on ANOVA of first two replicates (R=2; 1st experiment) and 224 genotypes for the trait no. of lesions per leaflet.

Table 1.1 Analysis of variance (by PLABSTAT) of 224 lines of 1st experiment (two replicates) (LNL; Figures are sums across eight scoring dates)

Source of variation	Degree of freedom	M.S.S.	Var.cp	F
Replications (R)	1	151531.13	672.8	184.27**
Genotypes (G)	223	1194.26	185.97	1.45**
RxG	214	822.31	822.31	
Total	438			

M.S.S: mean sum of squares, Var.cp: variance components

$$heritability\ (h^2) = \frac{genotypic\ variance}{phenotypic\ variance}$$

$$h^2 = \frac{\sigma_g^2}{\sigma_p^2} = \frac{\sigma_g^2}{\sigma_g^2 + (\sigma_{GxR}^2/\mathrm{R})} = \frac{185.9747}{185.9747 + 822.3188/\mathrm{R}}$$

R = no. of replications

By adjusting the actual number of replications R, the heritability was predicted e.g. for R=4 ; the resulting h^2 was calculated in that case as

$$h^2 = \frac{185.97}{185.97 + 822.31/4} = 0.47$$

The heritability is presented in percentage, so 47% heritability was predicted for four replications based on the ANOVA of first experiment (Table 1.1).

After the completion of data recording for all the R=12 replicates, heritability judgement was repeated, yet now based on the ANOVA of all replicates. Such predictions are so called retrospective predictions for heritability. In the same way some other heritability values were predicted. Such pre experiment and post experiment predictions allowed the comparison of different prediction methods and the appreciation of knowledge in the beginning and after finalization of the project.

What is termed heritability h^2 takes obviously the perspective of the given experiment. It tells, inasmuch this experiment is able to predict the results that would be expected if the same experiment was extended to a number of R=∞ replicates. It does of course not deal with genotype x environment and genotype x replications interaction in farmers' field situation, hence, heritability as assessed here is honestly a repeatability parameter (Bernardo, 2020).

1.2.2.5 Statistical analyses of phenotypic data

The statistical analyses were performed with PLABSTAT software (PLAnt Breeding STATistical program) version Dez 2012 (Utz, 1991). Each of the six experiment was laid out with R=2 as lattice design. The experiments were accordingly analysed as lattice, as implemented in PLABSTAT. Hence, lattice-adjusted values for each of the 12 replicates were calculated. Thereafter, analysis of variance was performed with these 12 replicates (using these lattices adjusted single-replicate values) by using the following linear model.

$$Y_{ij} = \mu + g_i + r_j + gr_{ij}$$

where Y_{ij} is the phenotypic value of a resistance-related trait for inbred line i in replicate j, μ is the general mean, g_i, r_j are the main effects of genotypes and replications, respectively; gr_{ij} is genotype × replication interaction of genotype i with replication j.

Repeatability of the genotypes was measured in percentage for each trait and was calculated for R=12 (with MS GxR being the means square of the genotype x replication interaction) as

$$h^2 = \frac{genotypic\ variance}{phenotypic\ variance} = \frac{variance\ component\ of\ genotypes}{variance\ component\ of\ genotypes + \frac{1}{12}(MS\ \mathrm{GxR})}$$

The Spearman rank correlation coefficient was calculated for the phenotypic correlation between resistance-related traits. Microsoft Office Excel 2010 was used for the graphical representation of findings displayed as scatter plots and several prominent genotypes were marked in such plots.

1.3 Results and discussion

1.3.1 Results

1.3.1.1 Analysis of variance of *Ascochyta* blight resistance-related traits measured in faba bean germplasm

As group, the 224 faba bean genotypes, including the 188 A-set lines, displayed highly significant variation for all traits; in addition, replications and genotype x replication interactions were highly significant sources of variation. High repeatability estimates (h^2) were observed for all traits of leaflet and stem except for the presence of pycnidia per leaflet (h^2= 54.83%) and number of lesions at stem (h^2= 65.6%) while the heritability estimates were above 60% in all the other traits (Table 1.2). The highest repeatability estimates were observed for the area covered by the lesion per leaflet (h^2= 88.57%) and number of lesions per leaflet (h^2= 87.62%). All traits displayed a wide range of variation of the genotypes, for the maximum and minimum average values of the traits. Number of lesions at leaflet varied from 1.18 to 93.92 with an average of 23.88, size of biggest lesion at stem varied from 0.00 to 52.25 mm with an average value of 12.81mm (Table 1.2). The least significant difference between means of inbred lines (LSD, for 5% error probability) for number of lesions was 18.44 (Table 1.2).

1.3.1.2 Correlation among the resistance-related traits of the leaflet and stem

All the resistance-related traits of leaflet were markedly and significantly correlated with each other (Table 1.3). Similar positive and markedly and highly significant correlations were found within the resistance-related traits of stem. However, comparatively weaker but significant correlations were observed between leaflet and stem traits. The highest correlation was observed between number of lesions per leaflet and the area covered by the lesions per leaflet (r=0.93**) and comparatively weak correlations were observed for presence or absence of pycnidia per leaflet with all the other traits (Table 1.3). The faba bean germplasm's results were displayed in scatter plots for highest correlation observed between number of lesions per leaflet and the area covered by the lesions per leaflet and different genotype groups are displayed with different colours (see Fig. 1.8a).

1.3.1.3 Method validation test

The pre experiments (prospective predictions) and post experiments (retrospective predictions) heritability values are presented in Fig. 1.9. Each experiment was performed with two replicates. The sequential numbers of the six experiments are presented on primary x-axis and the actual numbers of replicates are scaled on secondary x-axis, while the heritability estimates as depending upon the no. of replications are presented on y-axis Fig. 1.9. The lower red graph represents the prospective predictions of h^2 values based on ANOVA of first experiment (R=2 from experiment 1). Each blue data point for h^2 value was calculated for one experiment (Exp. 1 to Exp. 6 as shown on the primary x-axis, i.e. with R=2 throughout). Each h^2 value displayed in black graph is the 'real' h^2 values at that moment (primary x-axis) of progressing in the project; the chronological increase of h^2 by adding further experiments and by improving the management of the experiments is shown here. The h^2 values for green graph were estimated by making retrospective predictions based on the ANOVA of all 12 replicates. The retrospective value for having only one, yet average-quality experiment shows approximately the average h^2 value of the six single experiments. The prospective predictions (red graph) helped to anticipate that 12 replicates would be sufficient to achieve almost 70% of heritability. Therefore, a total of six experiments (each experiment with two replicates, R=12) were performed. After the completion of six experiments, the analysis as shown here showed the virtue of adding enough replications and the virtue of improving the management of the experiments.

1.3.2 Discussion

Ascochyta blight is a common destructive faba bean disease caused by the fungus *Ascochyta fabae*. After conditions favourable for the disease development, severe yield losses were reported in susceptible cultivars of faba beans. *Ascochyta* can efficiently be controlled by genetic resistance of cultivars. The improvement of such a polygenetic trait through conventional breeding is demanding and laborious, because of limited heritability and frequent calamities with inappropriate infection levels in the field situation. Several sources of *Ascochyta* resistance have already been used in breeding programs. However, the genes for resistance and their molecular mode of action are still not identified. A gene pyramiding approach would be helpful but relies on the identification of markers tightly linked to the resistance genes (or, better, on the identification of the causal genes). Such QTL identification heavily depends upon the accuracy of phenotypic

data. Therefore, we performed a detailed screening and phenotyping of 224 pure faba beans lines, including Göttingen Winter Bean Population (GWBP, 188 A-set) under controlled conditions for *Ascochyta* resistance. The A-set is further utilized for our 'guided' genome-wide association study (Chapter 2). A very high genetic diversity was observed within 224 pure inbred lines of faba beans for *Ascochyta fabae* resistance. The analysis of variance results for 224 faba bean lines were almost the same as observed for the analysis of variance of A-set (Chapter 2; Table 2.2). Repeatability was high for all the traits in 224 faba bean lines (Table 1.2) as well as for A-set (Table 2.2) denoting the reliability of data set for GWAS (Chapter 2).

To appreciate the realized h^2 values and compare with the initially predicted h^2 values and to judge the validation of method, pre experiments (prospective predictions) and post experiments (retrospective predictions) heritability values were displayed Fig. 1.9. The green graph (retrospective predicted values of heritability) was higher than the black graph (chronologically realized heritability values) and the red graph (prospective values of heritability). However, the values of black graph were very close to green graph as soon as two experiments had been conducted. The starting point of black and red graph is common as expected for two replicates similarly the ending point of black and green graph is common as expected for 12 replicates. The discrepancy between the black graph and the green graph from replication 2 to 4 (from experiment 1 to experiment 2) shows the improvement in the management of the experiment early in the project. Each blue data point represents h^2 value calculated for one of the six experiments (chronological features,). The blue data points are rising. Initially, this was unexpected; yet, this was firstly, the learning for data scoring improved gradually with the no. of experiments so the heritability also improved. Secondly, for the experiment 5 and later, the two replicates of each experiment were sown and scored at the same time; this was not the case for the earlier experiments. For the earlier experiments, their replications were conducted one after the other.

It has been suggested previously that different genetic systems control leaves and stem resistance in faba beans (Avila et al., 2004; Kaur et al., 2014; Kohpina et al., 2000). In our study, all the 8 traits of stem and leaflet were genetically correlated with each other, however the correlation strength was comparatively weaker for the traits of leaflet with stem traits. Similar high significant correlations were observed (P=0.01) among all the traits for A-set (Chapter 2). The highest correlation was observed for number of lesions per leaflet and area covered by the lesions per leaflet (r=0.093**). The faba bean germplasm is displayed as scatter plot and represented with different

colour marks according to their genotype groups (Fig. 1.8 (a)). Many data points were crowded near value 0 at x-axis, therefore the same figure is zoomed-in near the value 0 and displayed as Fig. 1.8 (b) for clear display of close data points. The display of the GWBP-derived lines (marked with light grey colour) around their founder lines (marked with dark grey colour) validate the process of the development of GWBP from the 11 founder lines (Fig. 1.8 (a)). A total of five lines out of 224 lines performed better than 29H according to x-axis. Out of these five lines, three lines were from GWBP namely S_162-1-1-2-2, S_009-1-1-4, and S_038-1-1-1-1-3-8 and two lines, namely [(Hiv/2-5 x 29H-2)-1,15413] and [(Hiv/2-5 x 29H-2)-1,15735] were from the group of those nine lines which were developed from a cross (Hiv./2 x 29H) (Fig. 1.8 (b)). The other seven lines of this group were dispersed between the Hiv. /2 (marked with yellow colour) and 29H lines (marked with red colour) (Fig. 1.8 (a)). Seemingly, the above mentioned two high performing lines of this group performed better because of being transgressive segregants of their parents (Hiv./2 and 29H). The display of five high performing lines is focused in Fig. 1.8 (b) for clear demonstration. Hiv./1 was placed twice in material. Both Hiv./1 lines (marked with arrow signs) were observed near to each other in Fig. 1.8 (a) which further validate the reliability of data. Hiv./1 (data labelled) was observed more resistant than Hiv./2 (marked with yellow colour) in Fig. 1.8 (a). The spring lines as well as the Australian lines (marked with pink and orange colour, respectively) presented diversity in display as expected for their phenotypic expression (see Fig.1.8 (a)). One of the Australian lines, the line 'Icarus', was mentioned as 'susceptible line' in bi-parental cross used to detect *Ascochyta* QTLs in literature (Kaur et al., 2014). During this study, a total of 106 lines were found more susceptible than 'Icarus' (chapter 3). The top three susceptible lines were Manafest-2, S_232-1-1-1-16-6, and S_060-1-7, respectively (Chapter 3, Table 3.1).

The top five resistant lines in this study are comparatively better yet, this was found in comparison with this material under the given, artificial inoculations and controlled conditions. Their result under uncontrolled field conditions is hard to predict. Secondly, the current study was performed with a mixture of two a-sexually reproduced isolates identified as isolate 50 and isolate 51. However, *Ascochyta fabae* can reproduce both sexually and asexually, so the results may differ by using different isolates or a mixture of more than two isolates. *Ascochyta* blight resistance is a polygenic trait so there is no high risk that *Ascochyta* would acquire novel, trailblazing genes of virulence and would overcome the resistance very soon.

Perhaps, some useful or unfortunate correlations may be discovered between *Ascochyta* blight resistance and other traits for example with yield, time to maturity and lodging. Such analyses may help in indirect selection but still need to be conducted based on the available data at Goettingen or elsewhere. The faba bean entries can be scored and selected for *Ascochyta* blight resistance in small plots under field conditions but the natural infection of faba bean is uneven. The spread of pathogen can be improved by repeatedly sowing a susceptible genotype within the trial. The artificial inoculation in field is possible by spray method but a large amount of spore solution would be required and a high level of humidity should be maintained (Rani et al., 2020).

In Europe, usually spring beans are grown because of having high productivity. Winter beans can give more yield by avoiding drought if their potentially early maturity is realized. These days the area of winter bean cultivation is increasing, but winter beans stay longer in juvenile stage and in colder conditions than spring beans, and *Ascochyta fabae* attack more virulently in juvenile stage of host plant under cold weather conditions. Therefore, the screening of winter faba bean germplasm for *Ascochyta* resistance is imperative. However, the resistance mechanism for winter beans may not work for spring beans and vice versa.

Table 1.2. Phenotypic results and analysis of variance of 224 lines for 12 replicates during greenhouse seasons 2017/2018 and 2018/2019. Figures are sums across eight scoring dates.

Trait	Min.	Max.	Mean	Var.cp.(G)	LSD (0.05)	Repeatability (h^2) %
			Leaflet			
No. of lesions per leaflet	1.18	93.92	23.88	313.00**	18.44	87.62
Size of the biggest lesion per leaflet	3.25	51.58	21.02	82.78**	9.98	86.48
Area covered by lesions per leaflet	0.68	37.53	11.38	59.39**	7.68	88.57
Presence of pycnidia per leaflet	0.00	1.58	0.33	0.074**	0.69	54.83
			Stem			
No. of lesions at stem	0.00	43.17	7.38	34.15**	11.74	65.60
Size of the biggest lesion at stem	0.00	52.25	12.81	72.68**	14.78	71.89
Area covered by lesions at stem	0.00	23.30	4.01	13.91**	6.7	70.46
Presence of pycnidia at stem	0.00	2.36	0.32	0.15**	0.71	69.53

**Significant based on F-test for P= 0.01

Table 1.3. Correlation coefficient for the traits of faba bean germplasm (224 lines) for means across 12 replicates during greenhouse seasons 2017/2018 and 2018/2019.

