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(54) **INCREASED FUNGAL RESISTANCE IN CROP PLANTS**

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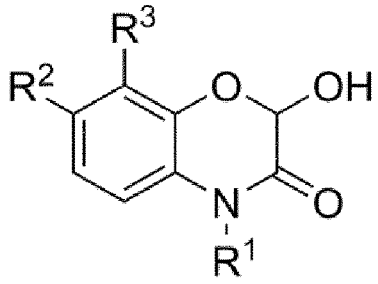
(57) **ABSTRACT**

The present invention relates to methods for producing plants with increased fungal resistance, preferably seedling resistance against Northern Corn Leaf Blight. Further provided are methods for introducing, modifying, or modulating at least one wall-associated kinase (WAK) in(to) a plant cell, tissue, organ, or whole plant and thereby causing a reduced synthesis of benzoxazinoid and in turn increased fungal resistance. There are further provided methods to identify and/or modify downstream effector molecules in a WAK signalling cascade. Finally, plant cells, tissues, organs or whole plants having increased fungal resistance and methods using substances to activate signalling pathways in a targeted way are provided. The present invention thus relates to WAKs as master regulators and crucial signaling mediators in plant defense against fungal disease and the regulation and cross-talk mechanisms in the WAK signaling cascade and further gives examples for establishing novel anti-fungal strategies relevant for a series of crop plants.

Specification includes a Sequence Listing.

A)



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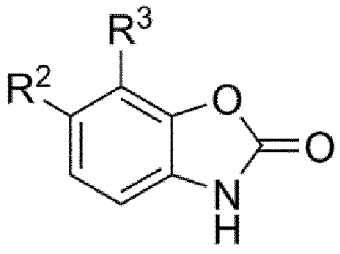
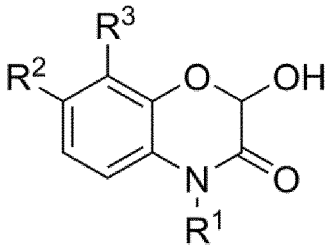


Figure 1

A)



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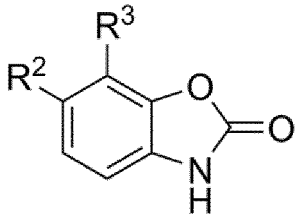

Figure 2

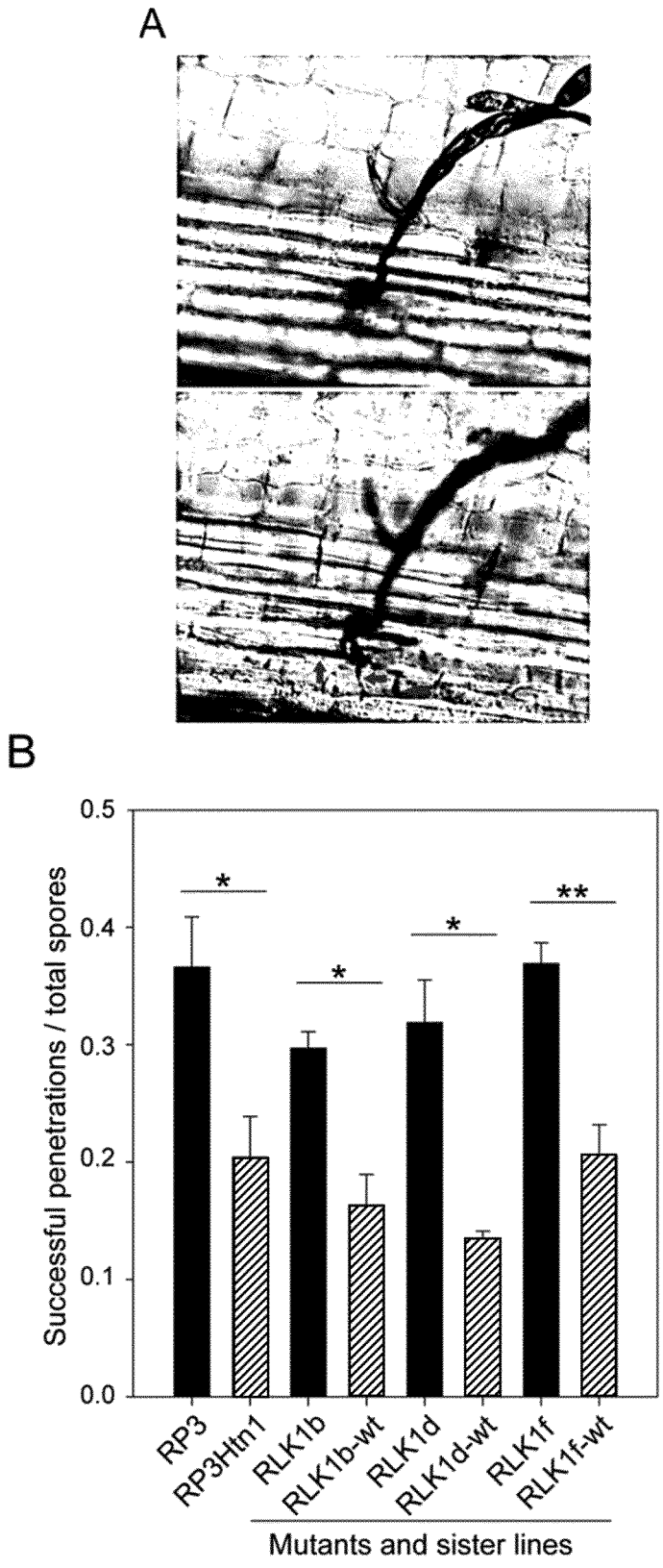
A
B
Successful penetrations / total spores
0.0
0.1
0.2
0.3
0.4
0.5
*
*
*
**
RP3
RP3Htn1
RLK1b
RLK1b-wt
RLK1d
RLK1d-wt
RLK1f
RLK1f-wt
Mutants and sister lines

Figure 3

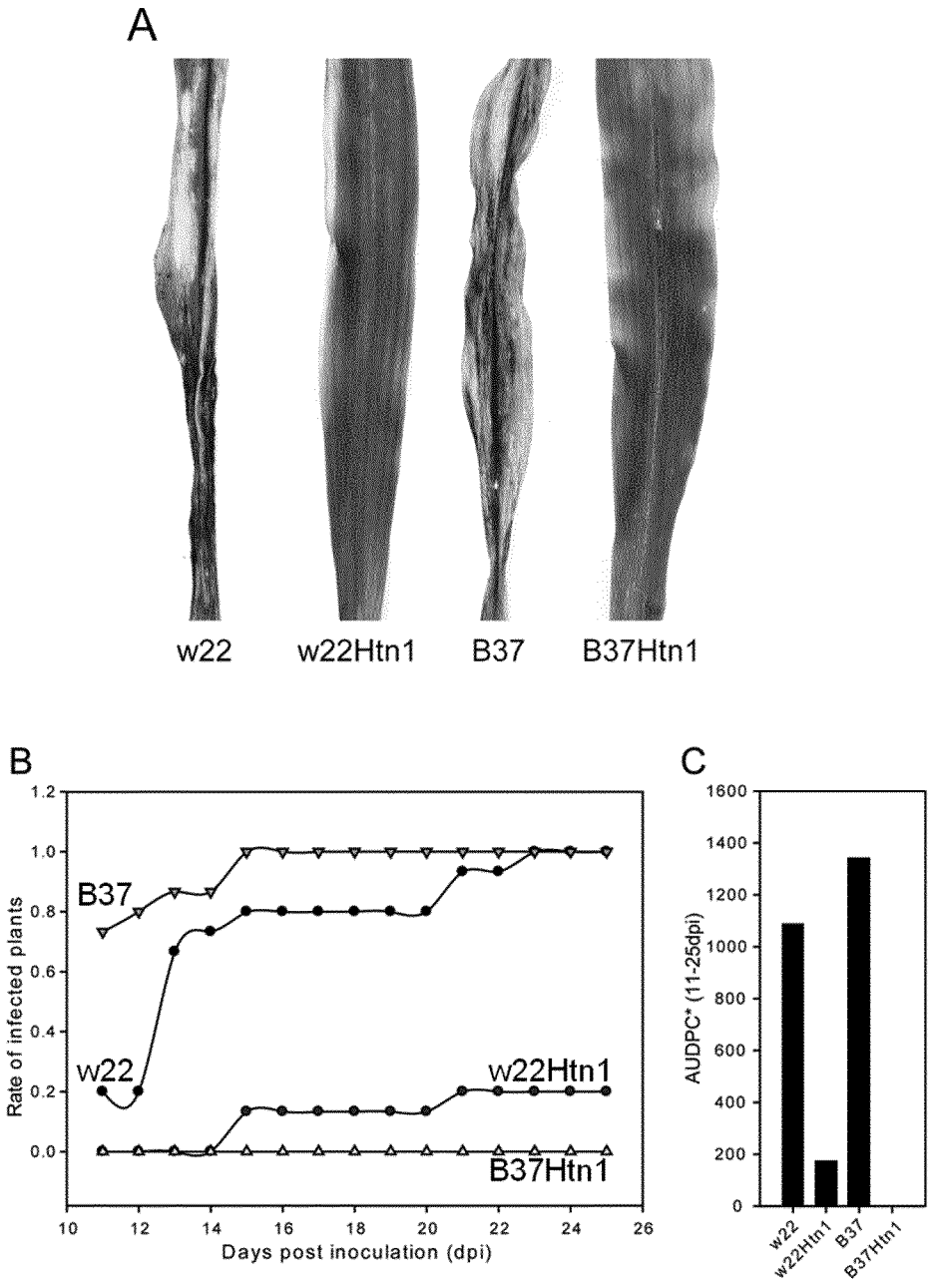
A
w22
w22Htn1
B37
B37Htn1
B
Rate of infected plants
Days post inoculation (dpi)
B37
w22
w22Htn1
B37Htn1
C
AUDPC* (11-25dpi)
w22
w22Htn1
B37
B37Htn1

Figure 4

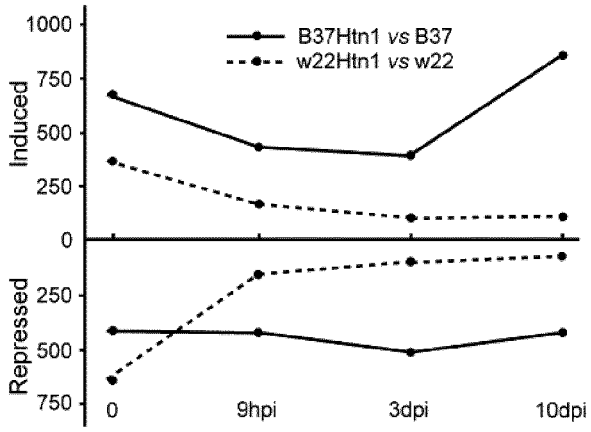
B37Htn1 vs B37
w22Htn1 vs w22
Induced
Repressed
1000
750
500
250
0
250
500
750
0
9hpi
3dpi
10dpi

Figure 5

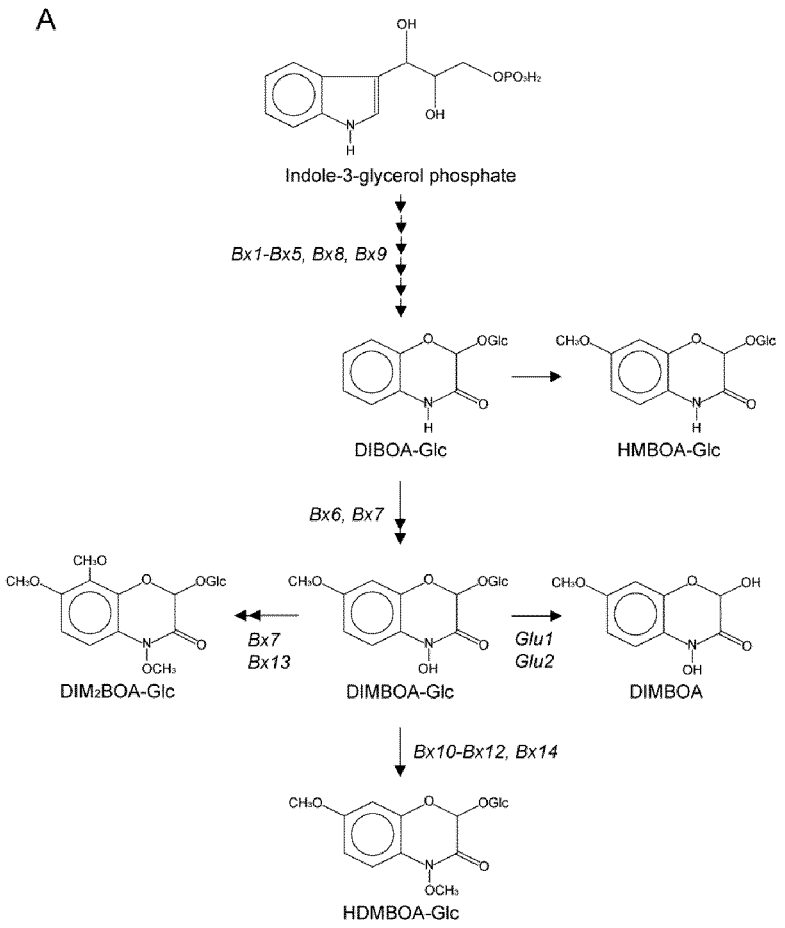
A
Indole-3-glycerol phosphate
Bx1-Bx5, Bx8, Bx9
DIBOA-Glc
HMBOA-Glc
Bx6, Bx7
DIM2BOA-Glc
Bx7
Bx13
DIMBOA-Glc
Glu1
Glu2
DIMBOA
Bx10-Bx12, Bx14
HDMBOA-Glc