Traits$	LNL	LSL	LAL	LPL	LNS	LSS	LAS
LSL	0.785**						
LAL	0.93**	0.89**					
LPL	0.56**	0.71**	0.69**				
LNS	0.65**	0.65**	0.69**	0.54**			
LSS	0.66**	0.68**	0.70**	0.54**	0.86**		
LAS	0.62**	0.64**	0.68**	0.54**	0.92**	0.92**	
LPS	0.58**	0.62**	0.67**	0.58**	0.79**	0.84**	0.86**

$ Abbreviations are mentioned in the material and methods section

** significant based on F test for p= 0.01 respectively

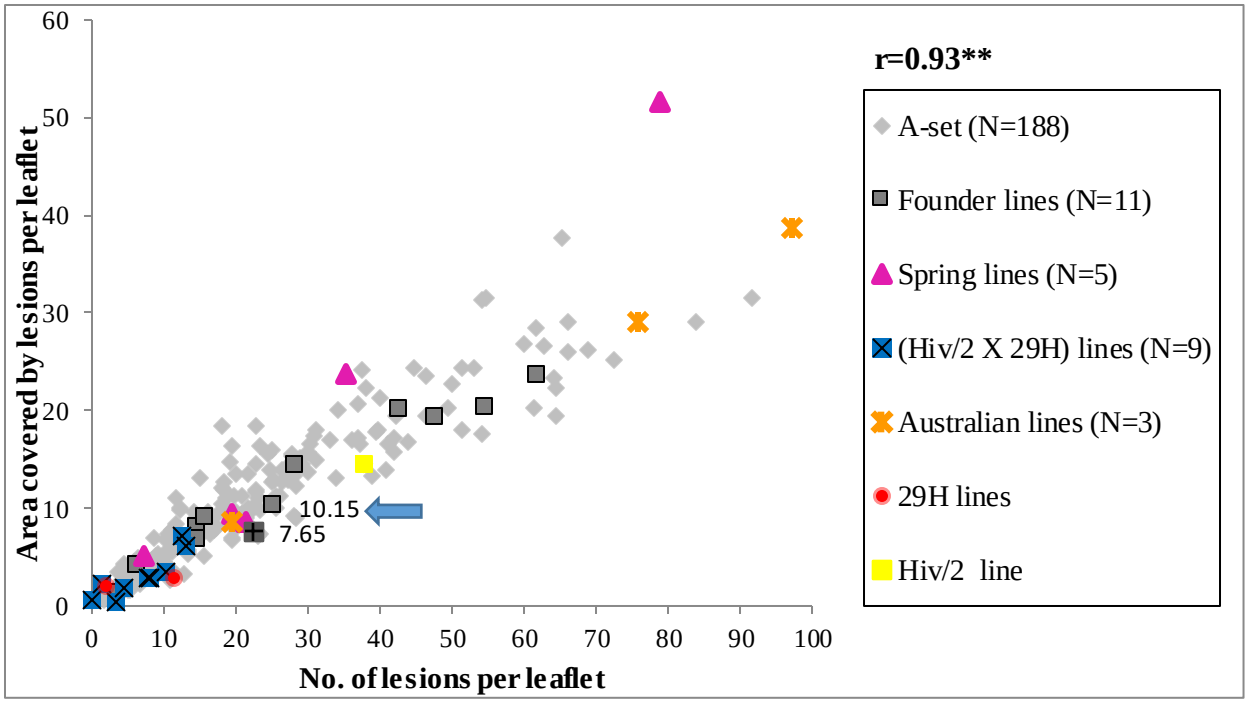


Fig. 1.8 (a) Correlation of no. of lesions per leaflet and area covered by the lesions per leaflet of 224 lines of faba bean germplasm (Figures are sums across eight scoring dates)

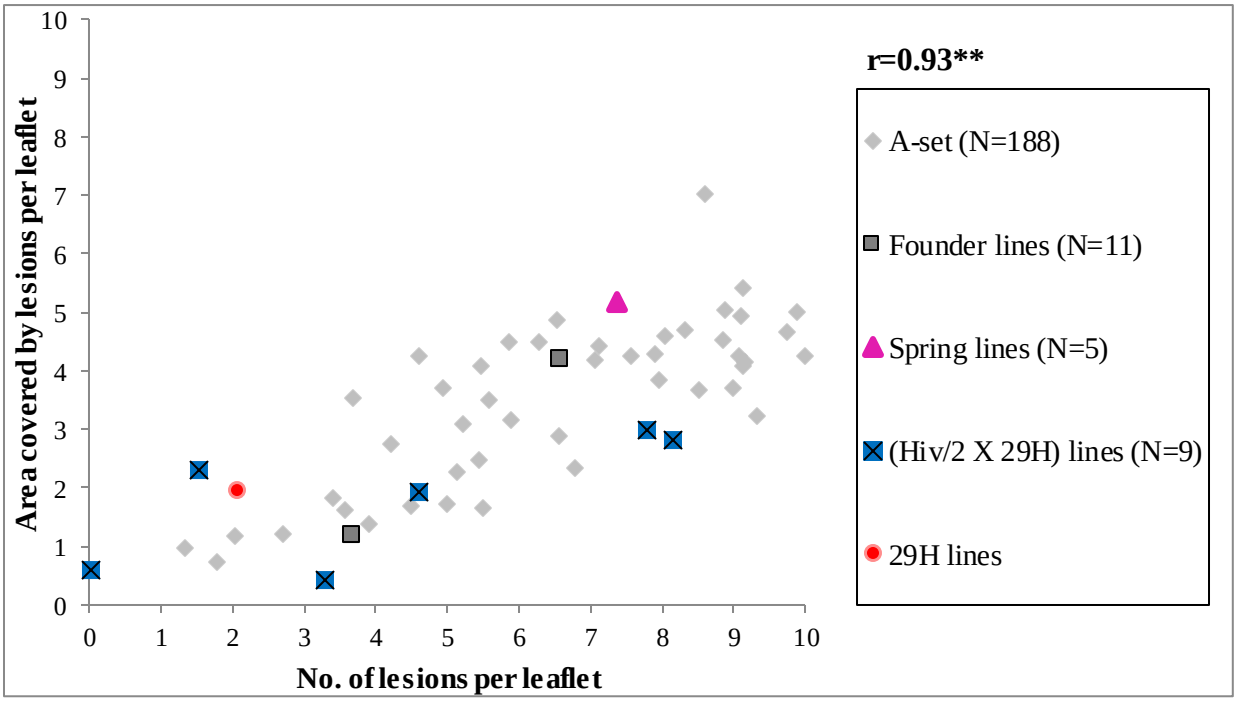


Fig. 1.8 (b) Correlation of no. of lesions per leaflet and area covered by the lesions per leaflet of 224 lines of faba bean germplasm (Figures are sums across eight scoring dates)

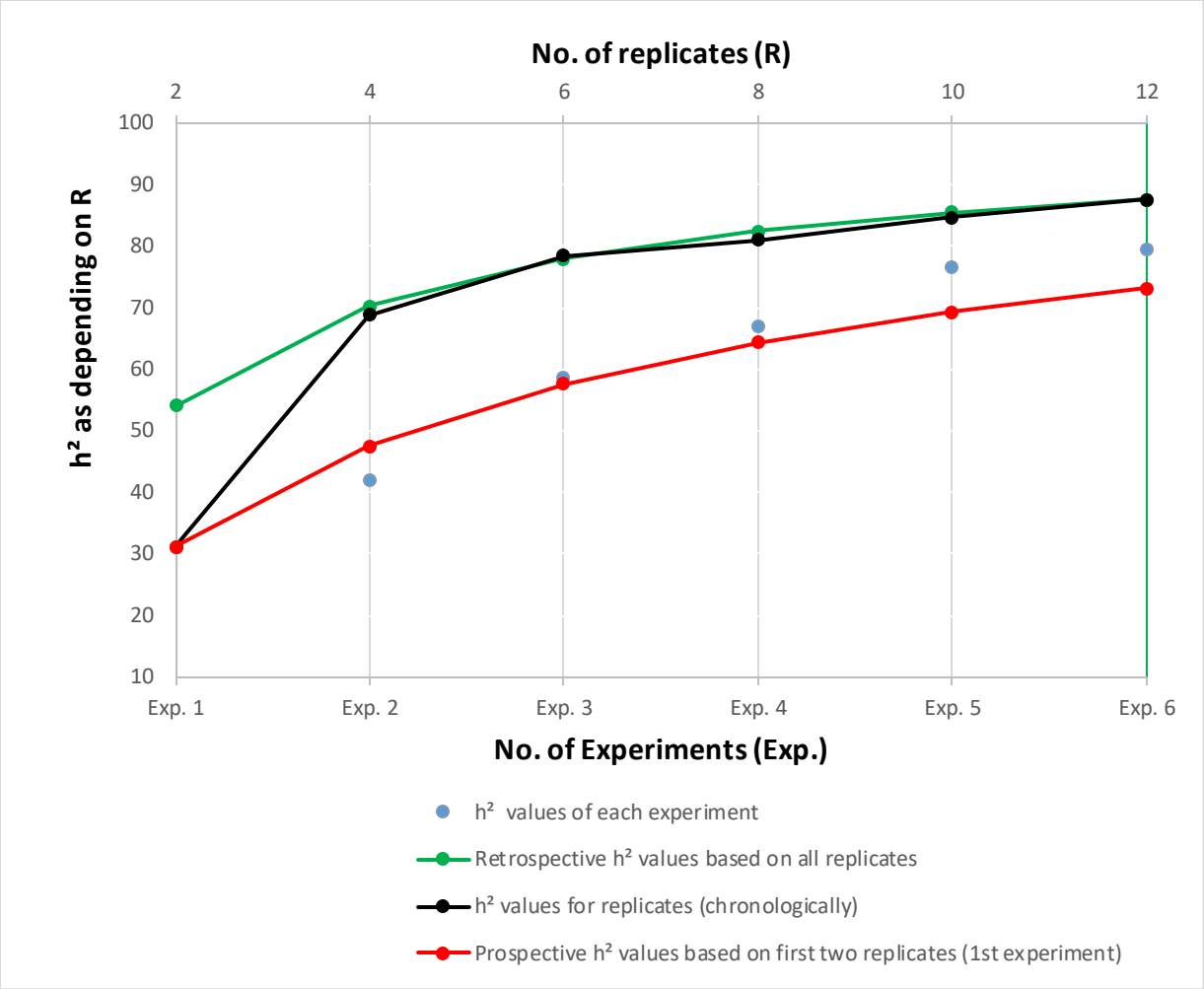


Fig. 1.9 Predicted vs real heritability

1.4 References

Abid, G., M., Hamdi, M., Mingeot, D., Aouida, M., Aroua, I., Muhovski, Y., Sassi, K., Souissi, F., Mannai, K., & Jebara, M. (2017). Effect of drought stress on chlorophyll fluorescence, antioxidant enzyme activities and gene expression patterns in faba bean (Vicia faba L.). *Archives of Agronomy and Soil Science*, *63*(4), 536–552.

Ahmed, S., Abang, M. M., & Maalouf, F. (2016). Integrated management of Ascochyta blight (Didymella fabae) on faba bean under Mediterranean conditions. *Crop Protection*, *81*, 65–69.

Ali, M., Welna, G. C., Sallam, A., Martsch, R., Balko, C., Gebser, B., Sass, O., & Link, W. (2016). Association analyses to genetically improve drought and freezing tolerance of faba bean (Vicia faba L.). *Crop Science*, *56*(3), 1036–1048.

Angus, J. F., Kirkegaard, J. A., Hunt, J. R., Ryan, M. H., Ohlander, L., & Peoples, M. B. (2015). Break crops and rotations for wheat. *Crop and Pasture Science*, *66*(6), 523–552.

Arbaoui, M., & Link, W. (2008). Effect of hardening on frost tolerance and fatty acid composition of leaves and stems of a set of faba bean (Vicia faba L.) genotypes. *Euphytica*, *162*(2), 211–219.

Atienza, S. G., Palomino, C., Gutiérrez, N., Alfaro, C. M., Rubiales, D., Torres, A. M., & Ávila, C. M. (2016). QTLs for ascochyta blight resistance in faba bean (Vicia faba L.): validation in field and controlled conditions. *Crop and Pasture Science*, *67*(2), 216–224.

Avila, C. M., Satovic, Z., Sillero, J. C., Rubiales, D., Moreno, M. T., & Torres, A. M. (2004). Isolate and organ-specific QTLs for ascochyta blight resistance in faba bean (Vicia faba L). *Theoretical and Applied Genetics*, *108*(6), 1071–1078.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society: Series B (Methodological)*, *57*(1), 289–300.

Bernardo, R. (2020). Reinventing quantitative genetics for plant breeding: something old, something new, something borrowed, something BLUE. *Heredity*, *125*(6), 375–385. https://doi.org/10.1038/s41437-020-0312-1

Bond, D. A., Jellis, G. J., Rowland, G. G., Le Guen, J., Robertson, L. D., Khalil, S. A., & Li-Juan, L. (1994). Present status and future strategy in breeding faba beans (Vicia faba L.) for

resistance to biotic and abiotic stresses. In *Expanding the production and use of cool season food legumes* (pp. 592–616). Springer.

Bond, D. A., & Pope, M. (1980). Ascochyta fabae on winter beans (Vicia faba): pathogen spread and variation in host resistance. *Plant Pathology*, *29*(2), 59–65.

Borevitz, J. O., & Nordborg, M. (2003). The impact of genomics on the study of natural variation in Arabidopsis. *Plant Physiology*, *132*(2), 718–725.

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. *Bioinformatics*, *23*(19), 2633–2635.

Burstin, J., Marget, P., Huart, M., Moessner, A., Mangin, B., Duchene, C., Desprez, B., Munier-Jolain, N., & Duc, G. (2007). Developmental genes have pleiotropic effects on plant morphology and source capacity, eventually impacting on seed protein content and productivity in pea. *Plant Physiology*, *144*(2), 768–781.

Cruz-Izquierdo, S., Avila, C. M., Satovic, Z., Palomino, C., Gutiérrez, N., Ellwood, S. R., Phan, H. T. T., Cubero, J. I., & Torres, A. M. (2012). Comparative genomics to bridge Vicia faba with model and closely-related legume species: stability of QTLs for flowering and yield-related traits. *Theoretical and Applied Genetics*, *125*(8), 1767–1782.

Cubero, J. I., Moreno, M. T., & Hernández, L. (1992). A faba bean (Vicia faba L.) cultivar resistant to broomrape (Orobanche crenata Forsk.). *Proceedings of the First European Conference on Grain Le Gumes, Association Europeenne Des Proté Agineux, Angers, France*, 41–42.

Davidson, J. A., & Kimber, R. B. E. (2007). Integrated disease management of ascochyta blight in pulse crops. In *Ascochyta blights of grain legumes* (pp. 99–110). Springer.

Díaz-Ruiz, R., Satovic, Z., Avila, C. M., Alfaro, C. M., Gutierrez, M. V., Torres, A. M., & Román, B. (2009). Confirmation of QTLs controlling Ascochyta fabae resistance in different generations of faba bean (Vicia faba L.). *Crop and Pasture Science*, *60*(4), 353–361.

Djemel, N., Guedon, D., Lechevalier, A., Salon, C., Miquel, M., Prosperi, J.-M., Rochat, C., & Boutin, J.-P. (2005). Development and composition of the seeds of nine genotypes of the Medicago truncatula species complex. *Plant Physiology and Biochemistry*, *43*(6), 557–566.

Duc. (2004). Seed composition of Medicago truncatula (line J5), compared to other seed

legumes. *Proceedings of the Fifth European Conference on Grain Legumes with the Second International Conference on Legume Genomics and Genetics, AEP-ICLGG, 2004*, 11–17.

Duc, Aleksić, J. M., Marget, P., Mikic, A., Paull, J., Redden, R. J., Sass, O., Stoddard, F. L., Vandenberg, A., & Vishnyakova, M. (2015). Faba bean. In *Grain Legumes* (pp. 141–178). Springer.

El-Rodeny, W., Kimura, M., Hirakawa, H., Sabah, A., Shirasawa, K., Sato, S., Tabata, S., Sasamoto, S., Watanabe, A., & Kawashima, K. (2014). Development of EST-SSR markers and construction of a linkage map in faba bean (Vicia faba). *Breeding Science*, *64*(3), 252–263.

Ellwood, S. R., Phan, H. T. T., Jordan, M., Hane, J., Torres, A. M., Avila, C. M., Cruz-Izquierdo, S., & Oliver, R. P. (2008). Construction of a comparative genetic map in faba bean (Vicia faba L.); conservation of genome structure with Lens culinaris. *BMC Genomics*, *9*(1), 380. https://doi.org/10.1186/1471-2164-9-380

Gasim, S. E. M. (2003). *Winter-hardy faba bean: Applied genetic research on the reproductive mode of the European gene pool*. Cuvillier.

Geard, I. D. (1961). *Studies on ascochyta, botrytis and seed'spot'of vicia faba in Tasmania*.

Gnanasambandam, A., Paull, J., Torres, A., Kaur, S., Leonforte, T., Li, H., Zong, X., Yang, T., & Materne, M. (2012). Impact of molecular technologies on faba bean (Vicia faba L.) breeding strategies. *Agronomy*, *2*(3), 132–166.

Gujaria, N., Kumar, A., Dauthal, P., Dubey, A., Hiremath, P., Prakash, A. B., Farmer, A., Bhide, M., Shah, T., & Gaur, P. M. (2011). Development and use of genic molecular markers (GMMs) for construction of a transcript map of chickpea (Cicer arietinum L.). *Theoretical and Applied Genetics*, *122*(8), 1577–1589.

Gutiérrez, N., Palomino, C., Satovic, Z., Ruiz-Rodríguez, M. D., Vitale, S., Gutiérrez, M. V, Rubiales, D., Kharrat, M., Amri, M., Emeran, A. A., Cubero, J. I., Atienza, S. G., Torres, A. M., & Avila, C. M. (2013). QTLs for Orobanche spp. resistance in faba bean: identification and validation across different environments. *Molecular Breeding*, *32*(4), 909–922. https://doi.org/10.1007/s11032-013-9920-2

Hanounik, S. B., & Robertson, L. D. (1989). Resistance in Vicia faba germ plasm to blight caused by Ascochyta fabae. *Plant Disease*, *73*(3), 202–205.

Hawtin, G. C., & Stewart, R. A. (1979). The development, production and problems of faba

beans (Vicia faba) in West Asia and North Africa. *FABIS Newsletter (ICARDA)*.

Hiremath, P. J., Farmer, A., Cannon, S. B., Woodward, J., Kudapa, H., Tuteja, R., Kumar, A., BhanuPrakash, A., Mulaosmanovic, B., & Gujaria, N. (2011). Large-scale transcriptome analysis in chickpea (Cicer arietinum L.), an orphan legume crop of the semi-arid tropics of Asia and Africa. *Plant Biotechnology Journal*, *9*(8), 922–931.

Jhanwar, S., Priya, P., Garg, R., Parida, S. K., Tyagi, A. K., & Jain, M. (2012). Transcriptome sequencing of wild chickpea as a rich resource for marker development. *Plant Biotechnology Journal*, *10*(6), 690–702.

Kang, H. M., Zaitlen, N. A., Wade, C. M., Kirby, A., Heckerman, D., Daly, M. J., & Eskin, E. (2008). Efficient control of population structure in model organism association mapping. *Genetics*, *178*(3), 1709–1723.