Figure 5 continued

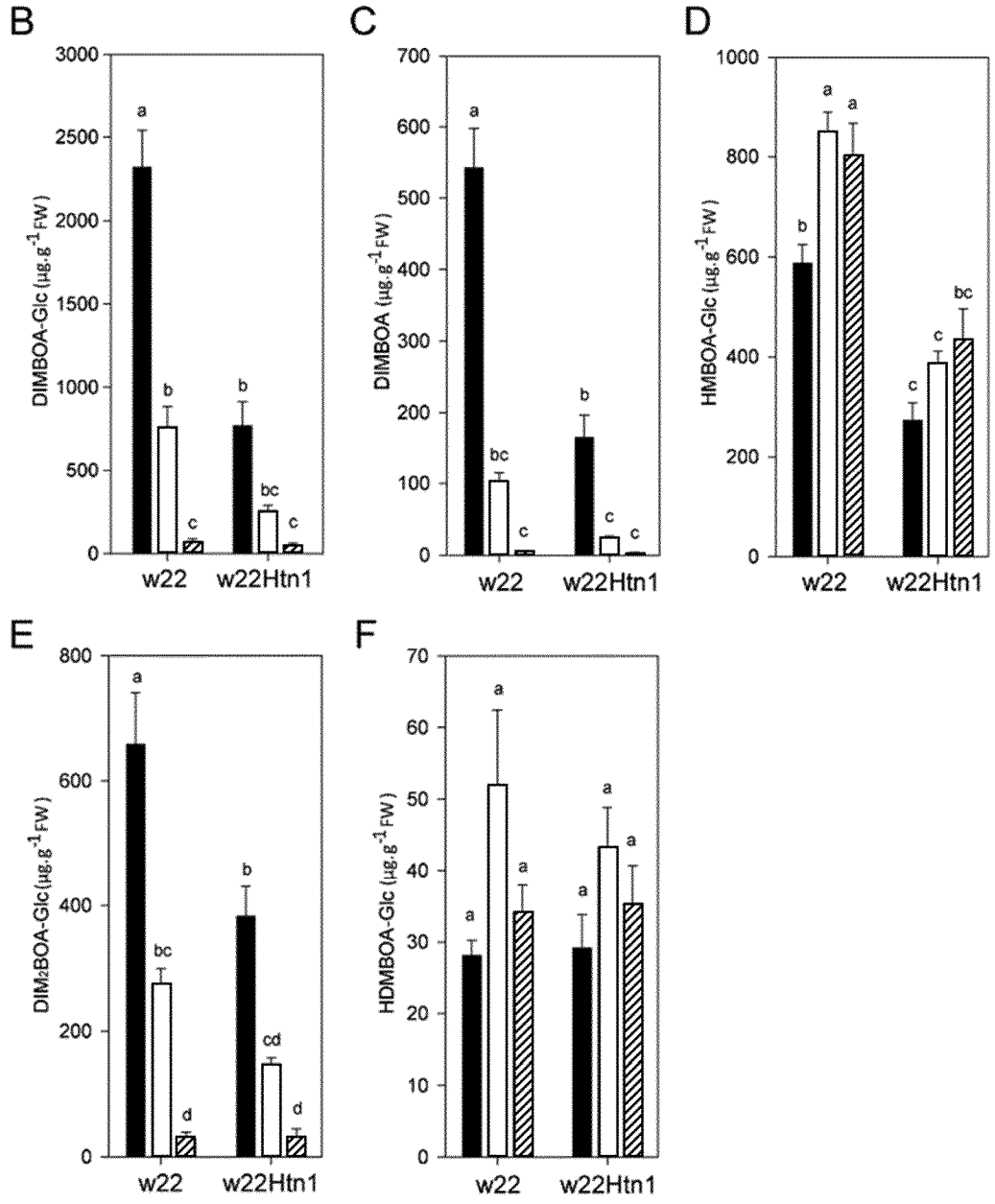

Figure 6

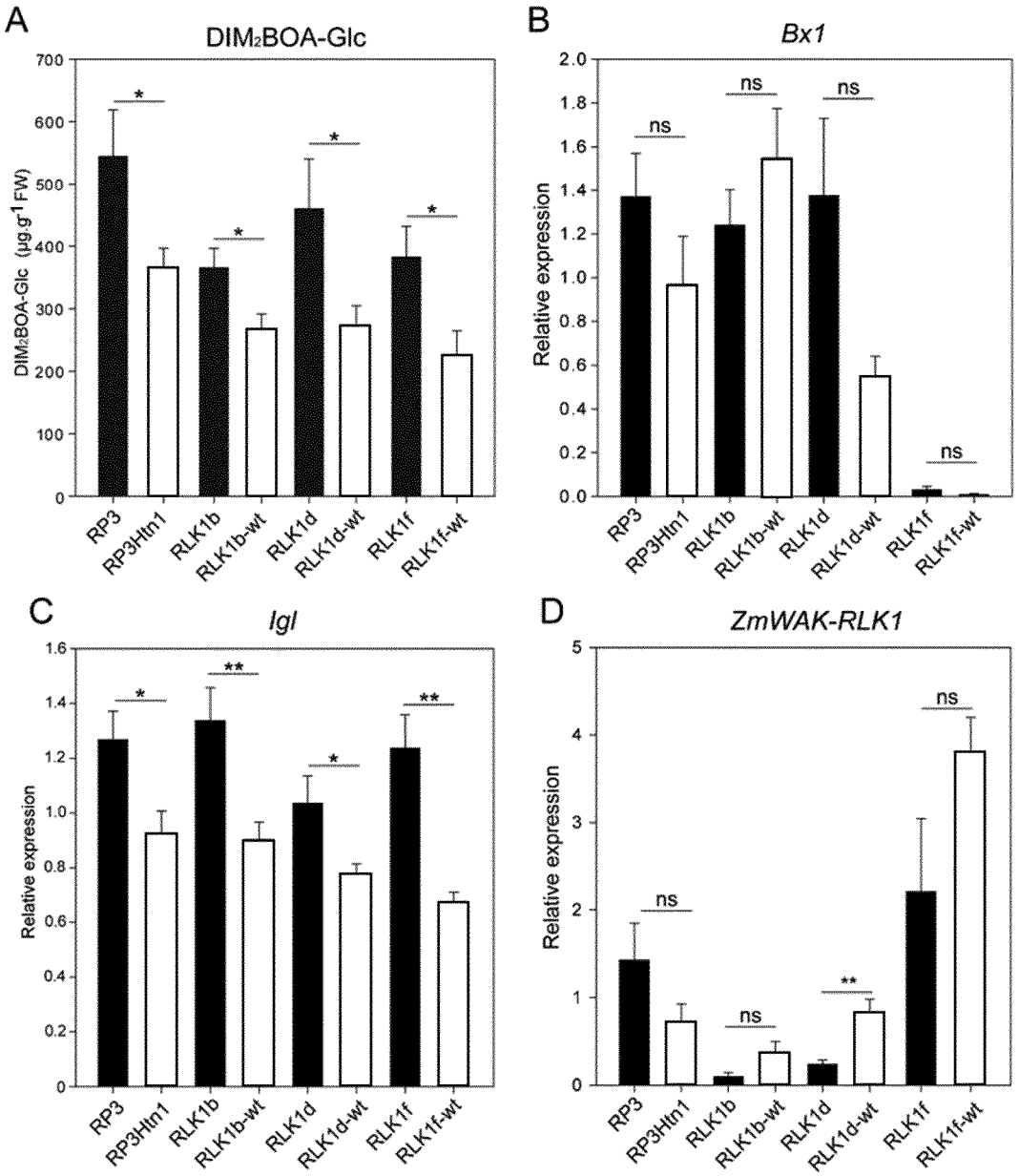
A
DIM2BOA-Glc
DIM2BOA-Glc (μg.g-1 FW)
700
600
500
400
300
200
100
0
*
*
*
RP3
RP3Htn1
RLK1b
RLK1b-wt
RLK1d
RLK1d-wt
RLK1f
RLK1f-wt
B
Bx1
Relative expression
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ns
ns
ns
ns
C
Igl
Relative expression
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0
*
**
*
**
D
ZmWAK-RLK1
Relative expression
5
4
3
2
1
0
ns
ns
**
ns

Figure 7

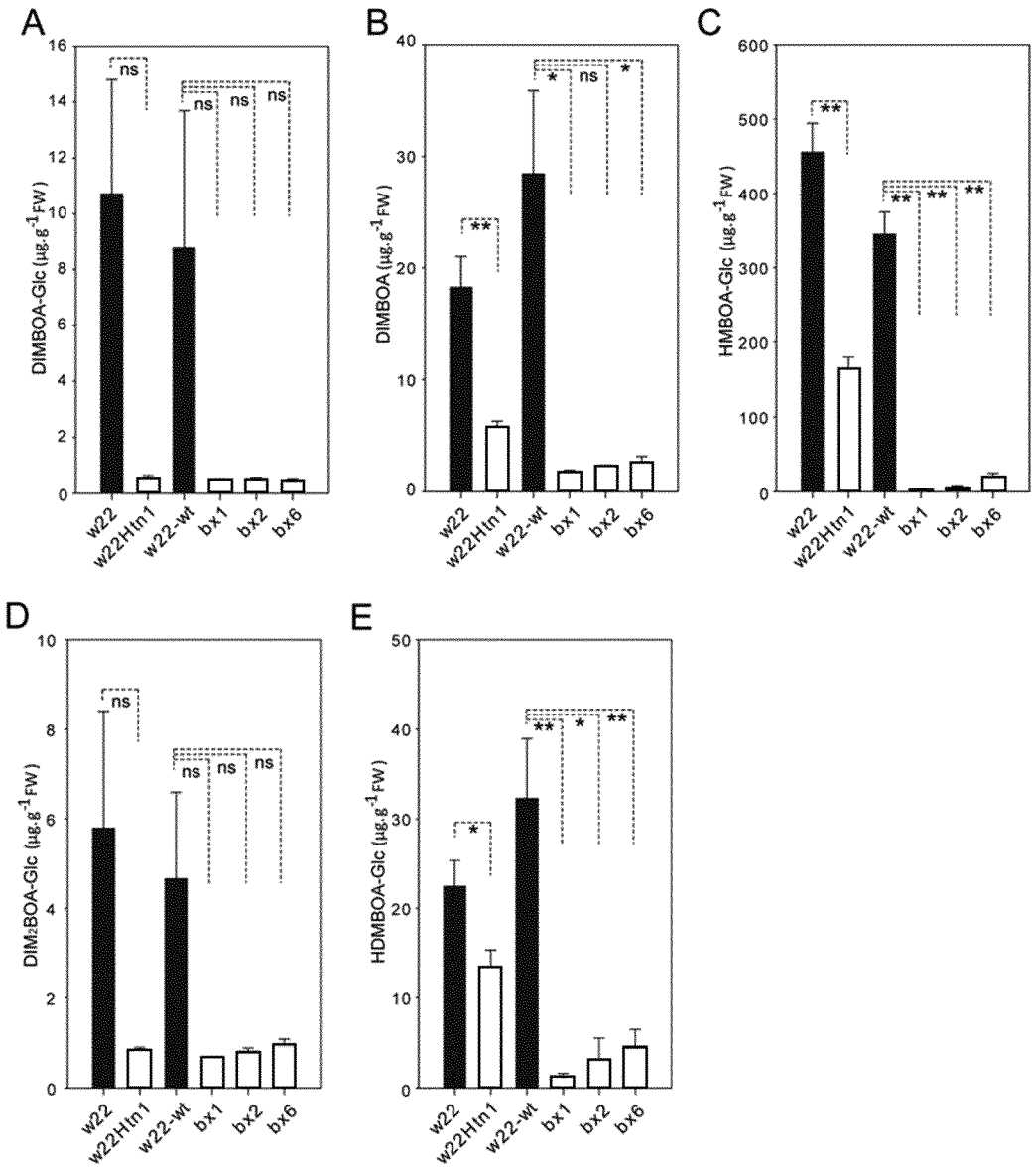
A
DIMBOA-Glc (µg.g-1FW)
ns
ns
ns
ns
w22
w22Htn1
w22-wt
bx1
bx2
bx6
B
DIMBOA (µg.g-1FW)
**
*
ns
*
C
HMBOA-Glc (µg.g-1FW)
**
**
**
**
D
DIM2BOA-Glc (µg.g-1FW)
E
HDMBOA-Glc (µg.g-1FW)

Figure 8

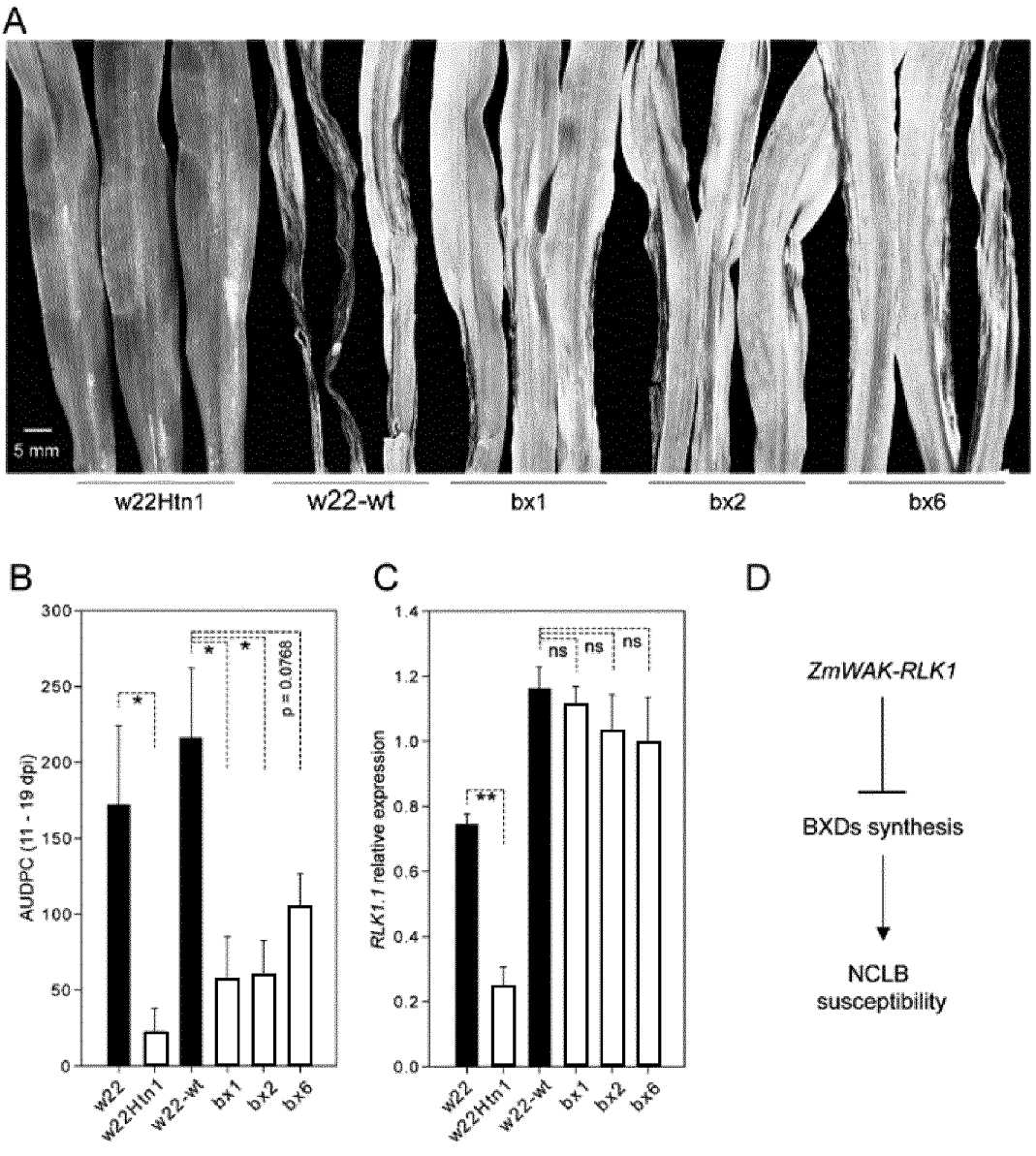
A
5 mm
w22Htn1
w22-wt
bx1
bx2
bx6
B
AUDPC (11 - 19 dpi)
p = 0.0766
w22
w22Htn1
w22-wt
bx1
bx2
bx6
C
RLK1.1 relative expression
ns
D
ZmWAK-RLK1
BXDs synthesis
NCLB
susceptibility

Figure 9

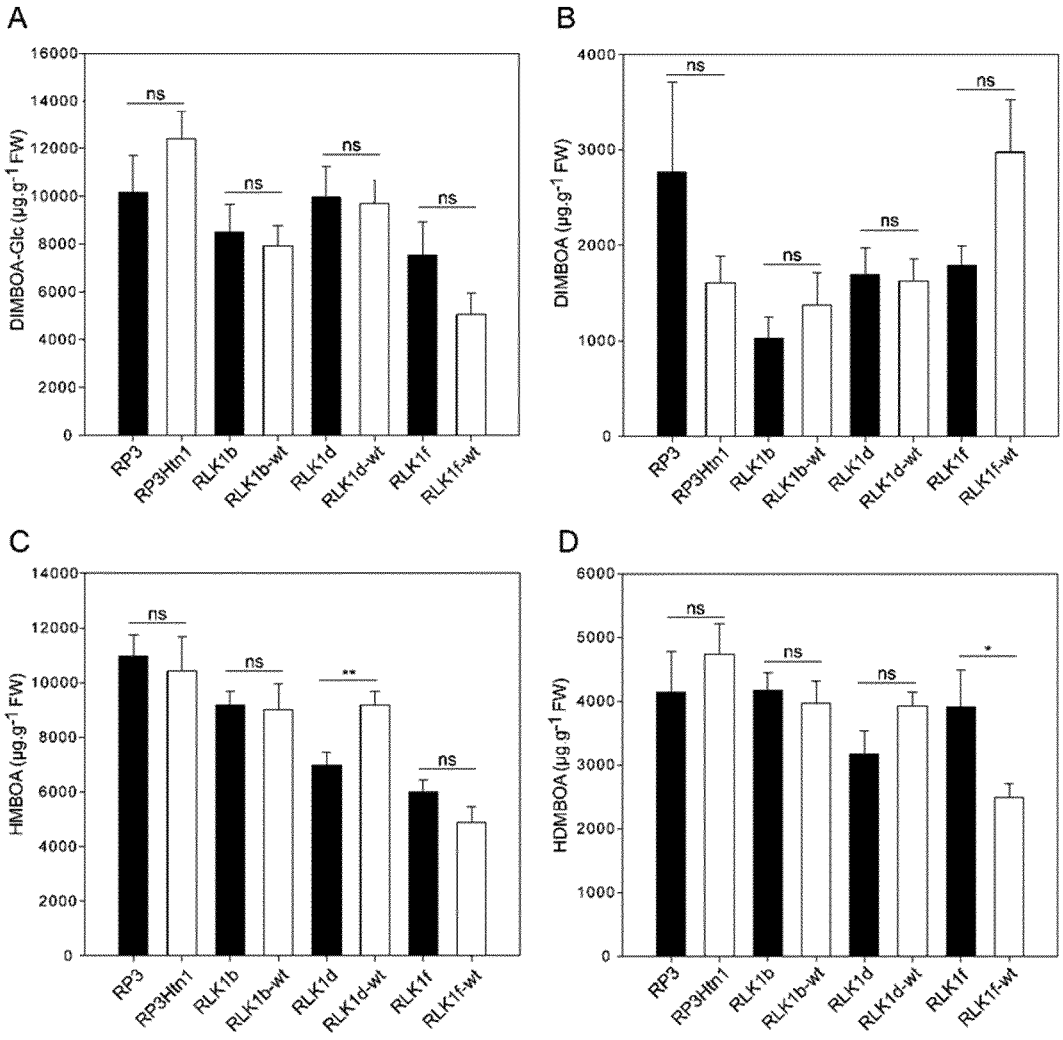
A
DIMBOA-Glc (µg.g-1 FW)
16000
14000
12000
10000
8000
6000
4000
2000
0
ns
RP3
RP3Htn1
RLK1b
RLK1b-wt
RLK1d
RLK1d-wt
RLK1f
RLK1f-wt
B
DIMBOA (µg.g-1 FW)
4000
3000
2000
1000
0
C
HMBOA (µg.g-1 FW)
14000
12000
10000
8000
6000
4000
2000
0
**
D
HDMBOA (µg.g-1 FW)
6000
5000
4000
3000
2000
1000
0
*

Figure 10

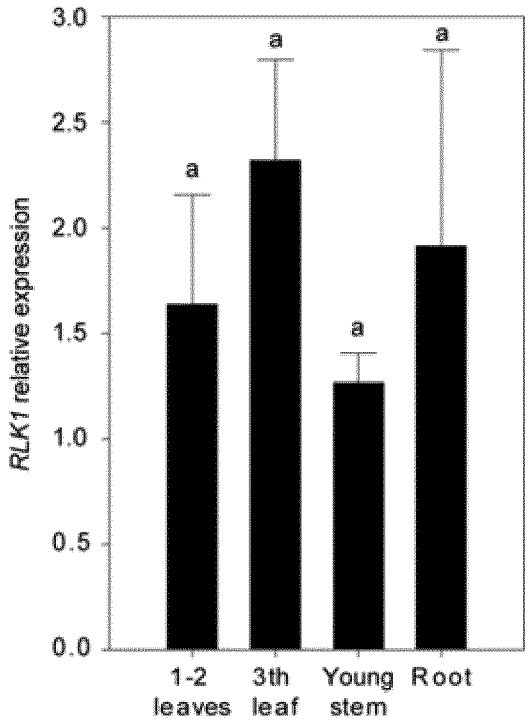
3.0
2.5
2.0
1.5
1.0
0.5
0.0
RLK1 relative expression
a
a
a
a
1-2 leaves
3th leaf
Young stem
Root

Figure 11

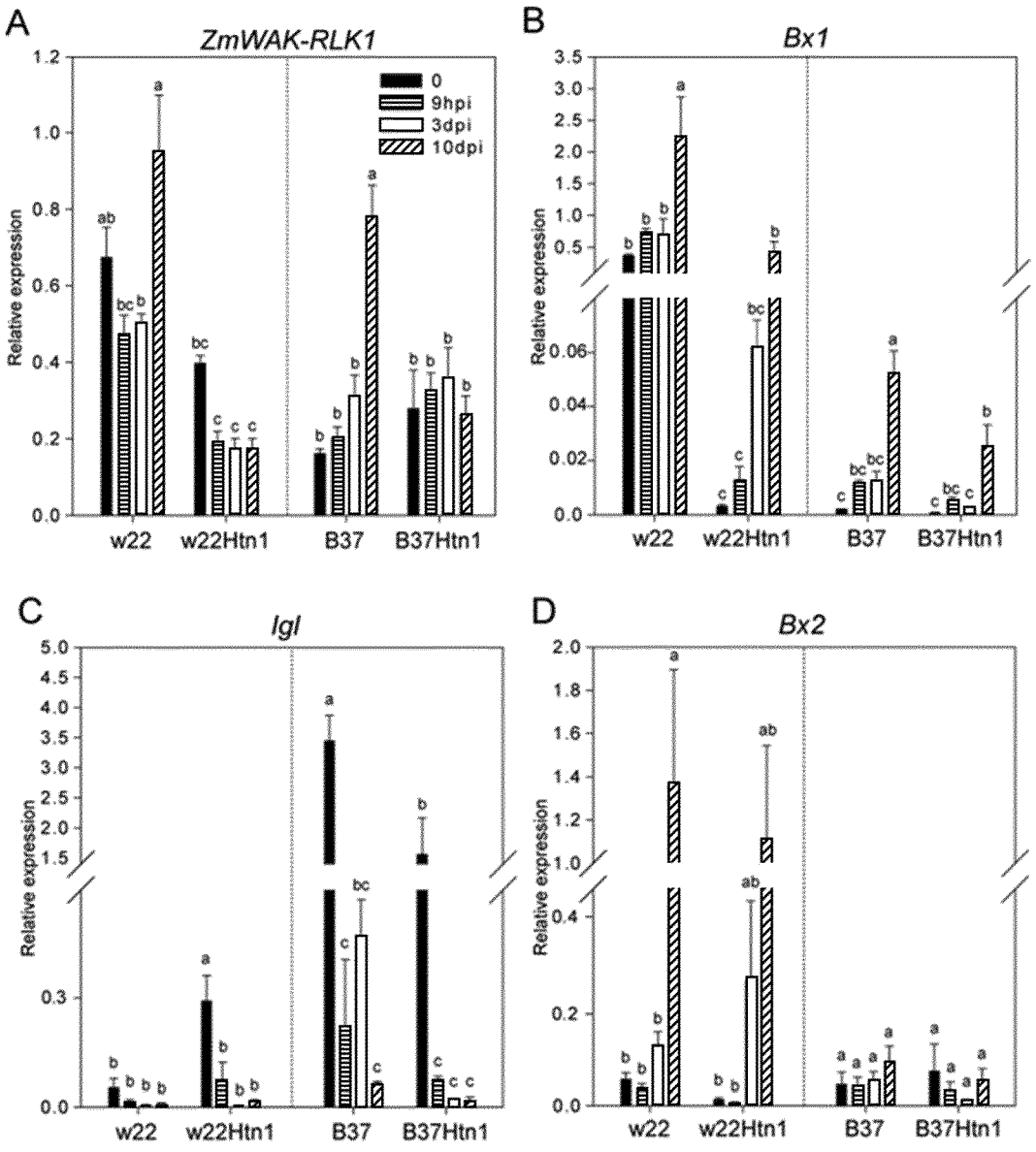
A
ZmWAK-RLK1
B
Bx1
C
Igl
D
Bx2
0
9hpi
3dpi
10dpi
Relative expression
w22
w22Htn1
B37
B37Htn1