Kaur, S., Cogan, N. O. I., Pembleton, L. W., Shinozuka, M., Savin, K. W., Materne, M., & Forster, J. W. (2011). Transcriptome sequencing of lentil based on second-generation technology permits large-scale unigene assembly and SSR marker discovery. *BMC Genomics*, *12*(1), 265.

Kaur, S., Kimber, R. B. E., Cogan, N. O. I., Materne, M., Forster, J. W., & Paull, J. G. (2014). SNP discovery and high-density genetic mapping in faba bean (Vicia faba L.) permits identification of QTLs for ascochyta blight resistance. *Plant Science*, *217*, 47–55.

Kaur, S., Pembleton, L. W., Cogan, N. O. I., Savin, K. W., Leonforte, T., Paull, J., Materne, M., & Forster, J. W. (2012). Transcriptome sequencing of field pea and faba bean for discovery and validation of SSR genetic markers. *BMC Genomics*, *13*(1), 104.

Kezeya, S. B., Muel, F., Smadja, T., & Stauss, W. (2020). *Report on legume markets in the EU Deliverable D3.1 of the EU-project LegValue*. www.fh-swf.de/FB/agrar

Khamassi, K., Jeddi, F. Ben, Hobbs, D., Irigoyen, J., Stoddard, F., O'Sullivan, D. M., & Jones, H. (2013). A baseline study of vicine--convicine levels in faba bean (Vicia faba L.) germplasm. *Plant Genetic Resources*, *11*(3), 250–257.

Kohpina, S., Knight, R., & Stoddard, F. L. (2000). Genetics of resistance to ascochyta blight in two populations of faba bean. *Euphytica*, *112*(2), 101–107.

Lander, E. S., & Schork, N. J. (1994). Genetic dissection of complex traits. *Science*, *12*(4), 265(5181). https://doi.org/10.1038/ng0496-355

Link, W, Hocking, T. J., Stoddard, F. L., & others. (2007). Evaluation of physiological traits for

improving drought tolerance in faba bean (Vicia faba L.). *Plant and Soil*, *292*(1), 205–217.

Link, Wolfgang. (2009). Züchtungsforschung bei der Ackerbohne : Fakten und Potentiale Themenheft Leguminosen Themen und Merkmale ohne nachhaltige Wirkung in. *Journal Für Kulturpflanzen*, *61*(9).

Maqbool, A., Shafiq, S., & Lake, L. (2010). Radiant frost tolerance in pulse crops: a review. *Euphytica*, *172*(1), 1–12.

Maurin, N., & Tivoli, B. (1992). Variation in the resistance of Vicia faba to Ascochyta fabae in relation to disease development in field trials. *Plant Pathology*, *41*(6), 737–744.

Mwanamwenge, J., Loss, S. P., Siddique, K. H. M., & Cocks, P. S. (1999). Effect of water stress during floral initiation, flowering and podding on the growth and yield of faba bean (Vicia faba L.). *European Journal of Agronomy*, *11*(1), 1–11.

Nozzolillo, C., Ricciardi, L., & Lattanzio, V. (1989). Flavonoid constituents of seed coats of Vicia faba (Fabaceae) in relation to genetic control of their color. *Canadian Journal of Botany*, *67*(5), 1600–1604. https://doi.org/10.1139/b89-200

Omeri, B. N., Le May, C., Mlayeh, O., & Kharrat, M. (2012). First report of Didymella fabae, teleomorph of Ascochyta fabae, on faba bean crop debris in Tunisia. *Phytopathologia Mediterranea*, 369–373.

Ondrej, M. (1993). Response of resistant lines of horse bean (Vicia faba) to pathogenous fungus Ascochyta fabae Speg. *Czechoslovak Board on Plant Genetic Resources, Annual Report 1992*.

Patrick, J., & Stoddard, F. (2010). Physiology of flowering and grain filling in faba bean. *Field Crops Research*, *115*, 234–242. https://doi.org/10.1016/j.fcr.2009.06.005

Phan, H. T. T., Ellwood, S. R., Hane, J. K., Ford, R., Materne, M., & Oliver, R. P. (2007). Extensive macrosynteny between Medicago truncatula and Lens culinaris ssp. culinaris. *Theoretical and Applied Genetics*, *114*(3), 549–558.

Rani, U., Singh, S., Basandrai, A. K., Rathee, V. K., Tripathi, K., Singh, N., Dixit, G. P., Rana, J. C., Pandey, S., Kumar, A., & others. (2020). Identification of novel resistant sources for ascochyta blight (Ascochyta rabiei) in chickpea. *Plos One*, *15*(10), e0240589.

Rashid, K. Y., Bernier, C. C., & Conner, R. L. (1991). Evaluation of faba bean for resistance to Ascochyta fabae and development of host differentials for race identification. *Plant Disease*, *75*(8), 852–855.

Rawal D. K., (eds), V. & N. (2019). The Global Economy of Pulses. In *FAO*. https://doi.org/https://doi.org/10.4060/I7108EN

Remer, S. (2016). *Evaluierung des Resistenzstatus verschiedener Winterackerbohnen-Genotypen (Vicia faba L.) gegenüber unterschiedlichen Isolaten bzw. Pathogenvarianten des Brennfleckenerregers Ascochyta fabae*. 1–110. http://sub.uni-goettingen.de

Román, B., Satovic, Z., Avila, C. M., Rubiales, D., Moreno, M. T., & Torres, A. M. (2003). Locating genes associated with Ascochyta fabae resistance in Vicia faba. *Australian Journal of Agricultural Research*, *54*(1), 85–90.

Rubiales, D., & Fondevilla, S. (2012). Future prospects for ascochyta blight resistance breeding in cool season food legumes. *Frontiers in Plant Science*, *3*, 27.

Sass, O. (2009). Market situation and breeding input in faba beans and field peas in the EU. *Journal Für Kulturpflanzen*, *61*(9), 306–308.

Satovic, Z., Avila, C. M., Cruz-Izquierdo, S., Díaz-Ruíz, R., García-Ruíz, G. M., Palomino, C., Gutiérrez, N., Vitale, S., Ocaña-Moral, S., & Gutiérrez, M. V. (2013). A reference consensus genetic map for molecular markers and economically important traits in faba bean (Vicia faba L.). *BMC Genomics*, *14*(1), 932.

Sharpe, A. G., Ramsay, L., Sanderson, L.-A., Fedoruk, M. J., Clarke, W. E., Li, R., Kagale, S., Vijayan, P., Vandenberg, A., & Bett, K. E. (2013). Ancient orphan crop joins modern era: gene-based SNP discovery and mapping in lentil. *BMC Genomics*, *14*(1), 192.

Shiran, B., Kiani, S., Sehgal, D., Hafizi, A., Hassan, T., Chaudhary, M., & Raina, S. (2014). Internal transcribed spacer sequences of nuclear ribosomal DNA resolving complex taxonomic history in the genus Vicia L. *Genetic Resources and Crop Evolution*, *61*. https://doi.org/10.1007/s10722-014-0086-5

Siddique, K. H. M., Erskine, W., Hobson, K., Knights, E. J., Leonforte, A., Khan, T. N., Paull, J. G., Redden, R., & Materne, M. (2013). Cool-season grain legume improvement in Australia—use of genetic resources. *Crop and Pasture Science*, *64*(4), 347–360.

Sillero, J. C., Avila, C. M., Moreno, M. T., & Rubiales, D. (2001). Identification of resistance to Ascochyta fabae in Vicia faba germplasm. *Plant Breeding*, *120*(6), 529–531.

Sillero, Villegas-Fernández, A. M., Thomas, J., Rojas-Molina, M. M., Emeran, A. A., Fernández-Aparicio, M., & Rubiales, D. (2010). Faba bean breeding for disease resistance. *Field Crops Research*, *115*(3), 297–307.

Singh, M., Upadhyaya, H. D., & Bisht, I. S. (2013). Introduction. *Genetic and Genomic Resources of Grain Legume Improvement*, 1–10. https://doi.org/10.1016/B978-0-12-397935-3.00001-3

Stephens, A., Lombardi, M., Cogan, N. O. I., Forster, J. W., Hobson, K., Materne, M., & Kaur, S. (2014). Genetic marker discovery, intraspecific linkage map construction and quantitative trait locus analysis of Ascochyta blight resistance in chickpea (Cicer arietinum L.). *Molecular Breeding*, *33*(2), 297–313.

Stoddard, F. L., Kohpina, S., & Knight, R. (1999). Variability of Ascochyta fabae in South Australia. *Australian Journal of Agricultural Research*, *50*(8), 1475–1481.

Stoddard, F. L., Nicholas, A. H., Rubiales, D., Thomas, J., & Villegas-Fernández, A. M. (2010). Integrated pest management in faba bean. *Field Crops Research*, *115*(3), 308–318.

Tivoli, B, Reynaud, B., Maurin, N., Berthelem, P., & Le Guen, J. (1987). Comparison of some methods for evaluation of reaction of different faba bean genotypes to Ascochyta fabae. *Fabis Newsletter*, *17*, 35–38.

Tivoli, Bernard, Baranger, A., Avila, C. M., Banniza, S., Barbetti, M., Chen, W., Davidson, J., Lindeck, K., Kharrat, M., & Rubiales, D. (2006). Screening techniques and sources of resistance to foliar diseases caused by major necrotrophic fungi in grain legumes. *Euphytica*, *147*(1–2), 223–253.

Torres, A M, Weeden, N. F., & Martin, A. (1993). Linkage among isozyme, RFLP and RAPD markers in Vicia faba. *Theoretical and Applied Genetics*, *85*(8), 937–945.

Torres, Ana Maria, Román, B., Avila, C. M., Satovic, Z., Rubiales, D., Sillero, J. C., Cubero, J. I., & Moreno, M. T. (2006). Faba bean breeding for resistance against biotic stresses: towards application of marker technology. *Euphytica*, *147*(1–2), 67–80.

Utz, H. F. (1991). A computer program for statistical analysis of plant breeding experiments. *Institute of Plant Breeding, Seed Science and Population Genetics. University of Hohenheim*.

Vilhjálmsson, B. J., & Nordborg, M. (2013). The nature of confounding in genome-wide association studies. *Nature Reviews Genetics*, *14*(1), 1–2.

Webb, A., Cottage, A., Wood, T., Khamassi, K., Hobbs, D., Gostkiewicz, K., White, M., Khazaei, H., Ali, M., & Street, D. (2016). A SNP-based consensus genetic map for synteny-based trait targeting in faba bean (Vicia faba L.). *Plant Biotechnology Journal*, *14*(1), 177–

185.

Woyengo, T. A., & Nyachoti, C. M. (2012). Ileal digestibility of amino acids for zero-tannin faba bean (Vicia faba L.) fed to broiler chicks. *Poultry Science*, *91*(2), 439–443.

Zerhusen-Blecher, P., Stevens, K., Schäfer, B. C., & Braun, J. (2018). *Peas and broad beans - crops worthwhile: Basis of calculation*. https://demoneterbo.agrarpraxisforschung.de/index.php?id=302

Zhang, Z., Ersoz, E., Lai, C.-Q., Todhunter, R. J., Tiwari, H. K., Gore, M. A., Bradbury, P. J., Yu, J., Arnett, D. K., & Ordovas, J. M. (2010). Mixed linear model approach adapted for genome-wide association studies. *Nature Genetics*, *42*(4), 355.

Zong, X., Liu, X., Guan, J., Wang, S., Liu, Q., Paull, J. G., & Redden, R. (2009). Molecular variation among Chinese and global winter faba bean germplasm. *Theoretical and Applied Genetics*, *118*(5), 971–978.

Chapter 2. Genetic study of the resistance of faba bean (*Vicia faba* L.) against the fungus *Ascochyta fabae* through a genome-wide association analysis

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2.1 Abstract

Ascochyta fabae is a fungal pathogen responsible for marked yield losses in spring and winter faba beans worldwide. The aim of this genome-wide association study (GWAS) using 188 diverse winter faba bean inbred lines was to exploit earlier *Ascochyta* blight resistance studies and to identify new resistance loci. Phenotyping after artificial inoculation under controlled conditions revealed significant variation for all eight scored disease traits. This GWAS was based on 1829 AFLP-marker and 229 SNP-marker, including 17 so-called 'guide' SNP-markers. The latter were identified by map fragment alignments between the consensus map of Webb et al. (2016) and three earlier published *Ascochyta* blight resistance studies. A total of 12 markers were

found significantly associated with six traits, explaining 5.6% to 21.7% of the phenotypic variance. One 'guide' SNP on chromosome III co-localizes with the known resistance QTL *Af1* on chromosome III. Probably nine new resistance trait associated marker loci were identified which will improve resistance breeding on winter faba beans and support a broader inclusion of the crop into rotations.

2.2 Introduction

Faba bean (*Vicia faba* L.) is a diploid legume crop adapted to cool-season regions. It offers nutritious seed (starch 45%, protein 30%) for humans and animals (e.g. Duc et al., 2015). Its agronomic benefits are supportive of a sustainable agriculture system as faba beans are capable of symbiotic fixation of atmospheric nitrogen and as being a useful break crop in cereal-dominated rotations (Angus et al., 2015). Major growing countries are China with about 933000 ha, Ethiopia (519000 ha), Australia (138000 ha), and France (68000 ha) (Rawal, 2019). In Germany, cultivation area steadily increasing during the last decade, from 16,300 hectares in 2010 up to 59,500 hectares in 2020 (Zerhusen-Blecher et al., 2018; Destatis, 2020). Yet faba bean production is affected by biotic stress as: *Ascochyta* blight (*Ascochyta fabae*), chocolate spot (*Botrytis fabae*), downy mildew (*Peronospora viciae*), rust (*Uromyces viciae-fabae*), foot rots (*Fusarium* spp.), and, in Mediterranean region, also by broomrape (*Orobanche crenata*) (Torres et al., 2006). *Ascochyta* blight is a serious disease, at times even a devastating threat to faba bean, the fungus *Ascochyta fabae* (its teleomorph is named *Didymella fabae*; Jellis and Punithalingam, 1991) attacks both spring beans and winter beans. *Ascochyta* blight is found globally (Bond & Pope, 1980; Geard, 1961; Hawtin & Stewart, 1979). The disease symptoms are observed on leaves, stems, pods and seeds. Faba bean yield losses may rise to 90% for susceptible cultivars, aggravated by wet weather conditions (Ahmed et al., 2016; Davidson & Kimber, 2007; Hanounik & Robertson, 1989; Omeri et al., 2012). *Ascochyta* spores are carried and distributed by infected faba bean seeds and crop debris; rain and wind disperse the spores (Hanounik & Robertson, 1989; Rashid et al., 1991). Fungicides, appropriate crop rotation and the use of clean seed help in reducing *Ascochyta* incidence (Ahmed et al., 2016; Davidson & Kimber, 2007; Hanounik & Robertson, 1989; Omeri et al., 2012). The development of resistant cultivars is the most effective control option. Reduced lesion numbers, lesion size, lesion area and absence of pycnidia in lesions are indicative of *Ascochyta* resistance mechanisms (Maurin & Tivoli, 1992;

Ondrej, 1993; Rashid et al., 1991). Several sources of partial resistance of faba bean against *Ascochyta* blight have been reported (Bond & Pope, 1980; Hanounik & Robertson, 1989; Rubiales & Fondevilla, 2012; Siddique et al., 2013; Sillero et al., 2001). There is limited, even conflicting information on the genetic basis of resistance against *Ascochyta* blight. Both polygenic (Román et al., 2003) and major gene inheritance (Rashid et al., 1991) have been reported (cf. Avila et al., 2004; Hanounik & Robertson, 1989; Kohpina et al., 2000; Román et al., 2003; Sillero et al., 2010; Stoddard et al., 1999). Moreover, the genetics of resistance in the stems and leaves of faba beans differ. Avila et al. (2004) described QTL specific to *Ascochyta* blight isolates and plant organs; and Kaur et al. (2014) found, in an artificial inoculation trial, four QTL for either leaf or stem necrosis. The small-seeded winter type, inbred line 29H, is highly resistant; *A. fabae* hardly penetrates its tissues (Bond et al., 1994; Maurin & Tivoli, 1992; Sillero et al., 2001; Sillero et al., 2010; Tivoli et al., 2006; Tivoli et al., 1987).

The big genome size of faba bean (13000 Mb) is a hurdle for genomic studies (Satovic et al., 2013). Actually, still no faba bean genome sequence is publicly available; but for the model legume *Medicago truncatula*, with its small genome of 500 Mb, sequence data is available (Burstin et al., 2007; Djemel et al., 2005; Duc, 2004; Gnanasambandam et al., 2012; Rispail et al., 2010). *M. truncatula* shows high synteny with faba bean; high synteny has also been reported from other legumes such as *Lens culinaris* , *Cicer arietinum*, *Lupinus albus* (Phan et al., 2007). Meanwhile, a large number of SNP and SSRs have been developed for *Lens culinaris* (Kaur et al., 2011; Sharpe et al., 2013), *Pisum sativum* (Kaur et al., 2012), *Cicer arietinum* (Gujaria et al., 2011; Hiremath et al., 2011; Jhanwar et al., 2012; Stephens et al., 2014).