Figure 11 continued

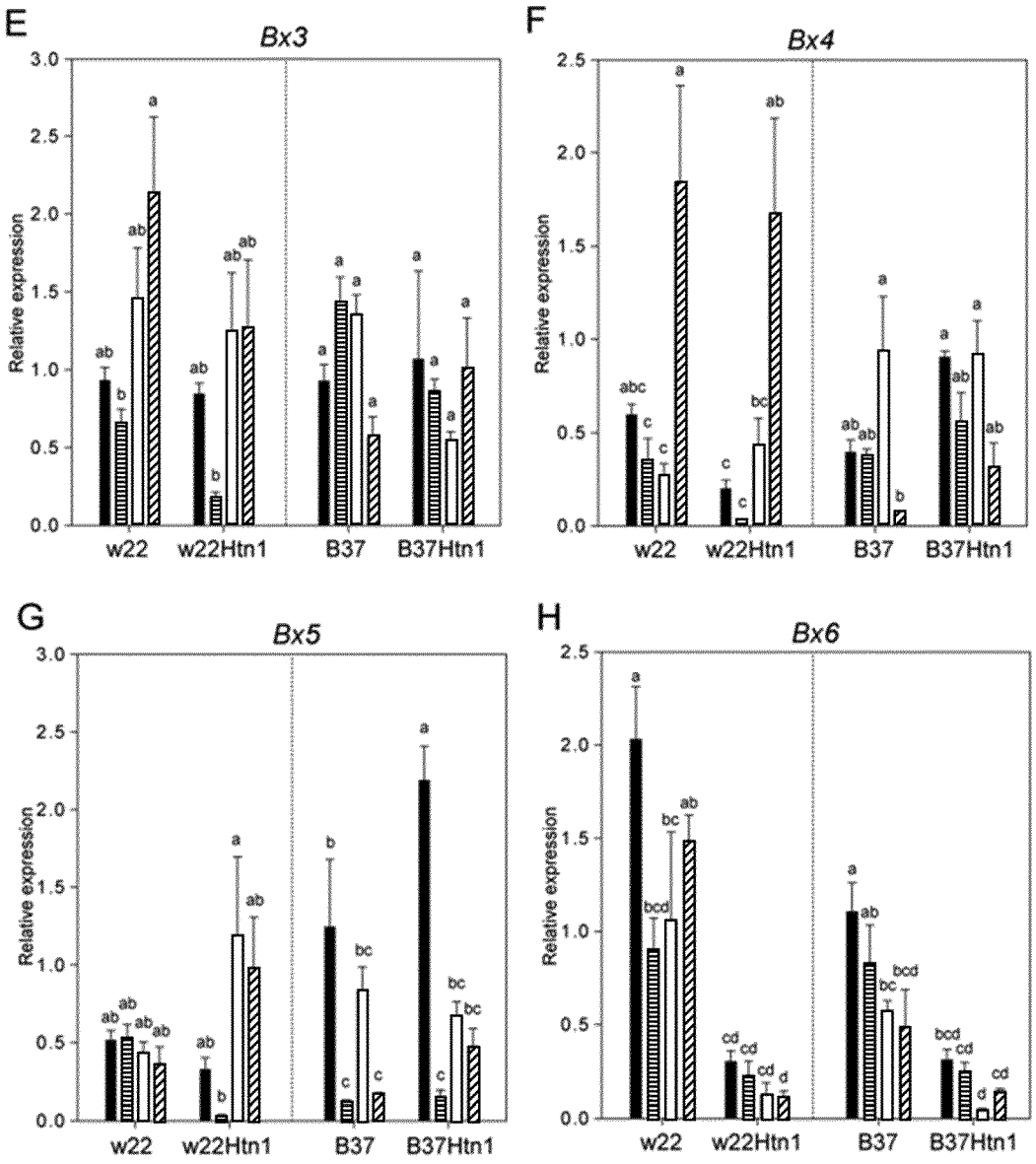

Figure 11 continued

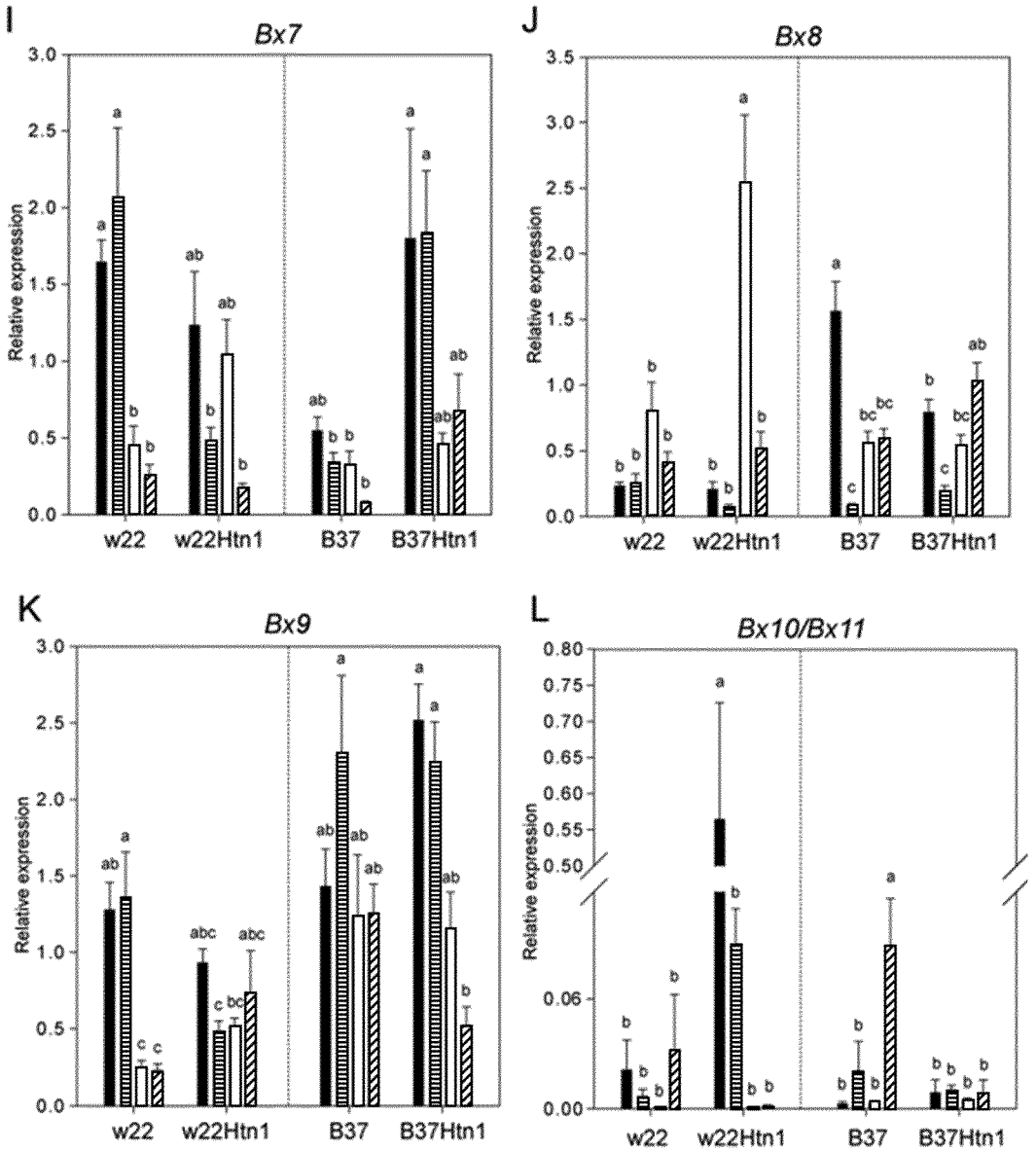

Figure 11 continued

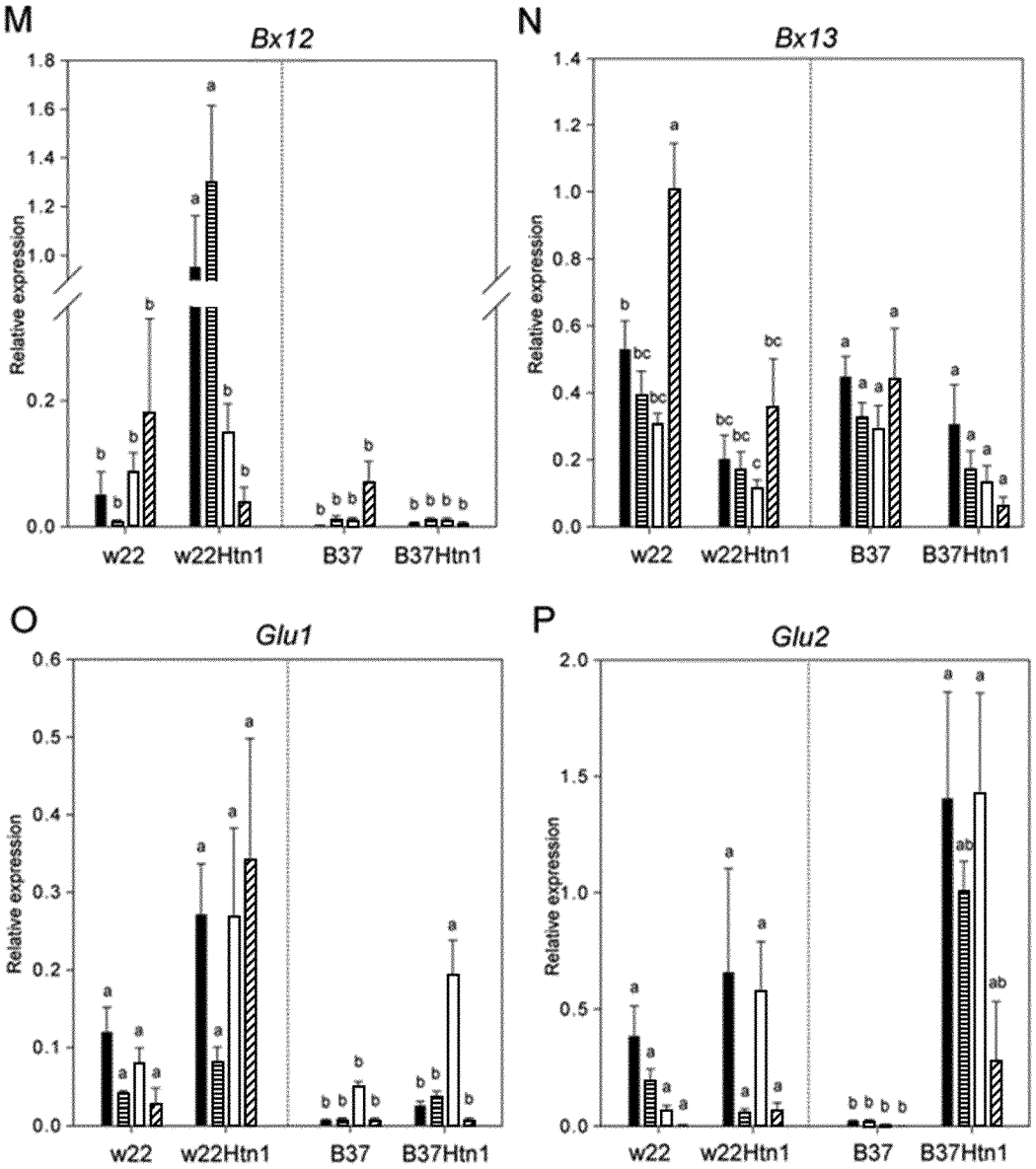

Figure 12

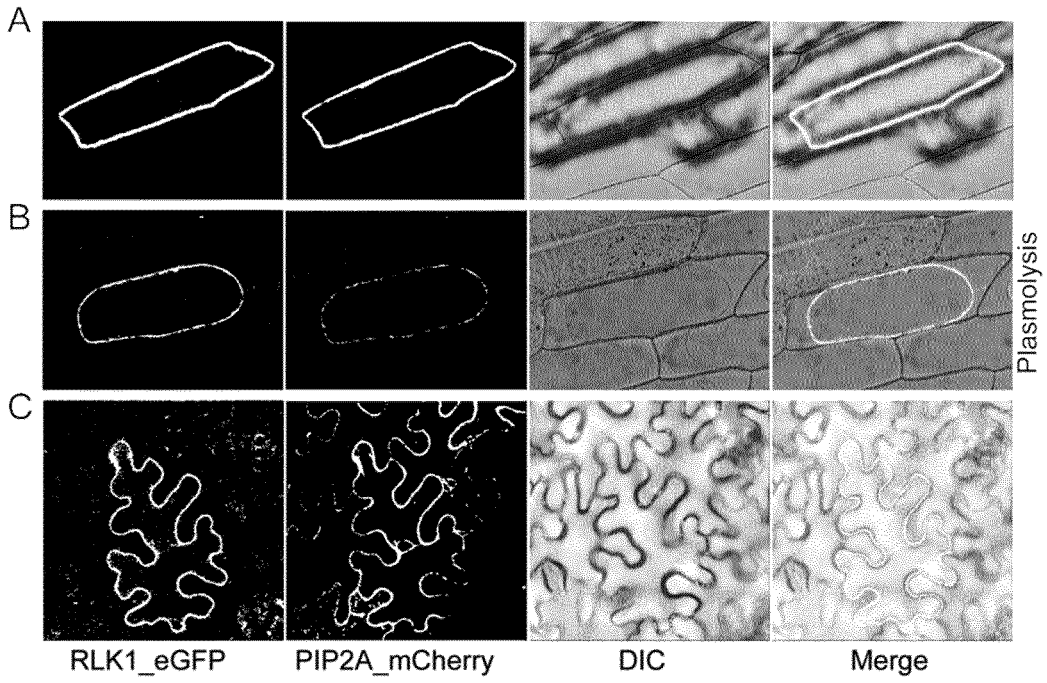

Figure 13

Number	DEGs	0		9 hpi		3 dpi		10 dpi		Annotation	Best hit in rice	Category
		W22Htn1 vs. W22	B37Htn1 vs. B37	W22Htn1 vs. W22	B37Htn1 vs. B37	W22Htn1 vs. W22	B37Htn1 vs. B37	W22Htn1 vs. W22	B37Htn1 vs. B37			
1	GRMZM2G085661			-6,35	-5,03					BX2 -- Benzoxazinone systhesis 2	LOC_Os03g58300.1	Benzoxazinoids pathway
2	GRMZM2G015892			3,96	4,52					Indole-3-glycerol phosphate lyase chloroplast precursor	LOC_Os03g58300.1	Benzoxazinoids pathway
3	GRMZM6G617209					-2,06	-2,13			BX6 - Benzoxazinone synthesis 6	LOC_Os03g48430.1	Benzoxazinoids pathway
4	GRMZM2G336824	11,02	4,32							BX11 - Benzoxazinone synthesis 11	LOC_Os12g25870.1	Benzoxazinoids pathway
5	GRMZM2G127418	6,93	5,84							BX14 - Benzoxazinone Synthesis 14	LOC_Os05g43940.1	Benzoxazinoids pathway
6	GRMZM2G000236			4,37	4,57					OPR2 - 12-oxo-phytodienoic acid reductase2	LOC_Os06g11290.1	JAs pathway
7	GRMZM2G109130	4,41	4,28							LOX3 - Lipoxygenase 3	LOC_Os03g49350.1	JAs pathway
8	GRMZM2G077316			-4,95	-2,51			-4,44	-3,91	AOC1 - Allene oxide cyclase 1	LOC_Os03g32314.1	JAs pathway
9	GRMZM2G166616	4,90	4,45							ACCO3 - 1-aminocyclopropane-1-carboxylate oxidase 3	LOC_Os05g05680.1	Ethylene pathways
10	GRMZM2G332423	6,46	3,88							ACCO4 - 1-aminocyclopropane-1-carboxylate oxidase 4	LOC_Os05g05680.1	Ethylene pathways
11	GRMZM2G054123	3,90	2,38							S-adenosylmethionine synthase	LOC_Os01g22010.1	Lignin pathway
12	GRMZM2G099363	2,72	3,19							OMT2 - Caffeoyl-CoA O-methyltransferase 2	LOC_Os06g06980.1	Lignin pathway
13	GRMZM2G127948	2,53	3,02							OMT1 - Caffeoyl-CoA O-methyltransferase 1	LOC_Os06g06980.1	Lignin pathway
14	AC209784.3_FG007			2,06	2,65					*DnaK family protein putative expressed	LOC_Os05g38530.1	Defense
15	GRMZM2G032856	2,68	2,11							Glutathione S-transferase GST 24	LOC_Os01g72150.1	Defense
16	GRMZM2G042639	6,65	6,69	3,03	4,75	2,71	3,78			SAF1 - Safener induced 1; Glutathione S-transferase	LOC_Os03g17480.1	Defense
17	GRMZM2G044383					3,47	2,55			Glutathione S-transferase GST 30	LOC_Os10g38740.1	Defense
18	GRMZM2G065585	3,32	4,23							GEB1 - Glucan endo-1,3-beta-glucosidase homolog 1	LOC_Os01g71340.1	Defense
19	GRMZM2G074743	5,92	5,39							AOX3 - Alternative Oxidase 3	LOC_Os04g57320.1	Defense
20	GRMZM2G145461	3,48	3,04			2,52	3,10			CHN2 - Chitinase 2	LOC_Os03g30470.1	Defense
21	GRMZM2G156632	3,91	3,79							WIP1 - wound induced protein1	LOC_Os01g04050.1	Defense
22	GRMZM2G162505	2,03	2,33							*Chitinase 2 putative expressed	LOC_Os10g28050.1	Defense
23	GRMZM2G150762			-5,63	-2,95					HIR2 - hypersensitive induced response 2	LOC_Os09g19710.1	Defense
24	GRMZM2G157869	-5,70	-3,88	-11,93	-9,71	-5,17	-3,41	-5,43	-6,05	*Hypersensitive-induced response protein putative expressed	LOC_Os09g19710.1	Defense
25	GRMZM2G063392	6,33	12,71							TKL_IRAK_DUF26-la.6 - DUF26 kinases	LOC_Os10g04730.1	Receptor like kinase
26	GRMZM2G303909	2,15	4,45							*Receptor-like protein kinase, putative expressed	LOC_Os04g12600.1	Receptor like kinase
27	GRMZM2G433684	4,68	12,64	5,67	13,12	5,00	11,08	3,80	11,24	*LRR receptor kinase putative expressed	LOC_Os02g40240.1	Receptor like kinase
28	GRMZM2G438871	2,70	3,94							*Lectin-like receptor kinase putative expressed	LOC_Os07g18240.1	Receptor like kinase
29	GRMZM2G466298	2,46	3,23							*Lectin-like protein kinase putative expressed	LOC_Os02g48210.1	Receptor like kinase
30	GRMZM2G009770	-5,50	-6,84							*receptor-like protein kinase precursor putative expressed	LOC_Os02g12400.1	Receptor like kinase

Figure 13 continued

31	GRMZM2G165387	-6,70	-3,46	-7,67	-3,20	-5,95	-2,59	-5,12	-2,61	*Receptor-like protein kinase 5 precursor	LOC_Os02g40180.1	Receptor like kinase
32	GRMZM2G436455	-6,82	-8,20	-4,40	-9,40	-2,71	-9,96	-2,76	-10,80	*Resistance-related receptor-like kinase putative expressed	LOC_Os01g02810.1	Receptor like kinase
33	GRMZM2G089836	3,28	3,40			2,98	5,03			Beta-fructofuranosidase 1; IVR2 - invertase 2	LOC_Os02g01590.1	Cell wall
34	GRMZM2G123107	3,17	4,69							Glycosyl hydrolases family 17 putative expressed	LOC_Os01g71410.1	Cell wall
35	GRMZM2G125032	2,89	5,88							Beta-1,3-glucanase	LOC_Os01g71670.1	Cell wall
36	GRMZM2G162359	4,34	2,46							*Glycosyl hydrolase putative expressed	LOC_Os06g25010.1	Cell wall
37	GRMZM2G135966	-2,72	-11,17	-3,90	-8,50	-5,99	-10,22			*Glycosyl hydrolase family 47 domain contain protein expressed	LOC_Os04g51690.1	Cell wall
38	AC214360.3_FG001			3,58	3,10					KS4 - Kaurene Synthase 4	LOC_Os02g36220.1	Secondary metabolism
39	GRMZM2G067225	2,06	3,84							Putative cytochrome P450 superfamily protein	LOC_Os03g12500.1	Secondary metabolism
40	GRMZM2G070304	8,75	5,70							Glycerol-3-phosphate acyltransferase putative expressed	LOC_Os11g45400.1	Secondary metabolism
41	GRMZM2G087875	2,96	3,84							Putative cytochrome P450 superfamily protein	LOC_Os03g55240.1	Secondary metabolism
42	GRMZM2G106950	3,11	3,13							Indole-3-glycerol phosphate synthase chloroplast precursor	LOC_Os09g08130.2	Secondary metabolism
43	GRMZM2G117878	3,35	4,60							*Cytokinin-N-glucosyltransferase putative expressed	LOC_Os07g13800.1	Secondary metabolism
44	GRMZM2G140817	3,13	2,92							Amino acid permease	LOC_Os04g35540.1	Secondary metabolism
45	GRMZM2G145242	2,36	2,79							Putative cytochrome P450 superfamily protein	LOC_Os05g41440.1	Secondary metabolism
46	GRMZM2G146206					-2,46	-2,81	-2,37	-5,28	TPI5 - triose phosphate isomerase 5	LOC_Os01g62420.4	Secondary metabolism
47	GRMZM2G132550	4,15	4,52							bHLH124 - bHLH-transcription factor 124	LOC_Os01g09990.1	Transcription factors
48	GRMZM2G395749	3,30	2,79							Putative MYB DNA-binding domain superfamily protein	LOC_Os01g09760.1	Transcription factors
49	GRMZM2G421256	2,11	2,03							MYB20 - MYB-related-transcription factor 20	LOC_Os04g49450.1	Transcription factors
50	GRMZM2G425430			5,64	4,38	6,06	3,86	4,81	4,84	*WRKY81	LOC_Os03g33012.1	Transcription factors
51	AC193786.3_FG005			-3,92	-3,17					bHLH54 - bHLH-transcription factor 54	LOC_Os01g72370.1	Transcription factors
52	GRMZM2G305901	-6,40	-11,29							Zinc finger C3HC4 type domain containing protein expressed	LOC_Os01g06590.1	Transcription factors
53	GRMZM2G326783	2,24	2,74	3,30	2,22	2,49	2,52	2,33	3,45	THXTrihelix-transcription factor 29	LOC_Os02g33610.1	Transcription factors
54	GRMZM2G016150	-4,86	-3,06							OHP1 - Opaque2 Heterodimerizing Protein1	LOC_Os03g58250.1	
55	AC196502.3_FG003	8,65	4,81							*Proline-rich family protein putative expressed	LOC_Os07g44830.1	
56	AC206788.3_FG015	2,19	2,23							*Anthocyanidin 5 3-O-glucosyltransferase putative expressed	LOC_Os05g45100.1	
57	GRMZM2G002173	3,25	5,27	2,88	3,96	2,88	2,82	5,22	3,31	Phosphopantothenate cysteine ligase	LOC_Os02g36550.1	
58	GRMZM2G004349	3,10	2,15							Seed maturation protein	LOC_Os05g45050.2	
59	GRMZM2G006219	3,38	2,54			3,82	2,88			*Major sperm protein (MSP) domain containing protein	LOC_Os02g42940.1	
60	GRMZM2G006878	4,86	3,69	5,70	4,37	5,86	2,78			Expressed protein	LOC_Os03g63870.1	
61	GRMZM2G009045	5,11	3,95	3,04	5,89					Phosphate transporter 3;2	LOC_Os09g28160.1	
62	GRMZM2G010048	5,55	2,92							Thaumatin family domain containing protein expressed	LOC_Os03g46060.1	
63	GRMZM2G010596	2,97	2,20							3-methyl-2-oxobutanoate hydroxymethyltransferase	LOC_Os01g12560.1	
64	GRMZM2G011253	3,33	2,10							60S ribosomal protein L31	LOC_Os08g39500.1	
65	GRMZM2G011523	3,41	4,92							BBT111 - Bowman-Birk type bran trypsin inhibitor precursor putative expressed	LOC_Os01g04040.1	
66	GRMZM2G011559	2,13	2,39							Alpha-soluble NSF attachment protein	LOC_Os08g18110.1	
67	GRMZM2G020471					2,40	8,94	2,19	9,24	Uncharacterized protein	n.a	
68	GRMZM2G022972	2,45	5,15							Calmodulin binding protein putative expressed	LOC_Os01g04280.1	

Figure 13 continued

69	GRMZM2G031177	2,50	2,18								*MATE efflux family protein putative expressed	LOC_Os03g08900.1		
70	GRMZM2G032910	2,24	2,29								*Hydrolase alpha/beta fold family domain containing protein expressed	LOC_Os10g35520.1		
71	GRMZM2G033226	2,36	2,60								*ATP-citrate synthase subunit 1 putative expressed	LOC_Os01g19450.1		
72	GRMZM2G039445	2,02	2,38			2,50	2,69	2,97	2,66		Uncharacterized protein	LOC_Os02g42730.1		
73	GRMZM2G039639	3,11	2,71								Protein P21	LOC_Os03g46070.1		
74	GRMZM2G043191	3,57	2,19								Inositol polyphosphate 5-phosphatase 11	LOC_Os03g42810.2		
75	GRMZM2G044128	2,88	2,35								*h/ACA ribonucleoprotein complex subunit 4 putative expressed	LOC_Os07g44190.1		
76	GRMZM2G045534	2,26	2,56								*ATP-citrate synthase subunit 1 putative expressed	LOC_Os01g19450.1		
77	GRMZM2G047187			3,08	6,92	4,59	3,50	2,97	4,61		*Transporter major facilitator family putative expressed	LOC_Os11g05390.1		
78	GRMZM2G048287	2,14	2,35								Uncharacterized protein	LOC_Os03g10410.1		
79	GRMZM2G054522	2,92	3,59			2,90	2,25	2,85	2,25		Uncharacterized protein	LOC_Os02g46956.1		
80	GRMZM2G054900	2,26	2,86			4,09	3,23				Putative calmodulin-binding family protein	LOC_Os01g38980.2		
81	GRMZM2G056513	2,27	5,14								Expressed protein	LOC_Os04g56390.1		
82	GRMZM2G057652	5,35	2,04					2,14	6,70		*OsRhmbd8 - Putative Rhomboid homologue expressed	LOC_Os03g24390.1		
83	GRMZM2G060210	5,26	2,55								Transferase family protein putative expressed	LOC_Os05g08640.1		
84	GRMZM2G064640	2,33	2,25								Ribosomal protein S9	LOC_Os03g05980.1		
85	GRMZM2G067242	2,35	2,19								*AAA-type ATPase family protein putative expressed	LOC_Os02g46990.1		
86	GRMZM2G074462	5,22	4,37	4,83	3,90	4,77	5,90	4,70	6,15		Putative starch binding domain containing family protein	LOC_Os01g63810.1		
87	GRMZM2G074611	2,28	2,70								Dirigent protein	LOC_Os01g25030.1		
88	GRMZM2G075505	3,98	2,75								*Nucleic acid binding protein putative expressed	LOC_Os03g06980.1		
89	GRMZM2G078725			3,35	2,69						Microtubule-associated protein TORTIFOLIA1	LOC_Os02g50640.1		
90	GRMZM2G078876	2,21	3,07								Xylogen protein 1	LOC_Os03g26820.1		
91	GRMZM2G080178			5,83	3,97						*Sulfate transporter putative expressed	LOC_Os08g31410.1		
92	GRMZM2G084825			2,08	2,30	2,69	2,10				Protein kinase	LOC_Os10g04720.1		
93	GRMZM2G090245	4,78	7,33								*Cupin domain containing protein expressed	LOC_Os08g35750.1		
94	GRMZM2G099092	2,47	7,68								Beta-expansin precursor putative expressed	LOC_Os06g47360.1		
95	GRMZM2G099467	3,32	2,21								Gibberellin 20 oxidase 2	LOC_Os10g39140.1		
96	GRMZM2G099491	2,43	2,28								Systemin receptor SR160	LOC_Os03g58110.1		
97	GRMZM2G104836	2,11	3,55								Glycine-rich protein A3	LOC_Os08g28560.1		
98	GRMZM2G112456	3,02	2,39								Oligopeptide transporter 4	LOC_Os02g46860.1		
99	GRMZM2G114692	2,53	3,59								Uncharacterized protein	LOC_Os03g61560.1		
100	GRMZM2G114775	2,72	2,29								GAGA18 - C2C2-GATA-transcription factor 18	LOC_Os03g61570.2		
101	GRMZM2G115422	9,65	3,08	2,98	2,96	6,70	6,51				*Transferase family protein putative expressed	LOC_Os01g63480.1		
102	GRMZM2G117707	6,18	2,78								Phospho-2-dehydro-3-deoxyheptonate aldolase 1	LOC_Os07g42960.1		
103	GRMZM2G120475	2,64	3,48			4,61	6,91				Uncharacterized protein	n/a		
104	GRMZM2G122126	2,85	2,37								6-phosphogluconolactonase putative expressed	LOC_Os08g43370.1		
105	GRMZM2G125196	5,99	4,72								NADP-dependent oxidoreductase putative expressed	LOC_Os11g14910.1		
106	GRMZM2G127328	3,76	4,49								LHT1, A Lysine- and Histidine-Specific Amino Acid Transporter	LOC_Os08g03350.1		

Figure 13 continued

107	GRMZM2G135834	2,42	3,13			2,63	2,31	2,81	5,44	DEK C terminal domain containing protein expressed	LOC_Os03g29960.1			
108	GRMZM2G136508	2,26	4,08							*DEK C terminal domain containing protein expressed	LOC_Os03g29960.1			
109	GRMZM2G148998	5,11	7,64							Uncharacterized protein	LOC_Os07g37156.1			
110	GRMZM2G149751	2,70	2,33							*Homocysteine methyltransferase putative expressed	LOC_Os12g42884.1			
111	GRMZM2G150876	2,60	4,37		3,10	3,40				*TBC domain containing protein expressed	LOC_Os02g56570.1			
112	GRMZM2G154223									Uncharacterized protein	LOC_Os04g33110.1			
113	GRMZM2G154523	8,09	9,36							Patatin putative expressed	LOC_Os11g39990.1			
114	GRMZM2G159636	6,92	10,73	9,92	10,05	10,67	11,04	7,06	10,88	*B12D protein	LOC_Os07g17310.1			
115	GRMZM2G161696	2,50	2,25			4,17	2,10			Lysosomal protective protein	LOC_Os02g42310.1			
116	GRMZM2G162697	2,41	2,15							DNA-directed RNA polymerase II 23 kDa polypeptide	LOC_Os12g04510.1			
117	GRMZM2G167875	2,20	2,35							*60S ribosomal protein L18a putative expressed	LOC_Os01g54870.1			
118	GRMZM2G168431	2,01	2,94							Aspartic proteinase nepenthesin-2 precursor putative expressed	LOC_Os01g42320.1			
119	GRMZM2G169261			2,83	4,24					Fatty acid desaturase putative expressed	LOC_Os07g23410.1			
120	GRMZM2G176301	4,95	6,94	7,66	7,28	4,93	8,04	4,06	7,28	*Fatty acid hydroxylase putative expressed	LOC_Os11g48020.1			
121	GRMZM2G176472	2,73	3,68							*Calmodulin binding protein	LOC_Os12g36110.1			
122	GRMZM2G178807	2,00	2,36							60S ribosomal protein L7 putative expressed	LOC_Os08g13690.1			
123	GRMZM2G179170	2,47	2,60							*ATP-citrate synthase subunit 1 putative expressed	LOC_Os01g19450.2			
124	GRMZM2G310795			2,12	2,35	2,09	2,44			*Glycosyltransferase family protein 1 putative expressed	LOC_Os01g43380.1			
125	GRMZM2G310947	3,08	4,84							Cell Division Protein AAA ATPase family	LOC_Os12g24320.1			
126	GRMZM2G319138			3,60	6,97					*Pleiotropic drug resistance protein putative expressed	LOC_Os11g37700.1			
127	GRMZM2G329737	9,32	10,30	9,93	10,57	6,62	10,68	9,84	10,62	*40S ribosomal protein S4 putative expressed	LOC_Os01g25610.1			
128	GRMZM2G339562			2,05	3,56					UP-9A putative expressed	LOC_Os10g36610.1			
129	GRMZM2G345700	2,16	5,99							36.4 kDa proline-rich protein	LOC_Os04g52250.1			
130	GRMZM2G354909	3,58	2,02							Short-chain dehydrogenase/reductase putative expressed	LOC_Os04g44980.1			
131	GRMZM2G365160	6,24	2,68							*Phospho-2-dehydro-3-deoxyheptonate aldolase chloroplast precursor	LOC_Os07g42960.1			
132	GRMZM2G368591	9,75	10,26	8,24	8,67	9,83	10,57	10,09	8,26	*Integral membrane protein DUF6 containing protein expressed	LOC_Os12g33300.1			
133	GRMZM2G373522			3,00	2,41					Dehydrin; Uncharacterized protein	LOC_Os02g44870.1			
134	GRMZM2G387227	5,55	7,50	5,64	7,67					Cyclin-A1 putative expressed	LOC_Os01g13260.1			
135	GRMZM2G387360	4,12	5,52	2,69	4,02					LTPL160 - Protease inhibitor/seed storage/LTP family protein precursor	LOC_Os10g36170.1			
136	GRMZM2G398781			2,90	9,72					Uncharacterized protein	n/a			
137	GRMZM2G403915			8,64	2,92	5,37	2,50	6,55	2,01	*Nucleotide-sugar transporter family protein putative expressed	LOC_Os02g36390.4			
138	GRMZM2G422240	2,97	9,26							Heat shock protein 17.2	LOC_Os01g04370.1			
139	GRMZM2G470882	3,33	5,50							Uncharacterized protein	LOC_Os04g37760.1			
140	GRMZM2G476762					2,12	8,60	2,93	5,04	Uncharacterized protein	n/a			
141	GRMZM2G495234					2,81	2,48	2,19	3,44	Uncharacterized protein	n/a			
142	GRMZM5G851266	3,43	8,72							Putative polyphenol oxidase family protein	LOC_Os01g58100.1			
143	GRMZM5G862540	3,03	2,16							UDP-glucose 6-dehydrogenase	LOC_Os12g25700.1			
144	GRMZM5G892675	7,30	5,21							ATPase 3	LOC_Os12g24320.1			

Figure 13 continued

145	GRMZMSG898668	4,69	2,94									*Exostosin family domain containing protein expressed	LOC_Os01g70200.1		
146	AC196158.3_FG002	-2,26	-2,92									Uncharacterized protein	n/a		
147	AC204539.4_FG003	-8,91	-3,39	-6,59	-4,65	-5,88	-3,79	-9,39	-3,31			*Profilin domain containing protein expressed	LOC_Os06g05880.1		
148	AC205886.3_FG001	-4,13	-2,51									*40S ribosomal protein S4 putative expressed	LOC_Os01g25610.1		
149	AC217050.4_FG007	-2,08	-6,76									TPS7 - terpene synthase 7	LOC_Os07g11790.1		
150	GRMZM2G005170	-2,36	-2,03	-2,19	-2,78							*MSP domain containing protein putative expressed	LOC_Os01g71050.1		
151	GRMZM2G005459	-3,70	-3,86			-2,96	-2,98					*RNA recognition motif containing protein putative expressed	LOC_Os01g71200.1		
152	GRMZM2G013920	-4,28	-6,84									Uncharacterized protein	n/a		
153	GRMZM2G021256	-5,10	-3,72	-4,60	-4,94	-3,49	-3,46	-3,29	-3,82			Oxygen evolving enhancer protein 3	LOC_Os02g36850.1		
154	GRMZM2G024738	-2,68	-2,01									Uroporphyrinogen decarboxylase	LOC_Os01g43390.1		
155	GRMZM2G028302	-6,18	-5,27	-4,98	-4,30	-3,24	-3,88	-3,80	-3,58			Uncharacterized protein	LOC_Os10g39150.1		
156	GRMZM2G028733	-3,88	-4,32	-3,23	-4,58	-3,71	-4,37	-3,20	-3,92			*Ubiquitin carboxyl-terminal hydrolase 6 putative expressed	LOC_Os01g36930.1		
157	GRMZM2G031204	-9,16	-9,58	-7,82	-5,31	-7,55	-7,36	-7,70	-8,79			Peptidyl-prolyl cis-trans isomerase	LOC_Os01g68710.1		
158	GRMZM2G036605	-3,59	-10,03	-3,79	-10,11	-4,70	-12,24	-4,79	-11,26			*Tetratricopeptide repeat domain containing protein expressed	LOC_Os02g01030.1		
159	GRMZM2G036880			-2,96	-3,13							Chlorophyll a-b binding protein 6A	LOC_Os06g21590.1		
160	GRMZM2G037334	-2,11	-8,58	-3,18	-4,75	-2,01	-6,02					*Remorin C-terminal domain containing protein putative expressed	LOC_Os02g44102.1		
161	GRMZM2G044851	-2,19	-2,45									*Peptide transporter PTR2 putative expressed	LOC_Os02g46460.1		
162	GRMZM2G045699	-4,29	-11,07									Uncharacterized protein	n/a		
163	GRMZM2G050875	-7,06	-11,33									Uncharacterized protein	n/a		
164	GRMZM2G056743	-2,39	-3,14									Uncharacterized protein	n/a		
165	GRMZM2G057491	-8,08	-9,59									*Glutamyl-tRNA synthetase cytoplasmic putative expressed	LOC_Os10g22380.1		
166	GRMZM2G060886			-2,70	-4,81							*CPuORF25 - conserved peptide uORF-containing transcript expressed	LOC_Os01g50030.1		
167	GRMZM2G061126	-9,07	-8,10	-7,92	-10,85	-7,66	-7,59	-5,51	-7,33			Uncharacterized protein	n/a		
168	GRMZM2G064374	-8,56	-6,22									Uncharacterized protein	n/a		
169	GRMZM2G067929	-10,74	-4,45	-11,02	-5,47			-10,56	-5,50			Uncharacterized protein	n/a		
170	GRMZM2G068707	-2,70	-5,44									Uncharacterized protein	n/a		
171	GRMZM2G072117	2,05	-2,53					-2,08	-2,62			AT-hook protein 1	LOC_Os10g42230.1		
172	GRMZM2G074083	-2,01	-2,43									*CBS domain containing membrane protein putative expressed	LOC_Os04g05010.1		
173	GRMZM2G074307	-10,26	-5,96	-10,71	-9,50	-10,68	-9,20	-10,38	-8,33			Uncharacterized protein	n/a		
174	GRMZM2G081192							-3,71	-2,68			Aquaporin PIP2-3	LOC_Os02g41860.2		
175	GRMZM2G082608			-2,32	-2,62							Uncharacterized protein	LOC_Os01g69840.1		
176	GRMZM2G091743	-2,85	-5,47	-5,31	-3,62							Uncharacterized protein	n/a		
177	GRMZM2G098460	-2,07	-3,24	-2,29	-2,38	-2,86	-2,72	-2,19	-3,30			Drought-induced protein 1	LOC_Os02g30320.1		
178	GRMZM2G100568	-4,53	-4,02									*Expressed protein	LOC_Os02g14500.1		
179	GRMZM2G106429							-2,11	-2,13			*DnaK family protein putative expressed	LOC_Os11g47760.1		
180	GRMZM2G106795			-2,34	-2,45	-2,74	-2,46	-2,19	-3,34			ADP-ribosylation factor	LOC_Os02g47110.1		
181	GRMZM2G107082							-2,27	-2,05			*ATP-grasp domain containing protein expressed	LOC_Os11g47330.1		
182	GRMZM2G108527	-3,88	-5,27									Uncharacterized protein	n/a		

Figure 13 continued

183	GRMZM2G110553	-2,67	-4,40							Uncharacterized protein	n/a		
184	GRMZM2G115491							-2,46	-2,74	Cupin domain containing protein expressed	LOC_Os02g32980.1		
185	GRMZM2G115698	-6,28	-7,70							*Expressed protein	LOC_Os02g14500.1		
186	GRMZM2G116966	-2,00	-3,84			-2,15	-4,38			Benzoate carboxyl methyltransferase	LOC_Os06g13350.1		
187	GRMZM2G131315	-3,41	-4,96			-5,38	-5,34	-7,57	-6,68	*OsSCP4 - Putative Serine Carboxypeptidase homologue expressed	LOC_Os01g43890.1		
188	GRMZM2G132577	-5,86	-3,99	-5,32	-5,11	-4,14	-3,93	-3,92	-3,51	Uncharacterized protein	LOC_Os01g38660.1		
189	GRMZM2G133203	-2,03	-2,05							Uncharacterized protein	LOC_Os02g40040.1		
190	GRMZM2G134340	-7,66	-7,73	-6,34	-8,71	-7,61	-7,53	-8,71	-6,36	Uncharacterized protein	n/a		
191	GRMZM2G135132					-3,47	-2,85	-3,88	-5,19	*Kinase pfkB family putative expressed	LOC_Os02g41590.1		
192	GRMZM2G137493			-8,63	-2,50					Uncharacterized protein	n/a		
193	GRMZM2G137696	-10,26	-12,41	-10,63	-8,46	-8,64	-13,35	-10,97	-12,55	*DnaK family protein putative expressed	LOC_Os02g48110.1		
194	GRMZM2G138248	-3,08	-6,81							CYP9 - Cytochrome P450 family 9	LOC_Os03g37290.1		
195	GRMZM2G157822	-6,70	-6,19	-11,88	-10,48	-7,65	-7,34	-5,96	-7,37	*Myosin putative expressed	LOC_Os03g64290.1		
196	GRMZM2G165308	-4,90	-5,04	-3,20	-6,36	-3,26	-5,29			Uncharacterized protein	n/a		
197	GRMZM2G318992	-6,56	-4,49	-4,34	-2,62	-6,08	-4,69	-5,46	-2,73	*KIP1 putative expressed	LOC_Os05g39000.1		
198	GRMZM2G338696	-5,64	-8,01							Cytochrome c oxidase subunit 5B mitochondrial precursor putative expressed	LOC_Os01g42650.1		
199	GRMZM2G343636	-5,19	-3,61			-3,39	-3,51	-2,94	-2,56	*NAD dependent epimerase/dehydratase family protein putative expressed	LOC_Os03g23980.1		
200	GRMZM2G351248	-2,70	-4,79							*NAD dependent epimerase/dehydratase family protein putative expressed	LOC_Os03g23980.1		
201	GRMZM2G356839	-2,79	-7,03							Uncharacterized protein	n/a		
202	GRMZM2G371079	-3,84	-3,12	-2,98	-3,35					Uncharacterized protein	LOC_Os01g69870.1		
203	GRMZM2G374302					-3,16	-3,42			Pyridoxal-dependent decarboxylase protein putative expressed	LOC_Os04g01690.1		
204	GRMZM2G400961		-2,24	-2,18						Uncharacterized protein	LOC_Os04g55610.1		
205	GRMZM2G411216	-5,24	-10,63							Uncharacterized protein	n/a		
206	GRMZM2G429972	-5,26	-12,55	-5,79	-10,32	-5,84	-10,81	-5,65	-10,37	Uncharacterized protein	n/a		
207	GRMZM2G457347	-9,63	-10,08	-10,63	-9,39	-6,75	-10,58	-8,05	-8,22	Uncharacterized protein	LOC_Os03g58590.1		
208	GRMZM2G587231	-2,32	-7,48							Uncharacterized protein	n/a		
209	GRMZM2G702522	-7,45	-6,77	-8,46	-5,74	-7,33	-5,42	-9,19	-7,68	Uncharacterized protein	n/a		
210	GRMZM5G815851	-3,49	-2,34							Uncharacterized protein	n/a		
211	GRMZM5G876518				-10,45	-5,16				40S ribosomal protein S4 putative expressed	LOC_Os01g25610.1		
212	GRMZM5G877929	-3,94	-11,40							Uncharacterized protein	n/a		
213	GRMZM5G883813	-5,90	-2,02							Uncharacterized protein	n/a		
214	GRMZM5G890040	-4,31	-6,61							Uncharacterized protein	n/a		
215	GRMZM5G897005	-3,87	-9,76	-3,86	-10,60	-3,37	-11,55	-3,70	-12,08	*Protein kinase domain containing protein expressed	LOC_Os01g02790.1		

INCREASED FUNGAL RESISTANCE IN CROP PLANTS

TECHNICAL FIELD

[0001] The present invention relates to methods for producing plants with increased fungal resistance, preferably seedling resistance against Northern Corn Leaf Blight. Further provided are methods for introducing, modifying, or modulating at least one wall-associated kinase (WAK) in(to) a plant cell, tissue, organ, or whole plant and thereby causing a reduced synthesis of benzoxazinoid and in turn increased fungal resistance. There are further provided methods to identify and/or modify downstream effector molecules in a WAK signalling cascade. Finally, plant cells, tissues, organs or whole plants having increased fungal resistance and methods using substances to activate signalling pathways in a targeted way are provided. The present invention thus relates to WAKs as master regulators and crucial signaling mediators in plant defense against fungal disease and the regulation and cross-talk mechanisms in the WAK signaling cascade and further gives examples for establishing novel anti-fungal strategies relevant for a series of crop plants.

BACKGROUND OF THE INVENTION

[0002] Infections and infestations of crop plants by pathogens encompassing viruses, bacteria, fungi, nematodes and insects and the resulting damages cause significant yield losses of cultivated plants. In maize (*Zea mays*) as one of the major crop plants worldwide there are a large number of fungal pathogens which cause leaf diseases. The fungus which can cause by far the most damage under tropical and also under temperate climatic conditions, such as those in large parts of Europe and North America as well as in Africa and India, is known as *Helminthosporium turcicum* or synonymously as *Exserohilum turcicum* (teleomorph: *Setosphaeria turcica*). *H. turcicum/E. turcicum* is the cause of the leaf spot disease known as "Northern Corn Leaf Blight" (NCLB), which can occur in epidemic proportions during wet years, attacking vulnerable maize varieties and causing a great deal of damage and considerable losses of yield of 30% and more over wide areas (Perkins, J. M., and W. L. Pedersen. "Disease development and yield losses associated with northern leaf blight on corn." Plant disease 71.10 (1987): 940-943; Raymundo, A. D., A. L. Hooker, and J. M. Perkins. "Effect of gene HtN on the development of northern corn leaf blight epidemics." Plant disease (1981); Ullstrup, A J, and Miles, S R 1957. The effects of some leaf blights of corn on grain yield. Phytopathology 47:331-336). Since the 1970s, then, natural resistance in genetic material has been sought.

[0003] The race for defining and establishing new resistance strategies against pathogens for major crop plants is more and more accelerated due to the increasing resistance breaking characteristics of pathogens, i.e., the evolutionary strategy of pathogens to adapt to and survive pressure of plant protective agents and/or to subvert the endogenous plant defense mechanisms.

[0004] Currently, quantitative and qualitative resistances are known. While the oligo- or polygenically inherited quantitative resistance appears incomplete and non-specific as regards race in the phenotype and is influenced by additional and partially dominant genes, qualitative resistance is typically race-specific and can be inherited through individual: For instances, mostly dominant resistance genes with regard to NCLB are Ht1, Ht2, Ht3, Htm, Htn1, or Htp (Lipps, P. E., R. C. Pratt, and J. J. Hakiza. "Interaction of Ht and partial resistance to *Exserohilum turcicum* in maize." Plant disease 81.3 (1997): 277-282; Wang, H., et al. "Expression of Ht2-related genes in response to the HT-Toxin of *Exserohilum turcicum* in Maize." Annals of applied biology 156.1 (2010): 111-120). Backcrosses in many frequently used inbred maize lines such as W22, A619, B37 or B73 have successfully brought about introgression of the HT genes, where they exhibit a partial dominance and expression as a function of the respective genetic background (Welz, H G (1998). "Genetics and epidemiology of the pathosystem *Zea mays/Setosphaeria turcica*." Habilitationsschrift Institut für Pflanzenzüchtung, Saatgutforschung and Populationsgenetik, Universität Hohenheim). WO 2011/163590 A1 annotated the presumed Htn1 gene in the resistance source PH26N and PH99N as a tandem protein kinase-like gene and disclosed its genetic sequence, but did not determine its functionality, for example in a transgenic maize plant. WO 2015/032494 A2 discloses the identification of another allelic variant of HTN1 gene derived from the donor Peptilla as well as a resistant maize plant into the genome of which a chromosome fragment from the donor Pepitilla has been integrated, which chromosome fragment comprises the resistance locus HTN1.