Various genetic faba bean maps have been published based on pertinent bi-parental mapping populations (Vf6 × Vf136, 29H × Vf136; Ascot × Icarus) (e.g. Gutiérrez et al., 2013; Satovic et al., 2013; Webb et al., 2016). Most SNP used in Webb et al. (2016) can be assigned to a gene of *M. truncatula*. Hence, this data allows synteny-based transfer of genetic information from the genomic sequence of *M. truncatula* to the consensus faba bean linkage map (Webb et al., 2016; Sullivan and Angra, 2016). At the start of this study, the densest map available for us was Webb et al. (2016), containing 687 SNP markers. Meanwhile further such consensus maps have become known (e.g. Ocaña-Moral et al., 2017; Sudheesh et al., 2019; Carrillo-Perdomo et al., 2020). Although previously various types of markers were employed to detect and map QTL for *Ascochyta* resistance in faba beans (Avila et al., 2004; Cruz-Izquierdo et al., 2012; Díaz-Ruiz et

al., 2009; El-Rodeny et al., 2014; Ellwood et al., 2008; Kaur et al., 2014; Román et al., 2003), today, mainly SNPs are used for such analyses.

Already in 2003, Román et al. detected two resistance QTL (Af1, Af2) in a faba bean study with artificial *Ascochyta fabae* inoculation in an F2 population derived from resistant line Vf6 and susceptible line Vf136. QTL Af1 showed additive gene action and was located on chromosome III. QTL Af2, located on chromosome II, displayed dominant gene action for resistance. QTL Af1 explained 25.2% and QTL Af2 as 21% of the phenotypic variation in that study.

Avila et al. (2004) continued the work of Román et al. (2003). They suggested the presence of six QTL (Af3 - Af8) based on offspring from the cross resistant line 29H × susceptible lineVf136. Their QTL Af3 was located on chromosome III, just as QTL (Af1) mentioned above. Later, Díaz-Ruiz et al. (2009) confirmed, based on informative, common markers, that QTL Af1 is located in the same chromosomal region as Af3. Furthermore, Díaz-Ruiz et al. (2009) confirmed the two QTL Af1 and Af2 in a recombinant inbred line (RIL) population derived from the material previously used by Román et al. (2003). Both QTL together explained 24% of the phenotypic variation for disease severity on leaves and 16% of the phenotypic variation for disease severity on stem.

Atienza et al. (2016) recently corroborated the QTL Af1 in a RIL population developed from the Avila et al. (2004) cross and judged it as identical to Af3. While Avila et al. (2004) assessed resistances after artificial inoculation in growth chamber, Atienza et al. (2016) tested in greenhouse and field. They re-identified Af2 on chromosome II and considered it the same as reported previously (Díaz-Ruiz et al., 2009; Kaur et al., 2014; Román et al., 2003).

Kaur et al. (2014) employed SNP markers and reported four *Ascochyta* QTL in a RIL population derived from crossing Icarus (susceptible) × Ascot (resistant) and tested under controlled conditions. Both belong to Australian-bred germplasm, seemingly different from the genotypes used in previous, Spanish studies. Their QTL-3 and the prior reported QTL Af2 (Díaz-Ruiz et al., 2009; Román et al., 2003) were both located on chromosome II; however, lack of common markers prevents more definite conclusions (Avila et al., 2004; Díaz-Ruiz et al., 2009; Román et al., 2003). *Ascochyta* blight resistance QTL-2 and QTL-4, detected first by Kaur et al. (2014), were confirmed in a biparental (Nurah × Farah) RIL population by Sudheesh et al. (2019), on chromosome I and VI, respectively. Since both parents, Nurah and Farah, were resistant to strain1 that RIL population specifically segregated for resistance to *Ascochyta* blight strain 2.

Breeding for improved resistance to *Ascochyta* blight is thought to significantly support winter faba beans in Central Europe, in Germany and neighbouring countries. In times of global warming, winter beans may offer advantages over spring beans and increase the diversity of crop types to choose from. The Göttingen Winter Bean Population is a promising and highly relevant, diverse germplasm pool for this objective; it is used for breeding and research (Ali et al., 2016). In the present study, a panel of 188 winter faba bean lines, derived from that germplasm pool, is tested for *Ascochyta* blight resistance under controlled conditions and artificial inoculation. Phenotypic data on symptom expression is used for genome wide association study to identify QTL for *Ascochyta* blight resistance in faba beans. Literature is thoroughly studied to exploit existing QTL data.

The study aimed to:
(1) exploit existing data on *Ascochyta* blight resistance QTL from earlier studies in Mediterranean types and spring types of faba bean and
(2) identify new markers associated with *Ascochyta* resistance in the Göttingen Winter Bean Population.

2.3 Material and methods

2.3.1 Genetic material

The plant material consisted of N=188 homozygous lines (association set; A-set). These lines were bred via single seed descent (SSD) without selection from the Göttingen Winter Bean Population (Link and Arbaoui, 2006) to generation F>9. The GWBP was created in 1989 by combining 11 founder lines: Webo/1, Wibo/1, Hiverna/1, L79/79, L977/88/S1wn and L979/S1/1/sn (German lines), Côte d'Or/1, Arrisot (French lines), Banner/1, Bourdon/1 and Bulldog/1 (UK lines), through eight generations of open pollination (Gasim, 2003). A total of 400 SSD lines were developed of which the A-set was randomly taken and used for earlier analyses (Ali et al., 2016) and for the current association study.

2.3.2 DNA markers and GWAS

Genotyping of the A-set lines as well as the 11 founder lines yielded a total of 2058 polymorphic markers, including 229 SNP. Judged from the SNPs, the average degree of homozygosity was 98.8%, very high as expected. A number of 1451 of these markers were mapped to 1159 loci by Welna (2014). The 12 linkage groups of this author could be unambiguously assigned (Welna,

2014; pages XXVII-XXXV; cf. Ali, 2015) to the six *Vicia faba* chromosomes following the notation of Webb et al. (2016). After deleting markers with allele frequencies (MAF) ≤ 5%, a total of 1355 markers were available for GWAS (1147 AFLPs, 208 SNPs). The AFLP bands were scored as present/absent; the very few expected cases of wrongly scoring a heterozygous ALFP locus as homozygous dominant were tolerated. All AFLP-markers and the majority of SNPs had been employed by Ali et al. (2016) for the same set of lines. All SNPs were chosen from the SNPs used by Webb et al. (2016). The SNPs contained 19 random SNPs and the so-called guide SNP marker set; these were not included in Ali et al. (2016). The 1355 markers thus dissociate into two sets: R-set (randomly chosen markers set) and G-set (guide markers set). The R-set enclosed all AFLPs and the SNPs as taken at the onset of this research from Ali et al. (2016), plus the 19 additional, random SNPs. The G-set contained, after deleting three markers for their MAF ≤ 5%, 14 of initially 17 so-called 'guide' SNP markers. The new SNPs (19 plus 17) were analysed as reported by Ali et al. (2016).

2.3.3 Development of the guide marker set (G-Set).

As all SNPs used here, the G-set markers can be found in Webb et al. (2016). The G-set markers were chosen during this study by exploiting prior literature about *Ascochyta* QTL (Table 1). DNA-markers linked to *Ascochyta* resistance QTL published in linkage maps (Kaur et al., 2014; Atienza et al., 2016; Satovic et al., 2013; more over Román et al., 2003; Díaz-Ruiz et al., 2009, and Avila et al., 2004) were noted. The guide SNP markers (Table 2.1) were defined by alignments of locus positions of the referenced maps with the map of Webb et al. (2016).

Five SNP markers were clearly identified in the map of Webb et al. (2016) as potentially being linked to two *Ascochyta* QTL in the map by Kaur et al. (2014), based on their sequence data and their BLAST-based physical position in the *Medicago truncatula* genome (Table1). Four guide markers were defined from cross-inspection of QTL-markers in the map of Atienza et al. (2016) and Webb et al. (2016) (Table 2.1), with 0.06cM to 1.07cM distances in Webb et al. (2016) between two initial marker (as reported by Atienza et al., 2016) and these four here-defined guide markers. Further guide markers were defined from alignment with the linkage map of Satovic et al. (2013). However, the distance between QTL and the common markers in the map of Satovic et al. (2013) was high and no direct picking was possible. Therefore, linear regression of cM data was applied with the positions of sets of common markers, to roughly predict the positions of

two of the QTL markers of Satovic et al. (2013) in the map of Webb et al. (2016). A total of eight further guide SNP markers (four predicted to be near to Af2 and the other four predicted to be near to Af1) were thus defined from the mapped SNPs at Webb et al. (2016). All were predicted to be between 0.2 and 4.2 cM from Af1 or Af2, respectively.

The investigations of the maps of Román et al. (2003), Díaz-Ruiz et al. (2009) and of Avila et al. (2004) did not reveal common markers thus no additional guide SNP. Our association study is thus a study in two layers: a genome-wide association study with all 1355 markers, and, included, a guided approach based on the 14 G-set markers.

Genome-wide association analyses were carried out using TASSEL version 3.0 (Bradbury et al., 2007). The mixed linear model (MLM) procedure of TASSEL was used with an optimum level of compression and re-estimation of the variance component estimates of each marker. A kinship matrix was employed, which was developed by using the average genetic similarity among the 11 founder lines as a threshold to define un-relatedness (Ali et al., 2016). A false discovery rate of 20% (FDR=0.20) was used to test the statistical significance of marker-trait associations (Benjamini & Hochberg, 1995; Benjamini and Yekutieli, 2005).

Based on GWAS results and marker genotype, a marker score was calculated for the trait 'number of lesions per leaflet'. For this, for each inbred line, the sum of the effects of its markers with favourable allele present was calculated.

Table 2.1: G-*set* of SNP (guide) markers as picked from the map by Webb et al. (2016)

No	Marker name in Web et al. (2016)	Chromo-some No.	Position (cM)*	Qualification of marker	
QTL assignment from Kaur et al. (2014)					
					QTL
1 ǂ	Vf_Mt5g098420_001	I	170.86	SNP_50000451	***QTL-2***
2 ǂ	Vf_Mt5g098060_001	I	171.49	SNP_50000451	***QTL-2***
3 ǂ	Vf_Mt4g091530_001	VI	90.81	SNP_50002192	***QTL-4***
4	Vf_Mt4g092850_001	VI	93.44	SNP_50002192	***QTL-4***
5 ǂ	Vf_Mt4g092750_001	VI	93.44	SNP_50001976	***QTL-4***
QTL assignment from Atienza et al. (2016), yet distances (cM) from Webb et al. (2016)					
				Distance	**QTL**
6 ǂ	Vf_Mt1g086810_001	III	95.81	0.65 cM from ♦LG31	***Af3***
7 ǂ	Vf_Mt1g088190_001	III	97.53	1.07 cM from ♦LG31	***Af3***
8 ǂ	Vf_Mt8g091280_001	VI	49.99	0.06 cM from *RNAR	***Field-DSP1***
9 ǂ	Vf_Mt8g093440_001	VI	50.55	0.50 cM from *RNAR	***Field-DSP1***
QTL assignment from Satovic et al. (2013), yet distances (cM) from Webb et al. (2016)					
				Distance	**QTL**
10 ǂ	Vf_Mt3g096560_001	II	107.21	0.33 cM	***Af2***
ǂ 11	Vf_Mt3g095660_001	II	106.66	0.86 cM	***Af2***
12	Vf_Mt3g094760_001	II	110.86	1.92 cM	***Af2***
13 ǂ	Vf_Mt3g098530_001	II	101.59	2.13 cM	***Af2***
14 ǂ	Vf_Mt1g012610_001	III	186.87	0.31 cM	***Af1***
15	Vf_Mt1g013400_001	III	189.72	2.54 cM	***Af1***
16 ǂ	Vf_Mt1g016390_001	III	195.46	2.99 cM	***Af1***
17 ǂ	Vf_Mt1g014230_001	III	188.26	4.21 cM	***Af1***

*RNAR; ♦LG31: common markers between map by Webb et al. (2016) and map by Atienza et al. (2016); ǂ 14 SNP markers available after applying limit of MAF(5%)

2.3.4 Phenotyping

The experiments were conducted under semi-controlled conditions in greenhouse in 2017 and 2018. The line 29H was included as phenotypic check. Plants were grown in pots of 13 x 13 cm^2 size filled with 1,6kg sand-soil mixture. One pot with one plant was the experimental unit. The

188 faba lines were grown in six experiments with two replications each. Plants were inoculated at four expanded leaf stage.

Fungal material had been collected and purified from leaf lesions of A-set lines grown in season 2015 at the local experimental faba bean nursery (Göttingen, Germany) and from a faba bean nursery of the breeding company NPZ Lembke near Eckernförde, Germany, yielding a total of 56 *Ascochyta* isolates. Leaf material showing typical lesions interspersed with pycnidia was air dried after sampling and subsequently incubated in humidity chambers for sporulation. Spores were picked from the ostiolum of a single pycnidium with the help of a sterile needle and transferred to V8-agar plates amended with 100 ppm streptomycin. In order to ensure to work with defined fungal genotypes, this procedure was once again repeated after one cycle of sub-cultivation. Two isolates named as number 50 and number 51 were used in the current study for being both, highly virulent yet differently responding to two rather susceptible and two rather resistant genotypes (Remer et al., 2016). The conidiospores were grown on V8 Agar media for the current analyses. Spore suspension was prepared with autoclaved tap water. The concentration of spore suspension was measured using a Fuchs Rosenthal hemocytometer under a microscope and further diluted to create the intended spore concentration ($1x10^6$ conidia spores per ml). Spore suspension of isolates 50 and 51 were mixed as 1:1 ratio for inoculation. A fresh spore suspension was prepared for each inoculation event. Plants were inoculated one by one by spraying with spore suspension until the level of run-off. Inoculated plants were enclosed in a plastic foliar tunnel for 72 h to maintain high humidity and favourable conditions for fungal growth. Afterwards, plants were visually scored eight times for each replication. The first scoring was performed seven days after inoculation. Further assessment followed alternately after three and four days, making a total scoring period of 30 days. A total of eight resistance-related traits were assessed at each scoring: 'number of lesions per leaflet', 'length of biggest lesion per leaflet' (mm; lesions are typically circular to oval), 'area covered by lesions per leaflet' (%), 'presence or absence of pycnidia per leaflet' (1 for yes, 0 for no), 'number of lesions at stem', 'length of biggest lesion at stem' (mm), 'area covered by lesions at stem' (%) and 'presence or absence of pycnidia at stem'. All leaf observations were recorded from the single, most infected leaflet per plant found at each visit for scoring, to efficiently differentiate between degrees of susceptibility. Only the main stem of plants was monitored while any tillers were removed. This pragmatic and efficient approach allowed conducting the experiments with its high number of genotypes and replicates.

The eight consecutive data points per trait were summed up into one aggregate value, basically following the concept of 'Area under Disease Progress Curve' (Campbell and Madden, 1990). Thus, for instance a value of 2.0 for 'presence of pycnidia per leaflet' accrued from finding pycnidia only at two of eight scoring dates (in such case at the ultimate and penultimate date).

The statistical analyses were performed with PLABSTAT (version Dez2012; Utz, 1991). The six experiments, each with its two replications, were randomized as an alpha lattice design and their results, as first step, analysed accordingly, to acquire lattice-adjusted values for the 12 replicates. The lattice-adjusted figures were then used for analysis of variance with the 12 replicates based on this linear model:

$Y_{ij} = \mu + g_i + r_j + gr_{ij}$

where Y_{ij} is the phenotypic value of a trait for inbred line i in replicate j, μ is the general mean, g_i, r_j are the main effects of genotypes and replications, respectively; gr_{ij} is genotype × replication interaction of genotype i with replication j.

This procedure allowed the software to estimate substitutes for the 0.92% of missing data points based on all 11 remaining replicates rather than based only on the one remaining of the two replicates per experiment. Variance components for genotypes were estimated from means squares of the analyses of variance as

Variance component for genotypes $= (MS_g - MS_{gr}) / 12$;

repeatability h^2 was estimated from mean squares (MS) and expressed in percent:

$h^2 = (MS_g - MS_{gr}) / MS_g$ .Spearman's rank correlation coefficients were used to examine correlations between traits.

2.4 Results

Data adjustments due to the randomization as alpha lattice were small, with lattice efficiencies between 100% (i.e. no adjustment) and 113% (across the eight traits and six experiments); the mean efficiency was 104%. Analyses of variance revealed highly significant variation ($p < 0.01$) for all eight traits, due to replicate, genotype and replicate x genotype effects. The genotype effect had F-values between 1.95 and 7.50, depending on trait (data not shown). Genetic variation within this panel of 188 inbred lines was high, as indicated by wide ranges of results (Table 2.2).

Repeatability of the variation among the 188 faba bean inbred lines was high for their leaf symptoms (84.15% < h^2 < 86.66%) except for 'presence of pycnidia per leaflet' (h^2= 48.70%). Repeatability of stem traits was lower than that of leaf traits (Table 2.2).

Highly significant correlations ($p < 0.01$) were observed among all traits (Table 2.3). Correlations within stem traits (0.72** < r < 0.91**) and within leaf traits (0.52** < r < 0.92**) were higher than between leaf and stem traits (0.44** < r < 0.61**) (Table2. 3). The highest correlation was found for 'number of lesions per leaflet' with 'area covered by the lesions per leaflet' (r=0.92**). However, moderate correlations were observed for the presence of pycnidia and other traits on leaflets (0.52** < r < 0.66**), whereas 'presence of pycnidia on stem' was highly correlated with the other stem traits (0.72** < r < 0.84**).

Association analysis was performed for the 188 A-set lines using their means across lattice-adjusted data from the 12 replications. The average LD among the 1355 markers employed for GWAS was r^2 = 0.0075 (*c.f.* Ali et al., 2016). Among the 12 markers that were significantly associated with traits, the averaged LD value was r^2 = 0.0067, ranging from 0.0000 < r^2 < 0.108. A total of 12 markers, including nine AFLP and three SNP markers, displayed a statistically significant association with six of the eight traits; four out of these 12 markers were associated with two or more traits (Table 2.4). One significantly associated SNP marker (marker 5; Vf-Mt1g014230-001) belongs to the G-set of markers. According to Satovic et al. (2013; Table 2.1), marker Mt1g014230-001 is located in the genomic vicinity of *Ascochyta* blight resistance *Af1*; here, it was significantly associated with two leaflet traits: 'number of lesions' and 'area covered by lesions', explaining 7.56% and 8.23% of the phenotypic variance, respectively. Four of the eleven further markers could not be mapped. Of the other mapped markers, AFLP marker E36M56-356 (marker 1; Table 4) was significantly associated with 'number of lesions per leaflet' and explained the highest percentage (21.71%) of the phenotypic variance of all associated markers. Most marker-trait associations were detected for leaf traits. For stem traits, only AFLP marker E40M59-281 (marker 8) appeared, being associated with 'length of the biggest lesion' and 'presence of pycnidia', two traits which were highly correlated (r=0.83**, Table 3). This one, marker 8 (E40M59-281), and the SNP Mt1g014230-001 (marker 5, associated with Af1) were in much higher LD than any other pair of markers (r^2=0.108). The AFLP marker E40M59-281 (marker 8) was furthermore associated with two leaf traits ('number of lesions per leaflet' and 'area covered by lesions per leaflet'; Table 2.4), which were as well highly correlated

(r=0.92**, Table 2.3). The explained phenotypic variance of marker 8 was, however, low; between 6.2 and 8.9% for its four associated traits. With the exception of marker 1 (E36M56-356), this range of explained variance is similar to that of other associated markers in the current study. For two stem-related traits (number of lesions and area covered by lesion), no significant marker-phenotype associations were detected.

The marker score for the trait 'number of lesions per leaflet' was correlated with the phenotypic result of this trait by r=0.295**.

Table 2.2: Phenotypic results of the 188 A-set lines across 12 replications (figures from aggregated values across eight consecutive scorings per trait)

Trait	Min.	Max.	Mean	Variance component	Least sign. difference ($p<0.05$)	Repeatability (h^2) %
Leaflet traits						
Number of lesions per leaflet	1.18	90.92	23.35	267.77**	17.87	86.57
Length of biggest lesion per leaflet (mm)	3.42	40.08	21.02	67.79**	9.91	84.15
Area covered by lesions per leaflet (%)	0.68	34.14	11.21	47.35**	7.49	86.66
Presence or absence of pycn. per leaflet (0 or 1)	0.00	1.58	0.31	0.05**	0.66	48.70
Stem traits						
Number of lesions at stem	0.00	33.08	6.99	23.14**	10.61	61.28
Length of biggest lesion at stem (mm)	0.00	50.08	12.53	55.26**	14.62	66.54
Area covered by lesions at stem (%)	0.00	23.30	3.78	10.73**	6.38	67.00
Presence or absence of pycn. at stem (0 or 1)	0.00	2.17	0.28	0.082**	0.67	58.31

** Significant based on F-test for $P < 0.01$

Table 2.3 Correlation coefficients between the eight traits of the 188 A-set lines (means across 12 replications)

Trait	LNL[a]	LLL	LAL	PPL	LNS	LLS	LAS
Length of biggest lesion per leaflet (LLL; mm)	0.74**						
Area covered by lesions per leaflet (LAL; %)	0.92**	0.87**					
Presence or absence of pycnidia per leaflet (0 or 1)	0.52**	0.66**	0.64**				
Number of lesions at stem (LNS)	0.55**	0.59**	0.59**	0.45**			
Length of biggest lesion at stem (LLS; mm)	0.56**	0.58**	0.61**	0.45**	0.83**		
Area covered by lesions at stem (LAS; %)	0.52**	0.56**	0.59**	0.44**	0.90**	0.91**	
Presence or absence of pycnidia at stem (0 or 1)	0.46**	0.51**	0.54**	0.48**	0.72**	0.83**	0.84**

[a] Number of lesions per leaflet; ** significant for $p<0.01$

Table 2.4 Association analysis results for *Ascochyta* blight resistance-related traits. Minimum minor allele frequency 5%, false discovery rate 20%. Chromosome number and cM position are according to Webb et al. (2016) except for five AFLP-marker with cM position according to Welna (2014; pages XXVIII-XXXV).

	DNA marker	Chrom.; position (cM) on map*	P-value[§]	BH-value[&]	Effect size[ł]	Good allele[‡]	No. of lines with good allele	R^2 (%)
Leaflet								
Number of lesions per leaflet								
1	E36M56-356	IV; (140.2)	$1.2x10^{-7}$	$1.48x10^{-4}$	12.19	1	27	21.71%
2	E44M56-174	I; (14.0)	$1.5x10^{-4}$	$4.43x10^{-4}$	8.54	0	56	9.08%
3	E39M60-165	V; (0.0)	$2.9x10^{-4}$	$7.38x10^{-4}$	10.37	0	125	8.00%

4	E44M58-177	III; (131.2)	$1.5x10^{-4}$	$2.95x10^{-4}$	11.45	1	24	7.80%
5♦	Vf-Mt1g014230	III; 188.3	$2.0x10^{-4}$	$5.90x10^{-4}$	9.78	A	101	7.56%
6	E40M54-328	I (192.2)	$5.4x10^{-4}$	$1.03x10^{-3}$	15.20	0	162	6.85%
7	E41M59-318	-[1]	$4.9x10^{-4}$	$8.86x10^{-4}$	8.90	1	64	6.64%
8	E40M59-281	-[1]	$6.7x10^{-4}$	$1.18x10^{-3}$	14.81	0	160	6.23%
9	Vf_Mt8g106690	VI; 6.0	$9.8x10^{-4}$	$1.33x10^{-3}$	12.05	A	158	6.10%
10	E41M55-177	-[1]	$1.3x10^{-3}$	$1.48x10^{-3}$	7.97	1	112	5.66%
Area covered by lesions per leaflet (%)								
8	E40M59-281	-[1]	$4.1x10^{-5}$	$1.48x10^{-4}$	7.37	0	160	8.95%
5♦	Vf-Mt1g014230	III; 188.3	$1.0x10^{-4}$	$2.95x10^{-4}$	4.31	A	101	8.23%
11	Vf_Mt8g086470	VI; 65.4	$2.2x10^{-4}$	$4.43x10^{-4}$	5.31	A	26	7.35%
Presence or absence of pycnidia per leaflet (0 or 1)								
12	E40M59-074	-[1]	$7.2x10^{-5}$	$1.48x10^{-4}$	0.20	0	115	8.41%
Length of the biggest lesion per leaflet (cm)								
4	E44M58-177	III; (131.2)	$6.8x10^{-5}$	$1.48x10^{-4}$	7.27	1	24	8.55%
Stem								
Length of biggest lesion at stem (cm)								
8	E40M59-281	-	$1.3x10^{-4}$	$1.48x10^{-4}$	8.07	0	160	7.85%
Presence or absence of pycnidia at stem (0 or 1)								
8	E40M59-281	-	$6.8x10^{-5}$	$1.48x10^{-4}$	0.34	0	160	8.46%

*For five AFLP markers, cM (in brackets) from Welna (2014), pages XXVII-XXXV

[ł]difference between the means of the two marker classes as calculated by TASSEL 3.0

[§]raw P-value as calculated by TASSEL 3.0

[&]Benjamini-Hochberg-criterion, calculated as: (rank of raw P-value · 0.2)/1355

[1] marker not mapped (Welna, 2014)

[‡]the AFLP alleles 1 (band present) or 0 (band absent) or the SNP allele A were the ones associated with decreasing (i.e. favourable) effect on trait

♦this G-set SNP marker is probably associated with known QTL *Af1*, based of inferred map position and cross-inspection of Satovic et al. (2013) and Webb et al. (2016)

2.5 Discussion

Ongoing climate warming, although a huge problem, may offer opportunities for new crop types. In Germany, where nearly exclusively spring faba beans are grown, winter faba bean is a novelty, a striking innovation for breeders and policymakers. The improvement of winter faba beans' genetic defence against biotic stresses such as *Ascochyta* blight will allow their inclusion into German crop rotations. This GWAS study is the first genome-wide association study with a focus on *Ascochyta* blight in *Vicia faba*. It revealed new putative resistance loci in the Göttingen Winter Bean Population, which recommends it as a possible germplasm source for resistance breeding.

The analyses were based on 188 faba bean inbred lines, two strains of *Ascochyta fabae*, and a total of 1355 DNA markers. Ali et al. (2016) certified the A-set of lines as showing no marked subgrouping. The P-values of the ten significant markers for 'number of lesions per leaflet', displayed as Q-Q plot (Fig. 2.1), deviated as expected from the identity line between observed and expected P-values. Below a value of 1.7 on the x-axis, 98.1% of the markers did not deviate markedly from the identity line. A total of 26 markers deviated visibly, and ten of them were significant in the GWAS analyses. The Q-Q plot supports the notion that the data base can be used for the applied analysis.

The average LD among all markers was very low (r^2 =0.0075). Given this very small LD, the available number of markers is likely a limiting factor. Indeed, two of eight traits were not associated with any of the marker even though literature had been exploited for so-called 'guide' SNP markers.

This GWAS could be carried out with phenotype data of, mostly, high repeatability. The highest values h^2 were found for 'area covered by lesions per leaflet' (h^2= 86.66 %) and 'number of lesions per leaflet' (h^2= 86.57 %) (Table 2.2). Accordingly, the highest number of marker associations was detected for these two traits. Three statistically significant markers were found for 'area covered by lesion per leaflet' and ten markers for 'number of lesions per leaflet' (Table

2.4). The AFLP locus E40M59-281 with its allele '0' (band absent) was associated with a decrease in 'number of lesions per leaflet', in 'area covered by lesion per leaflet', in 'length of the biggest lesion per leaflet', in 'length of biggest lesion at stem' and in 'presence of pycnidia at stem'. Another AFLP locus, E44M58-177, was, with its allele '1', associated with a decrease in 'number of lesions per leaflet' and 'length of the biggest lesion per leaflet'. This is in accordance with the strong correlation between these two traits. A statistically significant SNP (Vf-Mt1g014230-001) was shared by 'number of lesions per leaflet' and 'area covered by the lesion per leaflet', and explained 7 to 8 % of phenotypic variance. The exploitation of literature was successful insofar as this 'guide' SNP marker was picked for being listed in Webb et al. (2016) as near to the inferred position of a QTL marker of Satovic et al. (2013), when projecting that marker's position from the latter to the former map; even though the SNP was more than 4cM distant from that projected position. With one in 14 guide markers being significantly associated with resistance, this proportion was higher than for the genome-wide markers employed here.

Previously, QTL for *Ascochyta* blight have been reported on chromosomes II, III and VI. The QTL on chromosome III (*Af1*) has been reported and validated several times (Atienza et al., 2016; Avila et al., 2004; Díaz-Ruiz et al., 2009; Román et al., 2003; Satovic et al., 2013). Kaur et al. (2014) assumed that their QTL3 in cross (Icarus × Ascot) was identical with QTL *Af2* on chromosome II; *Af2* was reported by Díaz-Ruiz et al. (2009) and by Román et al. (2003). Despite a lacking physical *Vicia faba* map, Atienza et al. (2016) confirmed congruence of QTL3 and *Af2* on chromosome II, based on synteny to *Medicago truncatula*. Further QTL were reported on chromosome VI by Atienza et al. (2016) and by Avila et al. (2004). However, these QTL are not yet independently validated. Interestingly, the RNAR-marked QTL reported by Atienza et al. (2016) on chromosome VI shares this chromosome with our two significant SNP markers Vf_Mt8g106690_001 and Vf_Mt8g086470_001, which were located on that chromosome at 6.0 and 65.4 cM, at distances of 44.5 and 15.3 cM from the RNAR-marker (Webb et al., 2016) albeit with nearly zero LD to each other in the A-set of lines. Probably, these two SNPs do not mark the same QTL, yet Vf_Mt8g086470_001 (marker 11; Table 2.4) could still be associated to the RNAR-marked QTL (although the two employed G-set markers, at 0.06 and 0.50cM distance

from RNAR, were not significant). Altogether, for nine of the twelve markers, excluding markers 5, 8 and 11, there is currently no evidence of redundancy among them or of identity with known, published QTL for *Ascochyta* resistance.

For 'number of lesions per leaflet', the naïve sum of the ten R^2 values (Table 2.4) is 85.6%. This is probably overly high, although not higher than the repeatability of that trait (h^2=86.6%). Yet, one cannot expect that the current analyses detected all QTL for *Ascochyta* blight. A multiple regression of the phenotypic values of the lines on the ten significant markers resulted a multiple value of R^2 =29.7%; markedly lower than 85.6%. The sum of the effects of these ten significant markers was 111.3 lesions per leaflet, which is higher than the maximum numbers (72.6 to 91.5) found in the most susceptible lines S_232, S060, S_168; these are indications of overestimation in the data. Furthermore, the correlation between the marker score for 'number of lesions per leaflet' and the phenotype itself was small, r=0.295**, indicating that the marker score contains less information than what naïve interpretation suggests. The allele phases of two of the ten markers, marker 5 (presumably Af1) and marker 8 were associated (LD value of r^2=0.108), redundancy cannot fully be ruled out. With a false discovery rate of 20%, about 2 in ten markers are not expected to be sustained as positives, and with the limited number of 188 lines, overestimation of effect sizes has to be anticipated (Josephs et al., 2017; Vales et al., 2005). Epistasis, as it is statistically presented as interaction, might be a further explanation for shrinking effects when joining markers (unless epistasis is specifically implemented in the statistical model; Göring et al., 2001).

Although the findings here are bound to the greenhouse conditions and the two fungal strains used, the currently most promising parents among the A-set lines to combine in a cross for breeding seem to be line S_150 and line S_162. This is because S_150 is, except for marker 4 (E44M58-177), homozygous for the resistance-associated allele ('number of lesions per leaflet') at the other nine markers loci (Table 2.4); it is ranked as 26th best for the phenotypic trait value and on position 1 for marker score. The 25 lines that ranked phenotypically better than S_150 carried, at only three to eight marker loci, the resistance- associated allele; line S_150 was the highest-ranked line with nine. Line S_162 is, except for marker 5 (Vf-Mt1g014230-001) and marker 10 (E41M55-177), homozygous for the resistance-associated allele at the other eight loci. Line S_162 ranks 3rd-best for its marker score and 1^{rst} for its phenotypic value. Markers 4 and 5,

although mapped on the same chromosome (Table 2.4) are barely linked. Marker 10 is unmapped, yet with its LD-values of r^2=0.005 and r^2=0.013 to markers 4 and 5, it is probably not strongly linked to them. The cross of lines S_150 with S_162 would allow a complementation of the three genetic gaps (markers 4, 5, 10), thus roughly $(1/2)^3$ of the RIL lines from this cross should have all ten markers as desired.