[0005] The hemibiotrophic fungal pathogen *Exserohilum turcicum* (anamorph form of the fungus) causing NCLB is found in humid climates wherever corn is grown. *E. turcicum* survives in corn debris and builds up over time in high-residue and continuous corn cropping systems. High humidity and moderate temperatures favor the persistence of the *E. turcicum* fungus causing tremendous yield losses, e.g., due to decreased photosynthesis resulting in limited ear fill, or harvest losses if secondary stalk rot infection and stalk lodging accompany loss of leaf area.

[0006] As a natural defense mechanism against various kinds of pathogens, Plants have evolved multiple layers of defense against infection by pathogenic microbes (Jones, Jonathan DG, and Jeffery L. Dangl. "The plant immune system." Nature 444.7117 (2006): 323). The primary defense is based on the extracellular perception of pathogen-associated or host damage-associated molecular patterns or signatures (PAMPs/DAMPs) by plasma membrane-anchored pattern recognition receptors (PRRs). These receptor proteins monitor the extracellular space for the presence of microbial- or host-derived elicitors, i.e., PAMPs or DAMPs, respectively. These signatures can be highly conserved and characteristic for entire pathogen classes as in the case of the bacterial flagellin that is perceived by the leucine-rich repeat receptor kinase (LRR-RK) FLS2, which results in basal and broad-spectrum resistance against most bacteria (Macho, Alberto P., and Cyril Zipfel. "Plant PRRs and the activation of innate immune signaling." Molecular cell 54.2 (2014): 263-272). Other receptor kinases only confer resistance to certain races of a particular pathogen (Hu, Keming, et al. "Improvement of multiple agronomic traits by a disease resistance gene via cell wall reinforcement." Nature plants 3 (2017): 17009). Receptor kinases have different types of extracellular domains, including leucine-rich repeats (LRRs), lysine motifs (LysMs), lectin motifs or epidermal growth factor (EGF) like extracellular domains (Dardick, Chris, Benjamin Schwessinger, and Pamela Ronald. "Non-arginine-aspartate (non-RD) kinases are associated with

innate immune receptors that recognize conserved microbial signatures." Current opinion in plant biology 15.4 (2012): 358-366).

[0007] In grasses, there is emerging evidence that WAKs might be important players in fungal and bacterial disease resistance. The WAK genes qHSR1, Htn1 and OsWAK (Xa4) confer disease resistance (Hurni, Severine, et al. "The maize disease resistance gene Htn1 against northern corn leaf blight encodes a wall-associated receptor-like kinase." Proceedings of the National Academy of Sciences 112.28 (2015): 8780-8785., Hu et al. 2017), yet little is known about the underlying mechanisms and signaling cascades responsible for the observed phenotypes.

[0008] OsWAK underlying resistance involves strengthening of the cell intensity by enhancing cellulose biosynthesis (Hu et al. 2017). Interestingly, there is also one case described where the wheat WAK encoded by the Snn1 gene acts as a susceptibility factor. It has been shown that Snn1 perceives the SnTox1 toxin encoded by the fungal pathogen *Stagonospora nodorum*, which triggers cell death and allows the necrotrophic *S. nodorum* pathogen to proliferate on wheat (Shi, Gongjun, et al. "Marker development, saturation mapping, and high-resolution mapping of the *Septoria nodorum* blotch susceptibility gene Snn3-B1 in wheat." Molecular genetics and genomics 291.1 (2016): 107-119). In dicots, the *Arabidopsis* AtWAK1 was found to physically associate with and recognized cell wall-derived oligogalactouronides (OGs), which result from polysaccharide degradation (Kohorn, Bruce D., et al. "Pectin activation of MAP kinase and gene expression is WAK2 dependent." *The Plant Journal* 60.6 (2009): 974-982). In contrast to the *Arabidopsis* WAK gene family that consists of only five members, WAK genes in monocots belong to a large family. For instance, in rice >100 members were found (Kanneganti, Vydehi, and Aditya K. Gupta. "Wall associated kinases from plants an overview." *Physiology and Molecular Biology of Plants* 14.1-2 (2008): 109-118). The emergence of WAKs in monocots might be associated to several functional aspects, e.g. biotic diseases (Li, Hui, et al. "A novel wall-associated receptor-like protein kinase gene, OsWAK1, plays important roles in rice blast disease resistance." *Plant molecular biology* 69.3 (2009): 337-346; Hurni et al. 2015; Zuo, Weiliang, et al. "A maize wall-associated kinase confers quantitative resistance to head smut." *Nature genetics* 47.2 (2015): 151-157; Shi, Gongjun, et al. "The hijacking of a receptor kinase-driven pathway by a wheat fungal pathogen leads to disease." Science advances 2.10 (2016): e1600822; Hu et al. 2017), tolerance of phosphorus deficiency (Hufnagel, Barbara, et al. "Duplicate and conquer: Multiple homologs of PHOSPHORUS-STARVATION TOLERANCE1 enhance phosphorus acquisition and sorghum performance on low-phosphorus soils." *Plant physiology* 166.2 (2014): 659-677), root growth (Kaur, Ravneet, Kashmir Singh, and Jaswinder Singh. "A root-specific wall-associated kinase gene, HvWAK1, regulates root growth and is highly divergent in barley and other cereals." *Functional & integrative genomics* 13.2 (2013): 167-177) as well as gametophyte development (Wang, Na, et al. "The rice wall-associated receptor-like kinase gene OsDEES1 plays a role in female gametophyte development." *Plant physiology* 160.2 (2012): 696-707; Hu et al. 2017). Therefore, there is emerging evidence that wall-associated kinases (WAKs) could play a pivotal role in plant immunity specifically in cereal crops.

[0009] Still, presently little is known about the specific molecular mechanisms underlying plant immunity based on WAKs, said immune mechanisms being highly specific for each pathogen and thus PAMP/DAMP to be recognized and furthermore depending on the downstream signaling cascade and the subsequently initiated effector mechanisms. So far, rather the phenotypic outcome of a plant comprising or not comprising a receptor-like kinase on pathogen resistance has been matched with the genotype of a plant. The molecular mechanisms which are responsible for the action of the WAKs, the precise signaling pathways and also the crosstalk between different pathways remain elusive.

[0010] Chemical fungicides have long been utilised for controlling fungal diseases. A different approach relies on the examination and elucidation of the complex biosynthetic pathways involved in pathogen resistance causing a natural pathogen defence of plants by studying the resistance ability of an existing plant cultivar to inhibit or at least limit any infestation of a pathogen to provide new strategies to combat plant pathogens and to provide new plants carrying resistance traits of interest.

[0011] Benzoxazinoids (BXDs) were identified in the 1960s as secondary plant metabolites functioning as natural pesticides. BXDs are a class of secondary metabolites found in maize and other cereal species and certain dicots and contain a 2-hydroxy-2H-1,4-benzoxazin-3(4H)-one skeleton (Niemeyer, Hermann M. "Hydroxamic acids derived from 2-hydroxy-2 H-1, 4-benzoxazin-3 (4 H)-one: key defense chemicals of cereals." *Journal of Agricultural and Food Chemistry* 57.5 (2009): 1677-1696). BXDs are synthesized in seedlings and stored as glucosides. The main aglucone moieties are 2,4-dihydroxy-2H-1,4-benzoxazin-3 (4H)-one (DIBOA) and 2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one (DIMBOA). BXDs are synthesised in two subfamilies of the Poaceae and sporadically found in single species of the dicots. BXDs are predominantly stored as inactive glucosides, while upon biotic stress they are hydrolyzed to the respective toxic hydroxamic acids (e.g., DIMBOA). The first step in BXD biosynthesis converts indole-3-glycerol phosphate into indole. In maize (*Zea mays*), this reaction is catalyzed by either Benzoxazineless1 (BX1), or Indole glycerol phosphate lyase (IGL or Igl herein). The bx1 gene is under developmental control and is mainly responsible for BX production, whereas the Igl gene is inducible by stress signals, such as wounding, herbivory, or jasmonates. The enzymatic properties of IGL are similar to BX1, but the transcriptional regulation of their corresponding genes is different. Like other Bx genes, bx1 is constitutively expressed during the early developmental stages of the plant, which correlates with endogenous BX levels. The introduction of four oxygen atoms into the indole moiety that yields DIBOA is catalyzed by four cytochrome P450 monooxygenases termed BX2 to BX5. A further Bx enzyme, BX6, is responsible for the hydroxylation in position C-7 of the benzoxazinoids in maize (Frey, Monika, et al. "A 2-oxoglutarate-dependent dioxygenase is integrated in DIMBOA-biosynthesis." *Phytochemistry* 62.3 (2003): 371-376). Bx7, as further example, is an O-methyltransferase (OMT) catalyzing the formation of DIMBOA-glc from TRIBOA-glc.

[0012] Little is known about the role of these compounds in fungal disease resistance. Few field studies decades ago proposed a correlation between benzoxazinoid hydroxamic acids concentration and the disease resistance to maize stalk

rot, maize northern corn leaf blight and wheat stem rust (Long, B. J., G. M. Dunn, and D. G. Routley. "Relationship of hydroxamate concentration in maize and field reaction to *Helminthosporium turcicum.*" *Crop Science* 18.4 (1978): 573-575) without providing any molecular basis for this phenomenon. Other studies even found no effect of BXDs in fungal disease resistance, including maize stalk rot, Southern corn leaf blight, maize anthracnose, corn smut and head blight at all (Niemeyer, 2009).

[0013] Therefore, it was an outstanding aim of the present invention to provide new tools and methods to allow for a tight resistance management for major crop plants concerning the control of fungal plant pathogens.

[0014] Further, it was an object of the present invention to provide new strategies and methods to combat fungal leaf diseases based on exploiting plant-endogenous defense mechanisms as mediated by secondary metabolites and further to provide plants having a specific genotype as source of increased resistance or tolerance against fungal pathogens causing severe plant diseases. Basically, it was an object of the present invention to elucidate the molecular basis of the maize Htn1 northern corn leaf blight (NCLB) resistance that is cause by the WAK gene ZmWAK-RLK1 to establish molecular targets and cross-talk mechanisms between relevant biosynthesis pathways to provide new urgently needed resistance strategies for a variety of important crop plants by characterizing the molecular players involved in disease resistance in a plant.

SUMMARY OF THE INVENTION

[0015] The above object was achieved by identifying the molecular basis of the maize Htn1 northern corn leaf blight (NCLB) resistance that is caused by the WAK gene ZmWAK-RLK1. It was demonstrated that ZmWAK-RLK1 modulates, i.e. it functions upstream of the benzoxazinoids (BXD) biosynthesis pathway, resulting in reduced BXD concentrations. Furthermore, the interaction of WAK with downstream effectors, i.e., BX enzymes and Igl, in the regulation of BXD biosynthesis and fungal disease were demonstrated. Furthermore, the present invention builds on relevant information of the cross-talk and cross-regulation of the plant WAK signaling pathway with the jasmonic acid (JA) and further relevant plant biosynthesis pathways to elucidate the regulatory networks important to modulate and to induce plant defense against major pathogens.

[0016] Interestingly, maize plants with compromised BXD biosynthesis showed increased resistance against NCLB. Thus, these new insight into WAK-mediated quantitative disease resistance and for the first time provided a functional link between WAKs and the secondary metabolites BXDs which was used to define new fungal defense strategies going beyond the use of fungicides for disease control which are applicable for several associated fungal pathogens inducing comparable plant immune reactions by pathogen-associated PAMPs/DAMPs in several important crop plants.

[0017] The present invention thus transforms the information on molecular mechanisms, the effect of specific mutations in kinase domains on the signaling function of a WAK, and further on the cross-talk and interplay of signaling cascades to provide plants having a defined genetic background and thus to provide plants with increased resistance against fungal pathogens. Furthermore, the present invention provides methods to modulate (increase/decrease) or neutralize the action of plant secondary metabolites and the relevant genes responsible for the synthesis pathways of said secondary metabolites to enhance fungal pathogen resistance in a targeted way.

[0018] In one aspect, there is thus provided a method for producing a plant having increased fungal resistance as compared to a corresponding control plant, wherein the fungal resistance is regulated by at least one wall-associated kinase, the method comprising: (i) (a) providing at least one plant cell, tissue, organ, or whole plant having a specific genotype with respect to the presence of at least one gene encoding a wall-associated kinase in the genome of said plant cell, tissue, organ, or whole plant; or (i) (b) introducing at least one gene encoding at least one wall-associated kinase into the genome of at least one cell of at least one of a plant cell, tissue, organ, or whole plant; and (ii) (a) modifying at least one gene encoding at least one wall-associated kinase in the at least one plant cell, tissue, organ, or whole plant; and/or (ii) (b) modulating the expression level of at least one wall-associated kinase and/or the transcription level, the expression level, or the function of at least one molecule within the signalling pathway from the at least one wall-associated kinase to the synthesis of at least one benzoxazinoid or within the synthesis pathway of at least one benzoxazinoid in the at least one plant cell, tissue, organ, or whole plant; (iii) producing a population of plants from the at least one plant cell, tissue, organ, or whole plant, and (iv) selecting/identifying a plant having increased fungal resistance, from the population based on the determination of a reduced synthesis of at least one benzoxazinoid preferably in response to a fungal pathogen infection, wherein the selected plant have an increased fungal resistance based on the reduced synthesis of a benzoxazinoid, and/or wherein the synthesis of the at least one benzoxazinoid is regulated by the at least one wall-associated kinase.

[0019] In one embodiment of the various aspects of the present invention, the at least one wall-associated kinase is a WAK-RLK1 gene, preferably selected from Htn1, Ht2, or Ht3, or an allelic variant thereof, a mutant or a functional fragment thereof, or a gene encoding the same, preferably wherein the at least one wall-associated kinase a) is encoded by a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 or 7, or a functional fragment thereof, b) is encoded by a nucleic acid molecule comprising the nucleotide sequence having at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to nucleotide sequence of SEQ ID NO: 1 or 7, preferably over the entire length of the sequence, c) is encoded a nucleic acid molecule hybridizing with a complementary sequence to a) or b) under stringent conditions, d) is encoded by a nucleic acid molecule comprising the nucleotide sequence coding for an amino acid sequence of SEQ ID NO: 2 or 8, or a functional fragment thereof, e) is encoded by a nucleic acid molecule comprising the nucleotide sequence coding for an amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to amino acid sequence of SEQ ID NO: 2 or 8, preferably over the entire length of the sequence, f) comprising the amino acid sequence of SEQ ID NO: 2 or 8, or g) comprising an amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%,

90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to amino acid sequence of SEQ ID NO: 2 or 8, preferably over the entire length of the sequence, provided that the any sequence of a) to g), optionally after expression, still encodes at least one functional Htn1, Ht2, or Ht3, or an allelic variant, a mutant, or a functional fragment thereof. Preferably, the at least one wall-associated kinase of the invention causes after expression a reduced synthesis of at least one benzoxazinoid. More preferably, in *Zea mays* the gene or nucleic acid molecule encoding the at least one wall-associated kinase is located at or mapped on a locus in bin 5 or bin 6 on the long arm of chromosome 8.

[0020] In a further embodiment of the various aspects of the present invention, the benzoxazinoid whose biosynthesis is regulated by the at least one wall-associated kinase, is selected from at least one of DIM_2BOA, DIMBOA, HMBOA, HM_2BOA, HDMBOA, HDM_2BOA, HBOA, DHBOA, DIBOA or TRIBOA, the aforementioned benzoxazinoid being in the glucoside or aglucone form, or a benzoxazolinone, or any combination of the aforementioned benzoxazinoids, preferably wherein the benzoxazinoid whose biosynthesis is regulated by the at least one wall-associated kinase is selected from at least one of DIM_2BOA, DIMBOA, HMBOA or HDMBOA, the aforementioned benzoxazinoid being in the glucoside or aglucone form, or any combination of the aforementioned benzoxazinoids.

[0021] In another embodiment, there is provided a method, wherein the reduced synthesis of at least one benzoxazinoid is achieved by providing at least one wall-associated kinase, an allelic variant, a mutant or a functional fragment thereof, or a gene encoding the same, wherein the at least one wall-associated kinase comprises a sequence which can directly or indirectly influence the benzoxazinoid (synthesis) pathway and at least one further plant metabolic pathway, preferably a disease resistance associated pathway, wherein the plant metabolic pathway is selected from the group consisting of the jasmonic acid pathway, the ethylene pathway, the lignin synthesis pathway, a defense pathway, a receptor-like kinase pathway, and/or a cell wall associated pathway.

[0022] In yet a further embodiment of the various aspects of the present invention, the fungus resistance against which resistance is increased, or the disease caused by said fungus is selected from a fungus of the order of Pleosporales, comprising *E. turcicum/H. turcicum* causing northern corn leaf blight (NCLB), particularly affecting maize and wheat plants, southern corn leaf blight (*Bipolaris maydis*), the order of Pucciniales causing rust disease, comprising common rust (*Puccinia sorghi*), or *Diploida* leaf streak/blight (*Diploida macrospora*/Stenocarpella *macrospora*), or *Colletotrichum graminicola*, or *Fusarium* spp., preferably *Fusarium* verticilioides causing *Fusarium* stalk rot, or *Gibberella* spp., e.g., *Gibberella zeae* causing *Giberella* stalk rot, rust, stalk rot, maize head smut (*Sphacelotheca reiliana*), and *Diploida* leaf streak/blight.