Based on 'number of lesions per leaflet', three A-set lines (S_162, S_009; S_123) were more resistant than the highly resistant check line 29H showing 2.07 'lesions per leaflet'. Hence, novel promising donors of high resistance levels are available for validation and use.
Marker-assisted introgression of resistance into elite genetic material is supported by conversion of AFLP-derived results into SNP-supported data. Currently, an Affymetrix 50K chip is under development and will be publicly available on short notice (O'Sullivan, 2020; personal communication). Genotyping the A-set lines with that tool promises marked advance for applied breeding and for genetic analyses

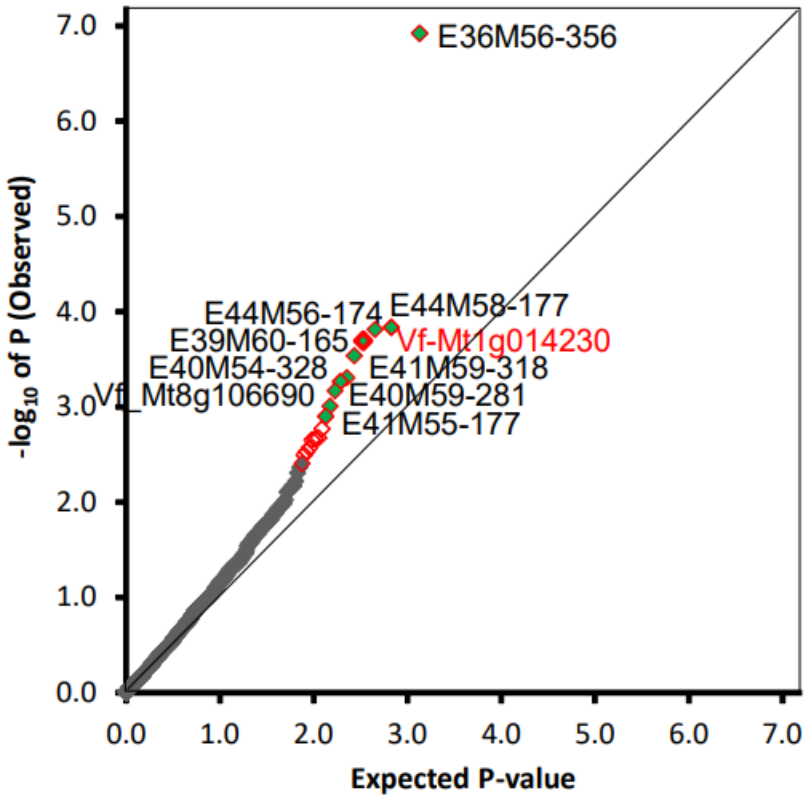

Fig. 2.1 P-value of significant markers displayed as Q-Q plot

2.6 Conclusion

Substantial and significant genetic variation for *Ascochyta*-resistance traits was detected, and all eight assessed traits were seemingly genetically related. LD in this set of Göttingen Winter Bean lines was very low; the number of markers probably did not match such high genetic resolution. To reduce these limitations and to steer the focus towards *Ascochyta* blight resistance genes, a so-called guided marker approach was conducted in addition to the default genome-wide analyses. A total of 12 markers, including nine AFLP and three SNP markers displayed significant associations with six traits; nine of these markers probably stand for new resistance genes. Significant SNP markers were found at chromosome III and VI in the descendants of the Göttingen Winter Bean Population. The guided approach was successful: one of 14 guide marker (Vf-Mt1g014230-001) was found significant and it is hypothesized that this SNP at chromosome III validates the previously reported QTL (*Af1*; chromosome III). The significant SNP found at chromosome VI should be validated in future studies. Applied marker-assisted selection for *Ascochyta*-resistance relies strongly on the transfer of genetic results among different faba bean populations, depending on further saturation of QTL bearing chromosomal regions.

2.7 References

Ahmed, S., Abang, M. M., & Maalouf, F. (2016). Integrated management of *Ascochyta* blight (*Didymella fabae*) on faba bean under Mediterranean conditions. Crop Protection, 81, 65–69.

Ali, M. (2015). Genetic linkage maps and homology study of backcross families of German faba bean (*Vicia faba* L.). Journal of Crop Improvement 29, 474-490.

Ali, M., Welna, G. C., Sallam, A., Martsch, R., Balko, C., Gebser, B., Sass, O., & Link, W. (2016). Association analyses to genetically improve drought and freezing tolerance of faba bean (*Vicia faba* L.). Crop Science, 56(3), 1036–1048.

Angus, J. F., Kirkegaard, J. A., Hunt, J. R., Ryan, M. H., Ohlander, L., & Peoples, M. B. (2015). Break crops and rotations for wheat. Crop and Pasture Science, 66(6), 523–552.

Atienza, S. G., Palomino, C., Gutiérrez, N., Alfaro, C. M., Rubiales, D., Torres, A. M., & Ávila, C. M. (2016). QTL for ascochyta blight resistance in faba bean (*Vicia faba* L.): validation in field and controlled conditions. Crop and Pasture Science, 67(2), 216–224.

Avila, C. M., Satovic, Z., Sillero, J. C., Rubiales, D., Moreno, M. T., & Torres, A. M. (2004). Isolate and organ-specific QTL for ascochyta blight resistance in faba bean (*Vicia faba* L). Theoretical and Applied Genetics, 108(6), 1071–1078.

Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the Royal Statistical Society: Series B (Methodological), 57(1), 289–300.

Benjamini, Y. & Yekutieli, D. (2005). Quantitative trait loci analyes using the false discovery rate. Genetics 171, 783-790.

Bond, D. A., Jellis, G. J., Rowland, G. G., Le Guen, J., Robertson, L. D., Khalil, S. A., & Li-Juan, L. (1994). Present status and future strategy in breeding faba beans (*Vicia faba* L.) for resistance to biotic and abiotic stresses. In: Muehlbauer, F., Kaiser, W.J. (eds). Expanding the production and use of cool season food legumes (pp. 592–616). Springer.

Bond, D. A., & Pope, M. (1980). *Ascochyta fabae* on winter beans (*Vicia faba*): pathogen spread and variation in host resistance. Plant Pathology, 29(2), 59–65.

Bradbury, P. J., Zhang, Z., Kroon, D. E., Casstevens, T. M., Ramdoss, Y., & Buckler, E. S. (2007). TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics, 23(19), 2633–2635.

Burstin, J., Marget, P., Huart, M., Moessner, A., Mangin, B., Duchene, C., Desprez, B., Munier-Jolain, N., & Duc, G. (2007). Developmental genes have pleiotropic effects on plant morphology and source capacity, eventually impacting on seed protein content and productivity in pea. Plant Physiology, 144(2), 768–781.

Campbell C.L., Madden L.V. (1990) Introduction to Plant Disease Epidemiology. New York, USA. John Wiley.

Carrillo-Perdomo, E., Vidal, A., Kreplak, J., Duborjal, H., Leveugle, M., Duarte, C., Desmetz, C., Deulvot, C., Raffiot, B., Marget, P., Tayeh, N., Pichon, J.P., Falque, M., Martin, O.C., Burstin, J., & Aubert, G. (2020) Development of new genetic resources for faba bean (*Vicia faba* L.) breeding through the discovery of gene-based SNP markers and the construction of a high-density consensus map. Scientific Reports 10, 6790. https://doi.org/10.1038/s41598-020-63664-7.

Cruz-Izquierdo, S., Avila, C. M., Satovic, Z., Palomino, C., Gutiérrez, N., Ellwood, S. R., Phan, H. T. T., Cubero, J. I., & Torres, A. M. (2012). Comparative genomics to bridge *Vicia faba* with model and closely-related legume species: stability of QTL for flowering and yield-related traits. Theoretical and Applied Genetics, 125(8), 1767–1782.

Davidson, J. A., & Kimber, R. B. E. (2007). Integrated disease management of *Ascochyta* blight in pulse crops. In: Tivoli, B., Baranger, A., Mehlbauer, F.J, Cooke, B.M. eds. *Ascochyta* blights of grain legumes (pp. 99–110). Springer.

Destatis, 2020. Statistisches Bundesamt. www.destatis.de.

Díaz-Ruiz, R., Satovic, Z., Avila, C. M., Alfaro, C. M., Gutierrez, M. V., Torres, A. M., & Román, B. (2009). Confirmation of QTL controlling *Ascochyta fabae* resistance in different generations of faba bean (*Vicia faba* L.). Crop and Pasture Science, 60(4), 353–361.

Djemel, N., Guedon, D., Lechevalier, A., Salon, C., Miquel, M., Prosperi, J.-M., Rochat, C., & Boutin, J.-P. (2005). Development and composition of the seeds of nine genotypes of the *Medicago truncatula* species complex. Plant Physiology and Biochemistry, 43(6), 557–566.

Duc, G. (2004). Seed composition of *Medicago truncatula* (line J5), compared to other seed legumes. Proceedings of the Fifth European Conference on Grain Legumes with the Second International Conference on Legume Genomics and Genetics, AEP-ICLGG, 2004, 11–17.

Duc, GH., Aleksić, J. M., Marget, P., Mikic, A., Paull, J., Redden, R. J., Sass, O., Stoddard, F. L., Vandenberg, A., Vishnyakova, M. & Torres, A.M. (2015). Chapter 5. Faba bean. In De Ron, A.M., (ed). Grain Legumes (pp. 141–178). Springer.

El-Rodeny, W., Kimura, M., Hirakawa, H., Sabah, A., Shirasawa, K., Sato, S., Tabata, S., Sasamoto, S., Watanabe, A., & Kawashima, K. (2014). Development of EST-SSR markers and construction of a linkage map in faba bean (*Vicia faba*). Breeding Science, 64(3), 252–263.

Ellwood, S. R., Phan, H. T. T., Jordan, M., Hane, J., Torres, A. M., Avila, C. M., Cruz-Izquierdo, S., & Oliver, R. P. (2008). Construction of a comparative genetic map in faba bean (*Vicia faba* L.); conservation of genome structure with *Lens culinaris*. BMC Genomics, 9(1), 380. https://doi.org/10.1186/1471-2164-9-380.

Gasim, S. E. M. (2003). Winter-hardy faba bean: Applied genetic research on the reproductive mode of the European gene pool. PhD thesis, University Göttingen. Cuvillier.

Geard, I.D. (1961). Studies on *Ascochyta*, *Botrytis* and seed 'spot'of *Vicia faba* in Tasmania. Thesis, University of Melbourne. http://hdl.handle.net/11343/114847.

Gnanasambandam, A., Paull, J., Torres, A., Kaur, S., Leonforte, T., Li, H., Zong, X., Yang, T., & Materne, M. (2012). Impact of molecular technologies on faba bean (*Vicia faba* L.) breeding strategies. Agronomy, 2(3), 132–166.

Göring, H.H.H, J.D. Terwilliger, J. Blangero (2001). Large upward bias in estimation of locus-specific effects from genomewide scans. Am. J. Hum. Genet. 69:1357–1369.

Gujaria, N., Kumar, A., Dauthal, P., Dubey, A., Hiremath, P., Prakash, A. B., Farmer, A., Bhide, M., Shah, T., & Gaur, P. M. (2011). Development and use of genic molecular markers (GMMs) for construction of a transcript map of chickpea (*Cicer arietinum* L.). Theoretical and Applied Genetics, 122(8), 1577–1589.

Gutiérrez, N., Palomino, C., Satovic, Z., Ruiz-Rodríguez, M. D., Vitale, S., Gutiérrez, M. V, Rubiales, D., Kharrat, M., Amri, M., Emeran, A. A., Cubero, J. I., Atienza, S. G., Torres, A. M., & Avila, C. M. (2013). QTL for *Orobanche* spp. resistance in faba bean: identification and validation across different environments. Molecular Breeding, 32(4), 909–922. https://doi.org/10.1007/s11032-013-9920-2.

Hanounik, S. B., & Robertson, L. D. (1989). Resistance in *Vicia faba* germ plasm to blight caused by Ascochyta fabae. Plant Disease, 73(3), 202–205.

Hawtin, G. C., & Stewart, R. A. (1979). The development, production and problems of faba beans (*Vicia faba*) in West Asia and North Africa. FABIS Newsletter 1, 7-9.

Hiremath, P. J., Farmer, A., Cannon, S. B., Woodward, J., Kudapa, H., Tuteja, R., Kumar, A., BhanuPrakash, A., Mulaosmanovic, B., & Gujaria, N. (2011). Large-scale transcriptome analysis in chickpea (*Cicer arietinum* L.), an orphan legume crop of the semi-arid tropics of Asia and Africa. Plant Biotechnology Journal, 9(8), 922–931.

Jellis, G. J. & Punithalingam, E. (1991) Discovery of *Didymella fabae* sp. nov., the teleomorph of *Ascochyta fabae*, on faba bean straw. Plant Pathology, 40 (1), 150-157.

Jhanwar, S., Priya, P., Garg, R., Parida, S. K., Tyagi, A. K., & Jain, M. (2012). Transcriptome sequencing of wild chickpea as a rich resource for marker development. Plant Biotechnology Journal, 10(6), 690–702.

Josephs, E.B, J.R. Stinchcombe, S.I Wright, 2017: What can genome-wide association studies tell us about the evolutionary forces maintaining genetic variation for quantitative traits? New Phytologist 214, 21-33.

Kaur, S., Cogan, N. O. I., Pembleton, L. W., Shinozuka, M., Savin, K. W., Materne, M., & Forster, J. W. (2011). Transcriptome sequencing of lentil based on second-generation technology permits large-scale unigene assembly and SSR marker discovery. BMC Genomics, 12(1), 265.

Kaur, S., Pembleton, L. W., Cogan, N. O. I., Savin, K. W., Leonforte, T., Paull, J., Materne, M., & Forster, J. W. (2012). Transcriptome sequencing of field pea and faba bean for discovery and validation of SSR genetic markers. BMC Genomics, 13(1), 104.

Kaur, S., Kimber, R. B. E., Cogan, N. O. I., Materne, M., Forster, J. W., & Paull, J. G. (2014). SNP discovery and high-density genetic mapping in faba bean (*Vicia faba* L.) permits identification of QTL for ascochyta blight resistance. Plant Science, 217, 47–55.

Kohpina, S., Knight, R., & Stoddard, F. L. (2000). Genetics of resistance to ascochyta blight in two populations of faba bean. Euphytica, 112(2), 101–107.

Link, W. & Arbaoui, M. (2006) Neues von der Göttinger Winterackerbohne. Berichte der 56. Arbeitstagung 2005 der AG Saatzuchtleiter BAL Gumpenstein, 31-38.

Maurin, N., & Tivoli, B. (1992). Variation in the resistance of *Vicia faba* to *Ascochyta fabae* in relation to disease development in field trials. Plant Pathology, 41(6), 737–744.

Ocaña-Moral, S., Gutiérrez, N., Torres, A.M. & Madrid, E. (2017). Saturation mapping of regions determining resistance to *Ascochyta* blight and broomrape in faba bean using transcriptome-based SNP genotyping. Theor Appl Genet 130, 2271–2282. doi.org/10.1007/s00122-017-2958-5.

Omeri, B. N., Le May, C., Mlayeh, O., & Kharrat, M. (2012). First report *of Didymella fabae*, teleomorph of *Ascochyta fabae*, on faba bean crop debris in Tunisia. Phytopathologia Mediterranea, 369–373.

Ondrej, M. (1993). Response of resistant lines of horse bean (*Vicia faba*) to pathogenous fungus *Ascochyta fabae* Speg. Czechoslovak Board on Plant Genetic Resources, Annual Report 1992.

Phan, H. T. T., Ellwood, S. R., Hane, J. K., Ford, R., Materne, M., & Oliver, R. P. (2007). Extensive macrosynteny between *Medicago truncatula* and *Lens culinaris* ssp. *culinaris*. Theoretical and Applied Genetics, 114(3), 549–558.

Rashid, K. Y., Bernier, C. C., & Conner, R. L. (1991). Evaluation of faba bean for resistance to Ascochyta fabae and development of host differentials for race identification. Plant Disease, 75(8), 852–855.

Rawal V, & Navarro, D.K., eds., (2019). The Global Economy of Pulses. Rome, FAO. https://doi.org/https://doi.org/10.4060/I7108EN.

Remer, S., Link W. & B. Koopmann B. (2016) Phänotypische Variation von Winterackerbohnen für ihre Resistenz gegen den Brennfleckenerreger *Ascochyta fabae*. Lecture at GFPi Summer Meeting, Pauliner Church Göttingen, May 31.

Rispail, N., Kaló, P., Kiss, G. B., Ellis, T. H. N., Gallardo, K., Thompson, R. D., Prats, E., Larrainzar, E., Ladrera, R., & González, E. M. (2010). Model legumes contribute to faba bean breeding. Field Crops Research, 115(3), 253–269.

Román, B., Satovic, Z., Avila, C. M., Rubiales, D., Moreno, M. T., & Torres, A. M. (2003). Locating genes associated with *Ascochyta fabae* resistance in *Vicia faba*. Australian Journal of Agricultural Research, 54(1), 85–90.

Rubiales, D., & Fondevilla, S. (2012). Future prospects for ascochyta blight resistance breeding in cool season food legumes. Frontiers in Plant Science, 3, 27.

Satovic, Z., Avila, C. M., Cruz-Izquierdo, S., Díaz-Ruíz, R., García-Ruíz, G. M., Palomino, C., Gutiérrez, N., Vitale, S., Ocaña-Moral, S., & Gutiérrez, M. V. (2013). A reference consensus genetic map for molecular markers and economically important traits in faba bean (*Vicia faba* L.). BMC Genomics, 14(1), 932.

Sharpe, A. G., Ramsay, L., Sanderson, L.-A., Fedoruk, M. J., Clarke, W. E., Li, R., Kagale, S., Vijayan, P., Vandenberg, A., & Bett, K. E. (2013). Ancient orphan crop joins modern era: gene-based SNP discovery and mapping in lentil. BMC Genomics, 14(1), 192.

Siddique, K. H. M., Erskine, W., Hobson, K., Knights, E. J., Leonforte, A., Khan, T. N., Paull, J. G., Redden, R., & Materne, M. (2013). Cool-season grain legume improvement in Australia—use of genetic resources. Crop and Pasture Science, 64(4), 347–360.

Sillero, J. C., Avila, C. M., Moreno, M. T., & Rubiales, D. (2001). Identification of resistance to *Ascochyta fabae* in *Vicia faba* germplasm. Plant Breeding, 120(6), 529–531.

Sillero, Villegas-Fernández, A. M., Thomas, J., Rojas-Molina, M. M., Emeran, A. A., Fernández-Aparicio, M., & Rubiales, D. (2010). Faba bean breeding for disease resistance. Field Crops Research, 115(3), 297–307.

Stephens, A., Lombardi, M., Cogan, N. O. I., Forster, J. W., Hobson, K., Materne, M., & Kaur, S. (2014). Genetic marker discovery, intraspecific linkage map construction and quantitative trait locus analysis of *Ascochyta blight* resistance in chickpea (*Cicer arietinum* L.). Molecular Breeding, 33(2), 297–313.

Stoddard, F. L., Kohpina, S., & Knight, R. (1999). Variability of *Ascochyta fabae* in South Australia. Australian Journal of Agricultural Research, 50(8), 1475–1481.

Sudheesh, S, Kimber, B. E., Braich, S., Forster J. W., Paull J. G., & Kaur S. (2019). Construction of an integrated genetic linkage map and detection of quantitative trait loci for ascochyta blight resistance in faba bean (*Vicia faba* L.) Euphytica 215, 42.

Sullivan, D. & D. Angra (2016) Advances in faba bean genetics and genomics. Frontiers in Genetics 7, 150. doi.org/10.3389/fgene.2016.00150.

Tivoli, B, Reynaud, B., Maurin, N., Berthelem, P., & Le Guen, J. (1987). Comparison of some methods for evaluation of reaction of different faba bean genotypes to *Ascochyta fabae*. Fabis Newsletter, 17, 35–38.

Tivoli, B., Baranger, A., Avila, C. M., Banniza, S., Barbetti, M., Chen, W., Davidson, J., Lindeck, K., Kharrat, M., & Rubiales, D. (2006). Screening techniques and sources of resistance

to foliar diseases caused by major necrotrophic fungi in grain legumes. Euphytica, 147(1–2), 223–253.

Torres, A. M., Román, B., Avila, C. M., Satovic, Z., Rubiales, D., Sillero, J. C., Cubero, J. I., & Moreno, M. T. (2006). Faba bean breeding for resistance against biotic stresses: towards application of marker technology. Euphytica, 147(1–2), 67–80.

Utz, H. F. (1991). A computer program for statistical analysis of plant breeding experiments. Institute of Plant Breeding, Seed Science and Population Genetics. University of Hohenheim.

Vales, M.I., C.C. Schön, F. Capettini, X.M. Chen, A.E. Corey, D.E. Mather, C.C. Mundt, K.L. Richardson, J.S. Sandoval-Islas, H.F. Utz, P.M. Hayes, 2005: Effect of population size in the estimation of QTL: a test using resistnce to barley stripe rust. Theoretical and Applied Genetics 111, 1260-1270.

Webb, A., Cottage, A., Wood, T., Khamassi, K., Hobbs, D., Gostkiewicz, K., White, M., Khazaei, H., Ali, M., & Street, D. (2016). A SNP-based consensus genetic map for synteny-based trait targeting in faba bean (*Vicia faba* L.). Plant Biotechnology Journal, 14(1), 177–185.

Welna, G. C. (2014). Genetic analysis for marker assisted improvement of drought tolerance in autumn sown faba bean. PhD thesis, University Göttingen, http://hdl.handle.net/11858/00-1735-0000-0022-5F43-2.

Zerhusen-Blecher, P., Stevens, K., Schäfer, B. C., & Braun, J. (2018). Peas and broad beans - crops worthwhile: Basis of calculation. https://demoneterbo.agrarpraxisforschung.de/index.php?id=302.

Chapter 3. Appendix:

Table 3.1 Details of *Ascochyta fabae* isolates used in the screening experiment

Isolate no.	Pedigree/faba bean genotypes	Origin
50	S_330-1-1-1	Hohenlieth
51	Hiverna/2	Hohenlieth

Table 3.2 Recipe for 750 ml of V8A media (vegetable media)

Components	Amount
Agar	11.25 g
Calcium Carbonate CaCO3	1.5 g
Vegetable juice	75 ml
Water	675 ml

g: grams, ml : milliliters

Table 3.3 List of faba bean genotypes and their ranking based on the number of lesions per leaflet (resistance to *Ascochyta* blight)

Serial	Genotypes	True pedigree no.	No. of lesions per leaflet	Ranking
	Founder lines (N=11)			
1	Webo/1-1-1	201	14.66	85
2	Wibo/1 -42370	202	62.01	210
3	CôteD`Or/1-1-3-1-2-1-1-2-2-3-1-6-4	203	42.85	192
4	L79/79/1 EP4-1-1-3	204	47.8	197
5	L977/88/S1wn-10-2	205	3.67	11
6	L979/S1/1/1sn EP10-1-1-1	206	54.72	206
7	Bourdon/1EP5-1-1-1-1	207	16.02	93
8	Arrisot/1 EP1-1-1-1	208	14.83	87
9	Banner/1 EP1-1-1-4	209	28.43	161
10	Bulldog/1-4 EP3-1-1-1-2	210	6.6	31
11	Hiverna/1-1-2 EP3-2-4	188	25.38	148

Table 3.3 continued

Göttingen Winter Bean Population (N=188)				
12	S_002-1-1-2	1	10	56
13	S_003-1-1-2	2	13.37	79
14	S_4-1-2	3	7.96	39
15	S_005-1-1-1	4	39.02	183
16	S_008-1-1-2	5	8.85	45
17	S_009-1-1-4	6	1.77	4
18	S_010-1-1-1-1	7	25.18	146
19	S_012-1-1-1	8	61.77	209
20	S_013-2-2	9	8.52	43
21	S_015-1-1-1-2	10	5.57	25
22	S_016-1-1-3-1	11	14.95	88
23	S_019-1-1-1-2	12	28.59	163
24	S_020-1-2-2	13	69.01	218
25	S_021-2-1	14	29.09	165
26	S_022-1-1-1-1	15	16.86	100
27	S_025-1-6	16	12.24	71
28	S_027-1-1-1	17	10.09	58
29	S_028-1-3-1-2	18	11.94	70
30	S_029-1-1-1-3	19	33.17	172
31	S_030-2-2	20	28.07	158
32	S_033-1-1	21	24.84	144
33	S_034-1-2	22	24.48	143
34	S_035-1-1-2-3	23	21.24	125
35	S_036-1-2-5	24	5.46	23
36	S_038-1-1-1-1-3-8	25	2.04	5
37	S_039-1-1-2	26	12.71	75
38	S_040-1-1-1-2	27	11.69	68
39	S_043-1-1-2	28	64.45	214
40	S_045-1-1-1	29	25.57	150
41	S_046-1-1-1-2-5	30	21.5	128
42	S_048-3-7	31	36.2	176
43	S_050-2-12	32	4.95	18
44	S_052-1-1-1-2	33	31.03	170
45	S_054-1-3-10-3	34	51.35	200
46	S_055-1-3-1-1	35	27.88	156
47	S_059-1-2-2-4	36	31.1	171
48	S_060-1-7	37	83.87	222

Table 3.3 continued

49	S_062-2-11	38	5.87	26
50	S_064-1-3-1-1	39	53.15	202
51	S_065-1-1-1	40	15.63	91
52	S_066-1-1-1-4	41	27.23	154
53	S_067-2-3	42	54.7	205
54	S_069-2-9	43	28.31	160
55	S_070-1-1-1	44	14.95	89
56	S_072-1-1-2	45	8.98	47
57	S_076-1-1-2	46	54.27	203
58	S_077-1-1-2	47	22.85	136
59	S_079-1-2-2-5	48	7.05	33
60	S_081-1-3-2-18	49	26.26	151
61	S_082-2-2-1-1-4	50	26.35	152
62	S_083-1-1-1-4	51	16.24	94
63	S_084-2-7	52	19.95	120
64	S_085-1-1-1	53	5	19
65	S_093-1-1-1-3	54	54.28	204
66	S_097-1-1-1-3	55	11.15	66
67	S_100-1-1-1	56	8.87	46
68	S_102-1-1-4	57	26.48	153
69	S_104-1-1-1-5	58	16.5	98
70	S_106-1-1-2-1	59	10.04	57
71	S_108-1-1-1	60	18.78	111
72	S_111-1-1-1-1	61	7.57	36
73	S_115-1-1-1	62	6.27	28
74	S_116-1-1-1-1	63	17.77	104
75	S_119-1-1-1-1	64	19.49	115
76	S_120-1-1-2	65	18.06	106
77	S_122-1-1-4-5	66	23.52	142
78	S_123-1-1-4	67	2.69	7
79	S_125-1-1	68	21.81	130
80	S_126-1-1-1	69	30.28	168
81	S_129-1-2-4	70	16.31	95
82	S_131-1-1-1	71	18.77	110
83	S_132-1-1-3	72	46.45	195
84	S_133-1-1-1	73	25.53	149
85	S_134-1-2-1-2	74	28.08	159
86	S_142-1-1-2	75	5.49	24

Table 3.3 continued

87	S_145-1-2-6	76	6.53	29
88	S_147-1-1-3	77	9.15	52
89	S_150-1-2-1-1	78	6.79	32
90	S_151-1-1-1-1-	79	10.58	61
91	S_153-1-1-1-2	80	28.43	162
92	S_158-1-1-1-1	81	17.14	101
93	S_160-1-1-1-1	82	9.1	49
94	S_161-2-1	83	4.48	15
95	S_162-1-1-2-2	84	1.32	2
96	S_163-1-1	85	11.74	69
97	S_165-1-1-2	86	16.31	96
98	S_166-1-1-2	87	8.33	42
99	S_167-2-5	88	49.47	198
100	S_168-1-1-1	89	72.61	219
101	S_169-1-1-5	90	20.08	121
102	S_170-1-1-1	91	18.51	109
103	S_172-1-1-1-1	92	3.57	10
104	S_173-1-1-1	93	9.12	50
105	S_174-1-1-1	94	30.99	169
106	S_175-1-1-4	95	13.39	80
107	S_176-1-1-1	96	14.27	83
108	S_177-1-1-2	97	15.16	90
109	S_181-1-1-5	98	12.27	73
110	S_182-1-1	99	9.34	53
111	S_185-1-1-1	100	3.67	12
112	S_186-1-1-2	101	62.71	211
113	S_189-1-1-2-3	102	51.48	201
114	S_190-1-1-1	103	43.96	193
115	S_191-1-3-1-5	104	22.8	134
116	S_192-1-1-2	105	37.31	179
117	S_194-1-1-2	106	17.25	102
118	S_195-1-1-2	107	23.29	140
119	S_196-1-1-1-2	108	34.17	174
120	S_197-1-1-1-	109	41.96	190
121	S_199-1-3-1-5	110	42.38	191
122	S_201-1-1-1-3	111	23.2	139
123	S_202-1-1-1	112	22.19	132
124	S_209-2-1	113	41.88	189
125	S_210-1-1-1-2	114	23.04	138

126	S_213-1-1-1-1-2	115	25.01	145

Table 3.3 continued

127	S_217-1-1-2-2	116	11.09	65
128	S_218-2-1	117	37.06	178
129	S_220-1-1-1	118	4.6	16
130	S_221-1-1-2-2	119	7.13	34
131	S_226-1-1-1-1	120	12.88	76
132	S_227-1-1-1-1-3	121	17.39	103
133	S_231-1-1-1-1	122	19.19	112
134	S_232-1-1-1-16-6	123	91.5	223
135	S_233-1-2-1-1	124	18.06	107
136	S_235-1-1-2-4	125	11.08	64
137	S_236-1-1-2	126	64.38	213
138	S_238-1-1-1	127	40.04	186
139	S_240-1-1-2-5	128	27.26	155
140	S_241-1-2	129	13.41	81
141	S_242-1-6	130	46.53	196
142	S_243-1-1-1	131	22.18	131
143	S_245-1-3	132	61.27	208
144	S_246-1-1-1-3	133	29.01	164
145	S_249-1-1-2-4	134	66.05	216
146	S_252-1-1-1-5	135	10.85	62
147	S_253-1-1-4-5	136	4.21	14
148	S_254-2-2-15-1	137	27.91	157
149	S_258-1-3-4	138	14.52	84
150	S_259-1-1-1	139	64.07	212
151	S_264-1-1-1-6	140	18.01	105
152	S_265-1-1-1-2	141	16.37	97
153	S_267-2-3	142	13.59	82
154	S_268-1-25	143	41.08	188
155	S_269-1-1	144	40.78	187
156	S_271-1-2-1-2	145	22.96	137
157	S_272-1-3-1-1	146	19.46	114
158	S_274-2-3	147	5.13	20
159	S_275-1-1-1	148	49.94	199
160	S_277-1-1-4	149	20.85	124
161	S_279-2-1-1	150	10.25	59
162	S_280-1-3-1-2	151	65.17	215
163	S_281-1-1-2	152	14.77	86
164	S_282-1-1-1-1	153	19.54	117

Table 3.3 continued

165	S_284-1-1-3	154	16.72	99
166	S_285-2-1	155	5.88	27
167	S_286-1-1-2	156	3.41	9
168	S_287-1-3	157	3.9	13
169	S_289-1-1-1-3	158	60.03	207
170	S_290-1-1-1	159	6.55	30
171	S_291-1-1-1	160	18.41	108
172	S_295-1-1	161	9.08	48
173	S_298-1-1-1-1	162	22.84	135
174	S_299-1-8	163	21.41	127
175	S_300-1-3-1-1	164	10.85	63
176	S_301-1-1-1-1	165	9.74	54
177	S_302-1-2-1-1	166	9.89	55
178	S_303-1-3	167	39.34	184
179	S_304-1-3-1-1	168	13.28	78
180	S_307-1-3	169	37.57	180
181	S_308-1-1-1-1	170	36.95	177
182	S_309-2-4	171	19.2	113
183	S_310-1-2-1-1	172	21.77	129
184	S_312-1-1	173	8.05	40
185	S_314-1-1-1	174	30.1	167
186	S_315-1-3	175	19.8	119
187	S_319-1-1-2-1	176	20.14	122
188	S_322-1-1-1	177	8.61	44
189	S_326-1-1-4-4	178	44.62	194
190	S_328-1-1-1-2	179	29.96	166
191	S_329-1-1-7	180	39.66	185
192	S_330-1-1-1	181	25.25	147
193	S_331-1-1-1	182	33.82	173
194	WAB_EP98_21-2-1 EP4-1-1-2-3	190	12.24	72
195	WAB_EP98_98-3-1 EP4-1-2-7	191	9.14	51
196	WAB98_98-4-1-2-1	192	5.43	22
197	WAB_EP98_267-11-1 EP5-16-7	193	23.34	141
198	WAB-EP02-Fam/S1_157-1-2-4-3-1-1-10	194	5.21	21
199	WAB-EP02-Fam/S1_159-1-2-4-1-1-3-1-3	195	15.68	92

Table 3.3 continued

	Spring lines (N=5)			
200	Limbo-7-4	197	35.32	175
201	Melodie-7-2	198	19.49	116
202	Hedin/2—4	199	78.91	221
203	Minica-5-1	200	21.4	126
204	ILB938/2-2	220	7.38	35
	(Hiv. X 29H) lines (N=9)			
205	(Hiv/2-5 x 29H-2)-1, 15402	212	12.99	77
206	(Hiv/2-5 x 29H-2)-1, 15413	213	0.01	1
207	(Hiv/2-5 x 29H-2)-1, 15419	214	10.48	60
208	(Hiv/2-5 x 29H-2)-2, 15470	215	12.46	74
209	(Hiv/2-5 x 29H-2)-4, 15581	216	4.6	17
210	(Hiv/2-5 x 29H-2)-4, 15612	217	8.16	41
211	(Hiv/2-5 x 29H-2)-4, 15622	218	7.79	37
212	(Hiv/2-5 x 29H-2)-5, 15694	219	3.29	8
213	(Hiv/2-5 x 29H-2)-5, 15735	221	1.52	3
	Australian lines (N=3)			
214	Icarus-1	222	19.58	118
215	Manafest-1	223	75.94	220
216	Manafest-2	224	97.14	224
	29H line (N=1, twice)			
217	29H (*Ascochyta*-resistant)-1-3-12	183	2.07	6
218	29H (*Ascochyta*-resistant)-2	211	11.52	67
	Additional lines from GWBP (N=6)			
219	Hiverna/2-5 EP1-1-8-1-3-3-2	187	37.86	181
220	(Cd`Or/1-1 x BPL4628/1521.1)-18-3-1	184	7.89	38
221	(Côte d`Or x BPL…)-95-4-1-1-3	185	20.78	123
222	CôteD`Or/1-1-3-1-2-1-1-2-2 -3-1	186	38.12	182
223	Webo/1-1-1 EP 10-1-1-2	196	66.11	217
224	Hiverna/1-1-2 EP3-2-4(2)	189	22.46	133