[0023] In one of the embodiments of the various aspects of the present invention, the at least one gene encoding at least one wall-associated kinase is stably integrated/introduced into the genome of the at least one plant cell, tissue, organ, or whole plant, or the at least one gene encoding at least one wall-associated kinase is transiently introduced into a plant cell, tissue, organ, or whole plant.

[0024] In a further embodiment of the various aspects of the present invention, the at least one molecule within the signalling pathway from the at least one wall-associated kinase to the synthesis of at least one benzoxazinoid or within the synthesis pathway of at least one benzoxazinoid is selected from the group consisting of the genes bx1 (SEQ ID NO: 10), bx2 (SEQ ID NO: 12), igl (SEQ ID NO: 14), bx6 (SEQ ID NO: 16), bx11 (SEQ ID NO: 18), bx14 (SEQ ID NO: 20), opr2 (SEQ ID NO: 22), lox3 (SEQ ID NO: 24) or aoc1 (SEQ ID NO: 26), or a homologous genes thereof, or the proteins BX1 (SEQ ID NO: 11), BX2 (SEQ ID NO: 13), IGL (SEQ ID NO: 15), BX6 (SEQ ID NO: 17), BX11 (SEQ ID NO: 19), BX14 (SEQ ID NO: 21), OPR2 (SEQ ID NO: 23), LOX3 (SEQ ID NO: 25) or AOC1 gene (SEQ ID NO: 27), or a homolog thereof.

[0025] In another embodiment of the various aspects of the present invention, the at least one gene encoding at least one wall-associated kinase is stably integrated into the genome of the at least one plant cell, tissue, organ, or whole plant, and the introduction of the at least one gene encoding at least one wall-associated kinase comprises the introgression of the at least one gene during plant breeding.

[0026] In a further embodiment of the of the various aspects of the present invention, the modification of the at least one gene encoding at least one wall-associated kinase within step (ii) (a) or (ii) (b) of the method of the above disclosed aspect is performed by at least one of a site-specific nuclease (SSN) or a catalytically active fragment thereof, or a nucleic acid sequence encoding the same, oligonucleotide directed mutagenesis, chemical mutagenesis, or TILLING.

[0027] In a further embodiment of the of the various aspects of the present invention, the at least one site-specific nuclease (SSN), or the nucleic acid sequence encoding the same, is selected from at least one of a CRISPR nuclease, including Cas or Cpf1 nucleases, a TALEN, a ZFN, a meganuclease, a base editor complex, a restriction endonuclease, including FokI or a variant thereof, or two site-specific nicking endonucleases, or a variant or a catalytically active fragment thereof.

[0028] According to one embodiment of the various aspects of the present invention, the at least one plant cell, tissue, organ, or whole plant provided in step (i) is selected from the group consisting of *Hordeum vulgare, Hordeum bulbusom, Sorghum bicolor, Saccharum officinarium, Zea* spp., including *Zea mays, Setaria italica, Oryza minuta, Oryza sativa, Oryza australiensis, Oryza alta, Triticum aestivum, Triticum durum, Secale cereale*, Triticale, *Malus domestica, Brachypodium distachyon, Hordeum marinum, Aegilops tauschii, Daucus glochidiatus, Beta* spp., including *Beta vulgaris, Daucus pusillus, Daucus muricatus, Daucus carota, Eucalyptus grandis, Nicotiana sylvestris, Nicotiana tomentosiformis, Nicotiana tabacum, Nicotiana benthamiana, Solanum lycopersicum, Solanum tuberosum, Coffea canephora, Vitis vinifera, Erythrante guttata, Genlisea aurea, Cucumis sativus, Marus notabilis, Arabidopsis arenosa, Arabidopsis lyrata, Arabidopsis thaliana, Crucihimalaya himalaica, Crucihimalaya wallichii, Cardamine nexuosa, Lepidium virginicum, Capsella* bursa *pastoris, Olmarabidopsis pumila, Arabis hirsute, Brassica napus, Brassica oleracea, Brassica rapa, Raphanus sativus, Brassica juncacea, Brassica nigra, Eruca vesicaria* subsp. *sativa, Citrus sinensis, Jatropha curcas, Populus trichocarpa, Medicago truncatula, Cicer yamashitae, Cicer bijugum, Cicer arietinum, Cicer reticulatum, Cicer judaicum, Cajanus cajanifolius, Cajanus scarabaeoides, Phaseo-*

lus vulgaris, Glycine max, Gossypium sp., *Astragalus sinicus, Lotus japonicas, Torenia fournieri, Allium cepa, Allium fistulosum, Allium sativum, Helianthus annuus, Helianthus tuberosus* and *Allium tuberosum*, or any variety or subspecies belonging to one of the aforementioned plants, preferably wherein the plant cell, tissue, organ, or whole plant in step (i) is selected from *Zea mays* or *Triticum* spp., or any variety or subspecies belonging to one of the aforementioned plants

[0029] In a further aspect, there is provided a plant cell, tissue, organ, whole plant or plant material, or a derivative or a progeny thereof, obtainable by a method according to any one of the embodiments of the methods of the present invention.

[0030] In a further aspect of the present invention, there is provided a method for identifying at least one gene involved in increased pathogen resistance, preferably increased fungal resistance, in a plant cell, tissue, organ, whole plant, or plant material as compared to a corresponding control plant cell, tissue, organ, whole plant, or plant material the method comprising: (i) determining the genotype of at least one plant cell, tissue, organ, whole plant, or plant material with respect to the presence of at least one gene encoding a wall-associated kinase in the genome of said plant cell, tissue, organ, whole plant or plant material; (ii) optionally: determining the benzoxazinoid signature of the at least one plant cell, tissue, organ, whole plant, or plant material of step (i); (iii) exposing the at least one plant cell, tissue, organ, whole plant, or plant material of step (i) or (ii) to a stimulus, optionally wherein the stimulus is correlated with the benzoxazinoid signature in the at least one plant cell, tissue, organ, whole plant, or plant material, preferably wherein the stimulus is associated with a fungal pathogen infection; (iv) performing an analysis of at least one analyte obtained from the at least one plant cell, tissue, organ, whole plant, or plant material of step (i) or (ii) after exposition to the stimulus; (v) determining at least one gene being regulated upon exposition to a stimulus according to step (iii) in at least one cell of the at least one plant cell, tissue, organ, whole plant, or plant material as derivable from the analysis of at least one analyte as defined in step (iv), (vi) subjecting the at least one gene as determined in step (v) to a functional characterization; and (vii) providing at least one gene involved in increased pathogen resistance, preferably increased fungal resistance, in a plant cell, tissue, organ, whole plant, or plant material.

[0031] Further provided is a plant cell, tissue, organ, whole plant or plant material, or a derivative or a progeny thereof, obtainable by introducing at least one gene as provided by the method for identifying at least one gene involved in increased pathogen resistance into at least one cell of at least one of a plant cell, tissue, organ, or whole plant.

[0032] In another embodiment of the present invention, there is provided a plant cell, tissue, organ, whole plant or plant material, or a derivative or a progeny thereof, obtainable by the methods of the present invention, wherein the introduction of at least one gene as provided by the method for identifying at least one gene involved in increased pathogen resistance is a stable introduction, preferably a stable introduction mediated by conventional plant breeding, or a stable introduction mediated by means of molecular biology, comprising genome editing, or a combination thereof.

[0033] In yet a further aspect of the present invention there is provided a method of increasing pathogen resistance, preferably fungal resistance, in a plant cell, tissue, organ, whole plant, or plant material as compared to a corresponding control plant cell, tissue, organ, whole plant, or plant material, the method comprising: (i) providing at least one plant cell, tissue, organ, whole plant or plant material; (ii) (a) treating the at least one plant cell, tissue, organ, whole plant or plant material according to step (i) with a substance neutralizing the effect of at least one benzoxazinoid, and/or (ii) (b) treating the at least one plant cell, tissue, organ, whole plant or plant material according to step (i) with a substance activating the signalling pathway downstream of at least one wall-associated kinase; and/or (ii) (c) modifying at least one promoter or at least one regulatory sequence of at least one gene of the at least one plant cell, tissue, organ, whole plant or plant material of step (i), wherein said at least promoter or at least one regulatory sequence is involved in the regulation of transcription of at least on gene involved in the signalling pathway of, or downstream of at least one wall-associated kinase; (iii) reducing the amount of at least one benzoxazinoid and thereby increasing pathogen resistance, preferably fungal resistance, in at least one plant cell, tissue, organ, whole plant, or plant material.

[0034] Further provided is a use of a substance as defined for the aspect of a method of increasing pathogen resistance for increasing pathogen resistance, preferably fungal resistance, in at least one plant cell, tissue, organ, whole plant, or plant material.

Definitions

[0035] The term "active fragment" or "functional fragment" as used herein referring to amino acid sequences denotes the core sequence derived from a given template amino acid sequence, or a nucleic acid sequence encoding the same, comprising all or part of the active site of the template sequence with the proviso that the resulting catalytically active fragment still possesses the activity characterizing the template sequence, for which the active site of the native enzyme or a variant thereof is responsible. Said modifications are suitable to generate less bulky amino acid sequences still having the same activity as a template sequence making the catalytically active fragment a more versatile or more stable tool being sterically less demanding. For amino acid sequences not representing enzymes, the term "functional fragment" can also imply that part or domain of the amino acid sequence involved in interaction with another molecule, and/or involved in any structural function within the cell.

[0036] An "allele" or "allelic variant" as used herein refers to a variant form of a given gene. As most multicellular organisms have two sets of chromosomes; that is, they are diploid (or, if more chromosome sets are present, they are polyploidy), these chromosomes are referred to as homologous chromosomes. If both alleles at a gene (or locus) on the homologous chromosomes are the same, they and the organism are homozygous with respect to that gene (or locus). If the alleles are different, they and the organism are heterozygous with respect to that gene. Alleles can result in the same, or a different observable phenotype. The term "allele" thus refers to one or two or more nucleotide sequences at a specific locus in the genome. A first allele is on a chromosome, a second on a second chromosome at the same position. If the two alleles are different, they are heterozy-

gous, and if they are the same, they are homozygous. Various alleles of a gene (gene alleles) differ in at least one SNP (single nucleotide polymorphism).

[0037] "Complementary" or "complementarity" as used herein describes the relationship between two DNA, two RNA, or, regarding hybrid sequences according to the present invention, between an RNA and a DNA nucleic acid region. Defined by the nucleobases of the DNA or RNA, two nucleic acid regions can hybridize to each other in accordance with the lock-and-key model. To this end the principles of Watson-Crick base pairing have the basis adenine and thymine/uracil as well as guanine and cytosine, respectively, as complementary bases apply. Furthermore, also non-Watson-Crick pairing, like reverse-Watson-Crick, Hoogsteen, reverse-Hoogsteen and Wobble pairing are comprised by the term "complementary" as used herein as long as the respective base pairs can build hydrogen bonding to each other, i.e., two different nucleic acid strands can hybridize to each other based on said complementarity.

[0038] The term "construct", especially "genetic construct", or "recombinant construct", or "expression construct" as used herein refers to a construct comprising, inter alia, plasmids or plasmid vectors, cosmids, artificial yeast chromosomes or bacterial artificial chromosomes (YACs and BACs), phagemides, bacterial phage based vectors, an expression cassette, isolated single-stranded or double-stranded nucleic acid sequences, comprising DNA and RNA sequences, or amino acid sequences, viral vectors, including modified viruses, and a combination or a mixture thereof, for introduction or transformation, transfection or transduction into a target cell or plant, plant cell, tissue, organ or material according to the present disclosure.

[0039] The term "delivery construct" or "delivery vector" as used herein refers to any biological or chemical means used as a cargo for transporting a nucleic acid, including a hybrid nucleic acid comprising RNA and DNA, and/or an amino acid sequence of interest into a target cell, preferably a eukaryotic cell. The term delivery construct or vector as used herein thus refers to a means of transport to deliver a genetic or a recombinant construct according to the present disclosure into a target cell, tissue, organ or an organism. A vector can thus comprise nucleic acid sequences, optionally comprising sequences like regulatory sequences or localization sequences for delivery, either directly or indirectly, into a target cell of interest or into a plant target structure in the desired cellular compartment of a plant. A vector can also be used to introduce an amino acid sequence or a ribonucleomolecular complex into a target cell or target structure. Usually, a vector as used herein can be a plasmid vector. Furthermore, according to certain preferred embodiments according to the present invention, a direct introduction of a construct or sequence or complex of interest is conducted. The term direct introduction implies that the desired target cell or target structure containing a DNA target sequence to be modified according to the present disclosure is directly transformed or transduced or transfected into the specific target cell of interest, where the material delivered with the delivery vector will exert its effect. The term indirect introduction implies that the introduction is achieved into a structure, for example, cells of leaves or cells of organs or tissues, which do not themselves represent the actual target cell or structure of interest to be transformed, but those structures serve as basis for the systemic spread and transfer of the vector, preferably comprising a genetic construct according to the present disclosure to the actual target structure, for example, a meristematic cell or tissue, or a stem cell or tissue. In case the term vector is used in the context of transfecting amino acid sequences and/or nucleic sequences, including hybrid nucleic acid sequences, into a target cell the term vector implies suitable agents for peptide or protein transfection, like for example ionic lipid mixtures, cell penetrating peptides (CPPs), or particle bombardment. In the context of the introduction of nucleic acid material, the term vector cannot only imply plasmid vectors but also suitable carrier materials which can serve as basis for the introduction of nucleic acid and/or amino acid sequence delivery into a target cell of interest, for example by means of particle bombardment. Said carrier material comprises, inter alia, gold or tungsten particles. Finally, the term vector also implies the use of viral vectors for the introduction of at least one genetic construct according to the present disclosure like, for example, modified viruses and bacterial vectors, like for example *Agrobacterium* spp., like for example *Agrobacterium tumefaciens*. Finally, the term vector also implies suitable chemical transport agents for introducing linear nucleic acid sequences (single- or double-stranded), or amino sequences, or a combination thereof into a target cell combined with a physical introduction method, including polymeric or lipid-based delivery constructs.

[0040] Suitable "delivery constructs" or "vectors" thus comprise biological means for delivering nucleotide and/or amino acid sequences into a target cell, including viral vectors, *Agrobacterium* spp., or chemical delivery constructs, including nanoparticles, e.g., mesoporous silica nanoparticles (MSNPs), cationic polymers, including PEI (polyethylenimine) polymer based approaches or polymers like DEAE-dextran, or non-covalent surface attachment of PEI to generate cationic surfaces, lipid or polymeric vesicles, or combinations thereof. Lipid or polymeric vesicles may be selected, for example, from lipids, liposomes, lipid encapsulation systems, nanoparticles, small nucleic acid-lipid particle formulations, polymers, and polymersomes.

[0041] The term "derivative" or "descendant" or "progeny" as used herein in the context of a prokaryotic or a eukaryotic cell, preferably a plant or plant cell or plant material according to the present disclosure relates to the descendants of such a cell or material which result from natural reproductive propagation including sexual and asexual propagation. It is well known to the person having skill in the art that said propagation can lead to the introduction of mutations into the genome of an organism resulting from natural phenomena which results in a descendant or progeny, which is genomically different to the parental organism or cell, however, still belongs to the same genus/species and possesses mostly the same characteristics as the parental recombinant host cell. Such derivatives or descendants or progeny resulting from natural phenomena during reproduction or regeneration are thus comprised by the term of the present disclosure. These terms, therefore, do not refer to any arbitrary derivative, descendant or progeny, but rather to a derivative, or descendant or progeny phylogenetically associated with, i.e., based on, a parent cell thereof, whereas this relationship between the derivative, descendant or progeny and the "parent" is clearly inferable by a person skilled in the art. "Progeny" comprises any subsequent generation of a plant, plant cell, plant tissue, or plant organ.

[0042] Furthermore, the term "derivative" can imply, in the context of a substance or molecule rather than referring to a cell or organism, directly or by means of modification indirectly obtained from another. This might imply a nucleic acid sequence derived from a cell or a plant metabolite obtained from a cell or material.

[0043] Furthermore, the terms "derived", or "derived from" as used herein in the context of a biological sequence (nucleic acid or amino acid) or a molecule or a complex imply that the respective sequence is based on a reference sequence, for example from the sequence listing, or a database accession number, or the respective scaffold structure, i.e., originating from said sequence, whereas the reference sequence can comprise more sequences, e.g., the whole genome or a full polyprotein encoding sequence, of a virus, whereas the sequence "derived from" the native sequence may only comprise one isolated fragment thereof, or a coherent fragment thereof. In this context, a cDNA molecule or a RNA can be said to be "derived from" a DNA sequence serving as molecular template. The skilled person can thus easily define a sequence "derived from" a reference sequence, which will, by sequence alignment on DNA or amino acid level, have a high identity to the respective reference sequence and which will have coherent stretches of DNA/amino acids in common with the respective reference sequence (>75% query identity for a given length of the molecule aligned provided that the derived sequence is the query and the reference sequence represents the subject during a sequence alignment). The skilled person can thus clone the respective sequences based on the disclosure provided herein by means of polymerase chain reactions and the like into a suitable vector system of interest, or use a sequence as vector scaffold. The term "derived from" is thus no arbitrary sequence, but a sequence corresponding to a reference sequence it is derived from, whereas certain differences, e.g., certain mutations naturally occurring during replication of a recombinant construct within a host cell, cannot be excluded and are thus comprised by the term "derived from". Furthermore, several sequence stretches from a parent sequence can be concentrated in a sequence derived from the parent. The different stretches will have high or even 100% homology to the parent sequence.

[0044] The term an "endogenous" in the context of nucleic acid and/or amino acid sequences refers to the nucleic acid and/or amino acid as found in a plant genome in its natural form and natural genetic context. As it is known to the skilled person, several variants, e.g., allelic variants, of a gene nucleic acid sequence may exist in a given species of plants.