Summary

Climate warming is a huge challenge for agronomists, although it may offer opportunities to new crops and new types of old crops, such as winter types in so far spring-sown species. In Germany, mostly spring faba beans (*Vicia faba* L.) are grown. The currently improved winter hardiness of winter faba beans catches up with the upcoming milder German winters. Such autumn-sown beans are a novelty for farmers. The improvement of winter faba beans' genetic defence against important biotic stresses such as *Ascochyta* blight will allow their inclusion into German crop rotations. *Ascochyta* blight is caused by the fungus *Ascochyta fabae*; severe yield losses were reported in susceptible cultivars of faba beans. Traditionally, the disease is controlled by avoiding sowing *Ascochyta*-infected seed. The genetic improvement of *Ascochyta* blight resistance through conventional breeding is demanding and laborious, because of complex inheritance, low heritability (h^2) and frequent calamities with uneven infection levels in the field situation. Several sources of *Ascochyta* resistance have already been described. However, the genes for resistance and their mode of action are still not identified. A resistance gene pyramiding approach would be helpful but relies on the identification of markers tightly linked to the resistance genes (or, better, on the identification of the causal alleles). Such QTL identification heavily depends upon the accuracy of phenotypic data. With this situation as background, we performed a detailed screening and phenotyping of 224 highly homozygous lines of faba beans, including the A-set lines of the Göttingen Winter Bean Population (GWBP, 188 inbred lines; cf. Ali et al., 2016) under controlled conditions for *Ascochyta* resistance. Two strains of *Ascochyta fabae* were spray-inoculated as mixture on potted juvenile plants in greenhouse; plants were visually scored eight times in a 30 day period on leaflets and on stem, for number and size (cm) of lesions, area (%) covered by lesions and for presence of *Ascochyta* pycnidia at leaflet and stem. The experimental unit was one plant. The accumulated (across the eight dates) and averaged (across 12 replicates) scores per trait, were used as phenotypic data. To judge the validity of the method and to employ estimates of h^2 for planning, predictions (prospective heritability values) from the first two replicates and post-experiments predictions (retrospective heritability values) from all 12

replicates were generated. Based on the prospective predictions, 12 replicates were required to achieve a value of heritability higher than h^2=70%, which was deemed as sufficient (the finally realized value was h^2=87%). Substantial and significant genetic variation for *Ascochyta*-resistance traits was detected, and all eight assessed traits were seemingly genetically correlated. The strongest correlation, r= 0.93** was between no. of lesions per leaflet and area covered by the lesions per leaflet. A total of five lines (S_162-1-1-2-2, S_009-1-1-4, S_038-1-1-1-1-3-8, (Hiv/2-5 x 29H-2)-15413 and (Hiv/2-5 x 29H-2)-1-15413)) out of 224 lines performed better than the resistant line 29H. The three most susceptible lines were Manafest-2, S_232-1-1-1-16-6 and S_060-1-7. The A-set with its 188 inbred lines was utilized for our 'guided' genome-wide association study. The GWAS was the first genome-wide association study with a focus on *Ascochyta* blight in *Vicia faba*. The analyses were thus based on 188 faba bean inbred lines, two strains of *Ascochyta fabae*, and a total of 2058 DNA markers (1829 AFLP-marker and 229 SNP-marker, including 17 so-called 'guide' SNP-markers). The average LD among all the markers was very low (r^2 = 0.0075); the number of markers probably did not match such high genetic resolution. To alleviate these limitations and to steer the focus towards *Ascochyta* blight resistance genes, the so-called guided marker approach was conducted in addition to the default genome-wide analyses. The 17 guide SNP markers were defined by alignments of locus positions of the previously published maps with our available map. In this way, nine of the markers were identified as potentially being linked to a published *Ascochyta* QTL. Further eight guide markers were not directly picked from a published map because the distance between the published QTL and the common markers was high. Therefore, linear regression of cM data was applied with the positions of sets of common markers, to roughly predict the positions of two of the published QTL markers in our available map. Our association study is thus a study in two layers: a genome-wide association study with all randomly chosen markers, and, included, a guided approach based on the 17 guide SNP markers. A total of 12 markers, including nine AFLP and three SNP markers displayed significant associations with six traits (number of lesions per leaflet, area covered by lesions per leaflet, presence of pycnidia per leaflet, length of the biggest lesion per leaflet, length of biggest lesion at stem, presence of pycnidia at stem; nine of these markers probably stand for new resistance genes. Significant DNA markers were found at chromosomes I, III, IV, V, and VI in the descendants of the Göttingen Winter Bean Population. The guided approach was success-

ful: one of our 17 guide marker (Vf-Mt1g014230-001) was found significant and it is hypothesized that this SNP at chromosome III validates the previously reported QTL (Af1; chromosome III). The guided marker approach for genome-wide analyses proved to be successful in a species, where no genome sequence was available.

Applied marker-assisted selection for *Ascochyta*-resistance relies strongly on the transfer of genetic results among different faba bean populations, depending on further marker-saturation of QTL bearing chromosomal regions. The new putative resistance loci in the Göttingen Winter Bean Population recommend this germplasm as a possible source for resistance breeding.

Zusammenfassung

Die Klimaerwärmung ist eine enorme agronomische Herausforderung, obwohl sie Chancen für neue Fruchtarten und neue Fruchtartentypen bereithält, wie für Wintertypen in bislang als Sommertypen gesäte Arten. In Deutschland werden zumeist Sommerackerbohnen (*Vicia faba* L.) angebaut. Die derzeit verbesserte Winterhärte von Winterackerbohnen trifft auf die schon kommenden milderen Winter in Deutschland. Solche Herbst-gesäten Bohnen sind für die Landwirte eine Neuheit. Die Verbesserung der genetischen Abwehr von Winterackerbohnen gegen wichtige biotische Stressoren wie die *Ascochyta*-Brennfleckenkrankheit wird ihre Hereinnahme in die Fruchtfolgen in Deutschland erlauben. Die Brennfleckenkrankheit wird durch den Pilz *Ascochyta fabae* verursacht; von anfälligen Ackerbohnensorten wurden schwere Ertragsverluste berichtet. Die Vermeidung von *Ascochyta*-infiziertem Saatgut ist eine traditionelle Bekämpfungsmaßnahme. Die erbliche Verbesserung der Brennfleckenkrankheits-Resistenz durch konventionelle Züchtung ist wegen der komplexen Vererbung, der niedrigen Erblichkeit (h^2) und wegen häufiger Kalamitäten mit inhomogenen Infektionsniveaus in der Feldsituation aufwendig und mühsam. Mehrere Quellen von *Ascochyta*-Resistenz sind schon beschrieben worden. Die Gene für Resistenz und ihre Wirkungsweise jedoch sind noch nicht identifiziert. Ein Ansatz zur Pyramidisierung von Resistenzgenen wäre hilfreich, aber dieses setzt die Identifikation von Markern mit enger Kopplung zu den Resistenzgenen voraus (oder, besser, die Identifikation der kausalen Allele). Solche QTL-Identifikation hängt sehr stark von akkuraten phänotypischen Daten ab. Vor diesem Hintergrund führten wir unter kontrollierten Bedingungen eine detaillierte Durchmusterung und Phänotypisierung auf *Ascochyta*-Resistenz von 224 hoch-homozygoten Ackerbohnen-Linien durch, einschließlich der A-Satz-Linien der Göttinger Winterackerbohnenpopulation (GWBP, 188 Inzuchtlinien; cf. Ali et al., 2016). Zwei *Ascochyte fabae*-Stämme wurden als Mischung per Sprühinokulierung auf junge, getopfte Pflanzen im Gewächshaus aufgebracht, und die Pflanzen wurden visuell zu acht Zeitpunkten bonitiert, über eine Periode von 30 Tagen: auf Fiederblatt- und Stängelsymptome, also auf Anzahl und Größe (cm) der Läsionen, auf die Fiederblattfläche (%), die von Läsionen bedeckt war und auf das Vorhandensein von *Ascochyta*-Pyknidien auf Fiederblatt und Stängel. Die experimentelle Einheit war eine Pflanze. Die akkumulierten (über die acht Termine) und gemittelten (über 12 Wiederholungen) Boniturwerte pro Merkmal wurden als phänotypische Daten benutzt. Um die Validität der

Methode einzuschätzen und um Schätzwerte für h^2 für die Planung zu nutzen, wurden Vorhersagen (prospektive Erblichkeitswerte) aus den ersten beiden Wiederholungen generiert, außerdem retrospektive Erblichkeitswerte (post-experimentelle Vorhersage) aus allen 12 Wiederholungen. Auf Basis der prospektiven Vorhersagen waren 12 Wiederholungen notwendig, um eine Erblichkeit von über h^2=70% zu erreichen, was als ausreichend betrachtet wurde (der schlussendlich erreichte Wert war h^2=87%). Es wurde eine substanzielle und signifikante erbliche Variation für *Ascochyta*-Resistenz detektiert, und alle acht erfassten Merkmale waren offensichtlich genetisch korreliert. Die engste Korrelation, r=0,93**, lag zwischen der Anzahl Läsionen pro Fiederblatt und der Läsions-bedeckten Fiederblattfläche vor. Insgesamt fünf Linien (S_162-1-1-2-2, S_009-1-1-4, S_038-1-1-1-1-3-8, (Hiv/2-5 x 29H-2)-15413 und (Hiv/2-5 x 29H-2)-1-15413)) der 224 Linien zeigten eine bessere Leistung als die resistente Linien 29H. Die drei anfälligsten Linien waren Manafest-2, S_232-1-1-1-16-6 und S_060-1-7. Der A-Satz mit seinen 188 Inzuchtlinien wurde für unsere ‚geführte' genomweite Assoziationsstudie genutzt. Die GWAS war die erste genomweite Assoziationsstudie mit einem Fokus auf *Ascochyta*-Brennflecken in *Vicia faba*. Die Analysen basierten somit auf 188 Ackerbohnenlinien, zwei *Ascochyta fabae*-Stämmen, und einer Gesamtzahl von 2058 DNS-Markern (1829 AFLP-Markern und 229 SNP-Markern, eingeschlossen 17 sogenannte ‚Führungs'-SNP-Marker). Das durchschnittliche LD unter allen Markern war sehr niedrig (r^2 = 0,0075); die Markeranzahl entsprach vermutlich nicht dieser hohen genetischen Auflösung. Um diese Einschränkung abzumildern und um den Fokus zu den *Ascochyta*-Resistenzgenen zu lenken, wurde zusätzlich zur standardmäßigen genomweiten Analyse der sogenannte geführte Markeransatz gewählt. Die 17 Führungsmarker wurden per Alignment von Locus-Positionen aus den vorher publizierten Karten mit unserer verfügbaren Karte definiert. Auf diesem Wege wurden neuen der Marker als potentiell mit einem publizierten *Ascochyta*-QTL gekoppelt identifiziert. Weitere acht Führungsmarker wurden nicht direkt aus einer publizierten Karte entnommen, weil eine hohe Distanz zwischen dem publizierten QTL und den gemeinsamen Markern vorlag. Daher wurde eine lineare Regression von cM-Daten mit den Positionen von Sätzen von gemeinsamen Markern angewendet, um annäherungsweise die Position von zwei publizierten QTL-Markern in unserer verfügbaren Karte vorherzusagen. Unsere Assoziationsanlayse ist somit eine Studie in zwei Schichten: eine genomweite Assoziationsstudie mit allen, zufälligen, Markern und, darin eingeschlossen, ein geführter Ansatz, der auf 17 Führungsmarkern beruhte. Insgesamt zeigten 12

Marker, darin neun AFLP und drei SNP, eine signifikante Assoziation mit sechs Merkmalen (Läsionsanzahl pro Fiederblatt, Läsionsflächenanteil pro Fiederblatt, Läsionslänge der größten Läsion auf Fiederblatt, auf Stängel, Vorkommen von Pyknidien auf Fiederblatt, am Stängel; neun dieser Marker stehen vermutlich für neue Resistenzgene. Signifikante DNS-Marker wurden auf den Chromosomen I, III, IV, V und VI in den Nachkommen der Göttinger Winteracker-bohnen-Population gefunden. Der geführte Ansatz war erfolgreich: einer der 17 Führungsmarker (Vf-Mt1g014230-001) war signifikant und die Hypothese steht, dass dieser SNP auf Chromosom III den früher berichteten QTL validiert (Af1, Chromosom III). Der Ansatz mit geführten Marker war erfolgreich in einer Art, für die keine Genomsequenz verfügbar war.

Angewandte markergestützte Auslese auf *Ascochyta*-Resistenz beruht stark auf dem Transfer von genetischen Ergebnissen zwischen verschiedenen Ackerbohnen-Populationen, was von weiterer Marker-Sättigung von QTL-führenden Chromosomenregionen abhängt. Die neuen, mutmaßlichen Resistenzloci in der Göttinger Winterackerbohnen-Population empfiehlt diese Population als mögliche Quelle für Resistenzzüchtung.

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Curriculum vitae of

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Biographical

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Education

2008-2012	:	B.Sc.(hons) in Major Plant Breeding and Genetics, University of Agriculture Faisalabad, Pakistan
2012-2014	:	M.Sc. (hons) in Plant Breeding and Genetics, University of Agriculture, Faisalabad. ***Title thesis*** Combining ability analysis of some morphological traits in Sunflower (*Helianthus annuus* L.)
2016-2022	:	PhD Program for Agricultural Sciences in Goettingen (PAG), Division of Plant Breeding, University of Göttingen, Germany. ***Title thesis*** Genetic analyses of the resistance of faba beans (*Vicia faba*) to the fungus *Ascochyta fabae*

Academic records

26.3.2012-15.6.2012	:	Internship training on "Chickpea germ plasm pool" at "AYYUB Agricultural Research Institute, Faisalabad
2015-Now (was on leave for the period of Oct. 2016-Aug. 2021)	:	Lecturer, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad, Pakistan

Academic Awards

2008-2009 :	Merit Scholarship by University of Agriculture Faisalabad on academic performance during studies in B.Sc. (hons) Agriculture Sciences.
2016-2020 :	50 overseas scholarships program for faculty development sponsored by University of Agriculture, Faisalabad for PhD studies in University of Goettingen, Germany

Extracurricular Awards

2014-2015 :	Appointed as Global youth ambassador at 'A World at School' campaign by United Nations.

22 Sep. 2014	:	Received a 'Youth Icon Award' and delivered a key note speech on 'Women Empowerment' in a side line program of 69th general assembly session, United Nations, New York, America

Attended workshops and training courses

01-03.06.2015	:	Workshop titled 'Active citizens Program in Universities', University of Engineering and Technology, Lahore, Pakistan
22-26.09.2017	:	Training course on Linear Statistical Models with R, University of Göttingen, Germany.
09-13.10.2017	:	Training course on Basic Laboratory Techniques, University of Göttingen, Germany
21-25.06.2018	:	Training course on Biotechnological Applications in Plant Breeding, University of Göttingen, Germany
23-25.11.2021	:	Professional development Workshop for young faculty. University of Agriculture, Faisalabad.

Attended conferences

13-17.July 2015	:	Global initiative symposium 'Individual to influential' National Taiwan University, Taipei, Taiwan
3-7.September 2017	:	4th International Symposium on Genomics of Plant Genetic Resources. Poster No.14, Giessen, Germany.
28.02-02.03.2018	:	German Plant Breeding Conference, Poster No.74, HKK Hotel, Wernigerode Germany.

Experiences

- *Methodology*: Plant breeding methods, DNA isolation, Gel documentation, Genetic engineering, transformation, Cytogenetic techniques, PCR, Electrophoresis, Molecular markers, Association analysis, Phenotyping techniques. Statistical analyses

- *Softwares:* PLABSTAT, PLABplan, R statistics, TASSEL 3.0, Mapmaker 3.0

Publications:

2015 : Sharmin, A., Ihsan, K., Qasrani, S. A., Sofia, J., Rabia, F., Ahamed, H. M. & Ahmad, R. (2015). Genetics of yield components for drought tolerant wheat (*Triticum aestivum* L.) genotypes. International Journal of Biosciences (IJB), 6(12), 21-34

2016 : Ali, Z., Sarwat, S. S., Karim, I., Faridi, R., Jaskani, M. J., & Khan, A. A. (2016). Functions of plant's bZIP transcription factors. Pakistan Journal of Agricultural Sciences, 53(2)

2017 : Xu, X., Song, W., Jinping, D., Ali, Z., Faridi, R., & Shao, H. (2017). Biochemical evaluation for weak-light tolerance of various tomato (*Solanum lycopersicum* L.) cultivars in China. Pak. J. Bot, 49(3), 913-919

2021 : Faridi, R., Koopman, B., Schierholt, A., Ali, M. B., Apel, S., & Link, W. (2021). Genetic study of the resistance of faba bean (*Vicia faba*) against the fungus *Ascochyta fabae* through a genome-wide association analysis. Plant Breeding, 140, 442-452

Field of study:

Molecular plant breeding, Genetics, and Cytogenetics

Language:

- Punjabi (mother language)
- Urdu
- English
- German (less proficient)