[0045] A "fungus" or "fungal pathogen" as used herein means any plant pathogenic fungus including oomycetes in any developmental stage, including spores, or any part of such a fungus, which can interact with a plant or plant part or cell to induce a response in said plant or plant part or cell.

[0046] As used herein, "fusion" can refer to a protein and/or nucleic acid comprising one or more non-native sequences (e.g., moieties). A fusion can be at the N-terminal or C-terminal end of the modified protein, or both, or within the molecule as separate domain. For nucleic acid molecules, the fusion molecule can be attached at the 5' or 3' end, or at any suitable position in between. A fusion can be a transcriptional and/or translational fusion. A fusion can comprise one or more of the same non-native sequences. A fusion can comprise one 10 or more of different non-native sequences. A fusion can be a chimera. A fusion can comprise a nucleic acid affinity tag. A fusion can comprise a barcode. A fusion can comprise a peptide affinity tag. A fusion can provide for subcellular localization of the site-specific effector or base editor (e.g., a nuclear localization signal (NLS) for targeting to the nucleus, a mitochondrial localization signal for targeting to the mitochondria, a chloroplast localization signal for targeting to a chloroplast, an endoplasmic reticulum (ER) retention signal, and the like). A fusion can provide a non-native sequence (e.g., affinity tag) that can be used to track or purify. A fusion can be a small molecule such as biotin or a dye such as alexa fluor dyes, Cyanine3 dye, Cyanine5 dye. The fusion can provide for increased or decreased stability. In some embodiments, a fusion can comprise a detectable label, including a moiety that can provide a detectable signal. Suitable detectable labels and/or moieties that can provide a detectable signal can include, but are not limited to, an enzyme, a radioisotope, a member of a specific binding pair; a fluorophore; a fluorescent reporter or fluorescent protein; a quantum dot; and the like. A fusion can comprise a member of a FRET pair, or a fluorophore/quantum dot donor/acceptor pair. A fusion can comprise an enzyme. Suitable enzymes can include, but are not limited to, horse radish peroxidase, luciferase, beta-25 galactosidase, and the like. A fusion can comprise a fluorescent protein. Suitable fluorescent proteins can include, but are not limited to, a green fluorescent protein (GFP), (e.g., a GFP from *Aequoria victoria*, fluorescent proteins from *Anguilla japonica*, or a mutant or derivative thereof), a red fluorescent protein, a yellow fluorescent protein, a yellow-green fluorescent protein (e.g., mNeonGreen derived from a tetrameric fluorescent protein from the cephalochordate *Branchiostoma lanceolatum*) any of a variety of fluorescent and colored proteins. A fusion can comprise a nanoparticle. Suitable nanoparticles can include fluorescent or luminescent nanoparticles, and magnetic nanoparticles, or nanodiamonds, optionally linked to a nanoparticle. Any optical or magnetic property or characteristic of the nanoparticle(s) can be detected. A fusion can comprise a helicase, a nuclease (e.g., Fok1), an endonuclease, an exonuclease (e.g., a 5' exonuclease and/or 3' exonuclease), a ligase, a nickase, a nuclease-helicase (e.g., Cas3), a DNA methyltransferase (e.g., Dam), or DNA demethylase, a histone methyltransferase, a histone demethylase, an acetylase (including for example and not limitation, a histone acetylase), a deacetylase (including for example and not limitation, a histone deacetylase), a phosphatase, a kinase, a transcription (co-) activator, a transcription (co-) factor, an RNA polymerase subunit, a transcription repressor, a DNA binding protein, a DNA structuring protein, a long non-coding RNA, a DNA repair protein (e.g., a protein involved in repair of either single- and/or double-stranded breaks, e.g., proteins involved in base excision repair, nucleotide excision repair, mismatch repair, NHEJ, HR, microhomology-mediated end joining (MMEJ), and/or alternative non-homologous end-joining (ANHEJ), such as for example and not limitation, HR regulators and HR complex assembly signals), a marker protein, a reporter protein, a fluorescent protein, a ligand binding protein (e.g., mCherry or a heavy metal binding protein), a signal peptide (e.g., Tat-signal sequence), a targeting protein or peptide, a subcellular localization sequence (e.g., nuclear localization sequence, a chloroplast localization sequence), and/or an antibody epitope, or any combination thereof.

[0047] The term "genetically modified" or "genetic manipulation" or "genetic(ally) manipulated" is used in a broad sense herein and means any modification of a nucleic acid sequence or an amino acid sequence, a target cell, tissue, organ or organism, which is accomplished by human intervention, either directly or indirectly, to influence the endogenous genetic material or the transciptome or the proteome of a target cell, tissue, organ or organism to modify it in a purposive way so that it differs from its state as found without human intervention. The human intervention can either take place in vitro or in vivo/in planta, or also both. Further modifications can be included, for example, one or more point mutation(s), e.g. for targeted protein engineering or for codon optimization, deletion(s), and one or more insertion(s) or deletion(s) of at least one nucleic acid or amino acid molecule (including also homologous recombination), modification of a nucleic acid or an amino acid sequence, or a combination thereof. The terms shall also comprise a nucleic acid molecule or an amino acid molecule or a host cell or an organism, including a plant or a plant material thereof which is/are similar to a comparable sequence, organism or material as occurring in nature, but which have been constructed by at least one step of purposive manipulation. A "targeted genetic manipulation" or "targeted (base) modification" as used herein is thus the result of a "genetic manipulation", which is effected in a targeted way, i.e. at a specific position in a target cell and under the specific suitable circumstances to achieve a desired effect in at least one cell, preferably a plant cell, to be manipulated, wherein the term implies that the sequence to be targeted and the corresponding modification are based on preceding sequence considerations so that the resulting modification can be planned in advance, e.g., based on available sequence information of a target site in the genome of a cell and/or based on the information of the target specificity (recognition or binding properties of a nucleic acid or an amino acid sequence, complementary base pairing and the like) of a molecular tool of interest.

[0048] The term "genome" refers to the entire complement of genetic material (genes and non-coding sequences) that is present in each cell of an organism, or virus or organelle, and/or a complete set of chromosomes inherited as a (haploid) unit from one parent. The genome thus also defines the "genotype" being the part of the genetic makeup of a given cell, and therefore of an organism or individual, which determines a specific characteristic (phenotype) of that cell/organism/individual.

[0049] The terms "genome editing", "genome engineering", or "gene editing/engineering" are used interchangeably herein and refer to strategies and techniques for the targeted, specific modification of any genetic information or genome of a living organism. As such, the terms comprise gene editing, but also the editing of regions other than gene encoding regions of a genome. It further comprises the editing or engineering of the nuclear (if present) as well as other genetic information of a cell. Furthermore, the terms "genome editing" and "genome engineering" also comprise an epigenetic editing or engineering, i.e., the targeted modification of, e.g., methylation, histone modification or of non-coding RNAs possibly causing heritable changes in gene expression.

[0050] "Germplasm", as used herein, is a term used to describe the genetic resources, or more precisely the DNA of an organism and collections of that material. In breeding technology, the term germplasm is used to indicate the collection of genetic material from which a new plant or plant variety can be created.

[0051] The terms "guide RNA", "gRNA" or "single guide RNA" or "sgRNA" are used interchangeably herein and either refer to a synthetic fusion of a CRISPR RNA (crRNA) and a trans-activating crRNA (tracrRNA), or the term refers to a single RNA molecule consisting only of a crRNA and/or a tracrRNA, or the term refers to a gRNA individually comprising a crRNA or a tracrRNA moiety. The tracr and the crRNA moiety thus do not necessarily have to be present on one covalently attached RNA molecule, yet they can also be comprised by two individual RNA molecules, which can associate or can be associated by non-covalent or covalent interaction to provide a gRNA according to the present disclosure. The terms "gDNA" or "sgDNA" or "guide DNA" are used interchangeably herein and either refer to a nucleic acid molecule interacting with an Argonaute nuclease. Both, the gRNAs and gDNAs as disclosed herein are termed "guiding nucleic acids" or "guide nucleic acids" due to their capacity to interacting with a site-specific nuclease and to assist in targeting said site-specific nuclease to a genomic target site.

[0052] The term "hybridization" as used herein refers to the pairing of complementary nucleic acids, i.e., DNA and/or RNA, using any process by which a strand of nucleic acid joins with a complementary strand through base pairing to form a hybridized complex. Hybridization and the strength of hybridization (i.e., the strength of the association between the nucleic acids) is impacted by such factors as the degree and length of complementarity between the nucleic acids, stringency of the conditions involved, the Tm of the formed hybrid, and the G:C ratio within the nucleic acids. The term hybridized complex refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bounds between complementary G and C bases and between complementary A and T/U bases. A hybridized complex or a corresponding hybrid construct can be formed between two DNA nucleic acid molecules, between two RNA nucleic acid molecules or between a DNA and an RNA nucleic acid molecule. For all constellations, the nucleic acid molecules can be naturally occurring nucleic acid molecules generated in vitro or in vivo and/or artificial or synthetic nucleic acid molecules. Hybridization as detailed above, e.g., Watson-Crick base pairs, which can form between DNA, RNA and DNA/RNA sequences, are dictated by a specific hydrogen bonding pattern, which thus represents a non-covalent attachment form according to the present invention. In the context of hybridization, the term "stringent hybridization conditions" should be understood to mean those conditions under which a hybridization takes place primarily only between homologous nucleic acid molecules. The term "hybridization conditions" in this respect refers not only to the actual conditions prevailing during actual agglomeration of the nucleic acids, but also to the conditions prevailing during the subsequent washing steps. Examples of stringent hybridization conditions are conditions under which primarily only those nucleic acid molecules that have at least at least 80%, preferably at least 85%, at least 90% or at least 95% sequence identity undergo hybridization. Stringent hybridization conditions are, for example: 4×SSC at 65° C. and subsequent multiple washes in 0.1×SSC at 65° C. for approximately 1 hour. The term "stringent hybridization conditions" as used herein may also

mean: hybridization at 68° C. in 0.25 M sodium phosphate, pH 7.2, 7% SDS, 1 mM EDTA and 1% BSA for 16 hours and subsequently washing twice with 2×SSC and 0.1% SDS at 68° C. Preferably, hybridization takes place under stringent conditions.

[0053] The term "introgression" as used herein refers to the transfer of at least one allele of a gene of interest on a genetic locus from one genetic background to another. For example, introgression can proceed through sexual crossing of two parents of the same species. Alternatively, the transfer of a gene allele can take place by recombination between two donor genomes, e.g., in a fused protoplast, wherein at least the donor protoplast carries the gene allele of interest in its genome. In any case, any progeny or derivatives comprising the gene allele of interest can then be subjected to repeated back-crossing steps with a plant line carrying a genetic background of interest to select for the gene allele of interest in the resulting derivatives or progeny. The result may be the fixation of the gene allele of interest such introgressed in a selected genetic background. The whole process of introgression can, for example, take place by a mixture of breeding strategies and techniques of molecular biology to achieve at a genotype/phenotype of interest for a given germplasm, plant, plant cell or plant material.

[0054] The term "locus" generally refers to a genetically defined region of a chromosome carrying a gene or, possibly, two or more genes so closely linked that genetically they behave as a single locus responsible for a phenotype.

[0055] As used herein, the terms "mutation" and "modification" are used interchangeably to refer to a deletion, insertion, addition, substitution, edit, strand break, and/or introduction of an adduct in the context of nucleic acid manipulation in vivo or in vitro. A deletion is defined as a change in a nucleic acid sequence in which one or more nucleotides is absent. An insertion or addition is that change in a nucleic acid sequence which has resulted in the addition of one or more nucleotides. A "substitution" or "edit" results from the replacement of one or more nucleotides by a molecule which is a different molecule from the replaced one or more nucleotides. For example, a nucleic acid may be replaced by a different nucleic acid as exemplified by replacement of a thymine by a cytosine, adenine, guanine, or uridine. Pyrimidine to pyrimidine (e.g., C to Tor T to C nucleotide substitutions) or purine to purine (e.g., G to A or A to G nucleotide substitutions) are termed transitions, whereas pyrimidine to purine or purine to pyrimidine (e.g., G to T or G to C or A to T or A to C) are termed transversions. Alternatively, a nucleic acid may be replaced by a modified nucleic acid as exemplified by replacement of a thymine by thymine glycol. Mutations may result in a mismatch. The term mismatch refers to a non-covalent interaction between two nucleic acids, each nucleic acid residing on a different nucleotide sequence or nucleic acid molecule, which does not follow the base-pairing rules. For example, for the partially complementary sequences 5'-AGT-3' and 5'-AAT-3', a G-A mismatch (a transition) is present.

[0056] "Near isogenic lines" or "NILs" as used herein are useful for identifying genes responsible for a phenotypic trait by mapping them to genetic chromosomes by analyzing NILs. To create a near isogenic line, an organism with the phenotype of interest, often a plant, is crossed with a standard line of the same plant. The F1 generation is selfed to produce the F2 generation. F2 individuals with the target trait are selected for crossing with the standard line (the recurrent parent). This process is repeated for several generations. The genetic make-ups of sister lines can be compared. Alleles derived from the donor parent that can be found in all sister lines are said to be associated with the trait.

[0057] The terms "nucleotide" and "nucleic acid" with reference to a sequence or a molecule are used interchangeably herein and refer to a single- or double-stranded DNA or RNA of natural or synthetic origin. The term nucleotide sequence is thus used for any DNA or RNA sequence independent of its length, so that the term comprises any nucleotide sequence comprising at least one nucleotide, but also any kind of larger oligonucleotide or polynucleotide. The term(s) thus refer to natural and/or synthetic deoxyribonucleic acids (DNA) and/or ribonucleic acid (RNA) sequences, which can optionally comprise synthetic nucleic acid analoga. A nucleic acid according to the present disclosure can optionally be codon optimized "Codon optimization" implies that the codon usage of a DNA or RNA is adapted to that of a cell or organism of interest to improve the transcription rate of said recombinant nucleic acid in the cell or organism of interest. The skilled person is well aware of the fact that a target nucleic acid can be modified at one position due to the codon degeneracy, whereas this modification will still lead to the same amino acid sequence at that position after translation, which is achieved by codon optimization to take into consideration the species-specific codon usage of a target cell or organism. Nucleic acid sequences according to the present application can carry specific codon optimization for the following non limiting list of organisms: *Hordeum vulgare, Sorghum bicolor, Secale cereale, Saccharum officinarium, Zea mays, Setaria italic, Oryza sativa, Oryza minuta, Oryza australiensis, Oryza alta, Triticum aestivum, Triticum durum*, Triticale, *Hordeum bulbosum, Brachypodium distachyon, Hordeum marinum, Aegilops tauschii, Ma/us domestica, Beta vulgaris, Helianthus annuus, Daucus glochidiatus, Daucus pusillus, Daucus muricatus, Daucus carota, Eucalyptus grandis, Erythranthe guttata, Genlisea aurea, Nicotiana sylvestris, Nicotiana tabacum, Nicotiana tomentosiformis, Nicotiana benthamiana, Solanum/ycopersicum, Solanum tuberosum, Coffea canephora, Vitis vinifera, Cucumis sativus, Marus notabilis, Arabidopsis thaliana, Arabidopsis lyrata, Arabidopsis arenosa, Crucihimalaya himalaica, Crucihimalaya wallichii, Cardamine flexuosa, Lepidium virginicum, Capsella bursa-pastoris, Olmarabidopsis pumila, Arabis hirsuta, Brassica napus, Brassica oleracea, Brassica rapa, Brassica juncacea, Brassica nigra, Raphanus sativus, Eruca vesicaria sativa, Citrus sinensis, Jatropha curcas, Glycine max, Gossypium* ssp., or *Populus trichocarpa*

[0058] The term "particle bombardment" as used herein, also named "biolistic transfection" or "microparticle-mediated gene transfer", refers to a physical delivery method for transferring a coated microparticle or nanoparticle comprising a nucleic acid or a genetic construct of interest into a target cell or tissue. The micro or nanoparticle functions as projectile and is fired on the target structure of interest under high pressure using a suitable device, often called gene-gun. The transformation via particle bombardment uses a microprojectile of metal covered with the gene of interest, which is then shot onto the target cells using an equipment known as "gene gun" (Sanford, John C., et al. "Delivery of substances into cells and tissues using a particle bombardment

process." *Particulate Science and Technology* 5.1 (1987): 27-37) at high velocity fast enough (~1500 km/h) to penetrate the cell wall of a target tissue, but not harsh enough to cause cell death. For protoplasts, which have their cell wall entirely removed, the conditions are different logically. The precipitated nucleic acid or the genetic construct on the at least one microprojectile is released into the cell after bombardment, and integrated into the genome. The acceleration of microprojectiles is accomplished by a high voltage electrical discharge or compressed gas (helium). Concerning the metal particles used it is mandatory that they are non-toxic, non-reactive, and that they have a lower diameter than the target cell. The most commonly used are gold or tungsten. There is plenty of information publicly available from the manufacturers and providers of gene-guns and associated system concerning their general use.

[0059] A "pathogen" as used herein refers to an organism which can infect a plant, or which can cause a disease in a plant. Pathogens which can infect a plant, or which can cause a disease in a plant, include fungi, oomycetes, bacteria, viruses, viroids, virus-like organisms, phytoplasmas, protozoa, nematodes and parasitic plants. Plant parasites can cause damage by feeding on a plant and can be selected from ectoparasites like insects, comprising aphids and other sap-sucking insect, mites, and vertebrates.

[0060] The term "plant" as used herein is to be construed broadly and refers to a whole plant organism, a plant organ, differentiated and undifferentiated plant tissues, plant cells, seeds, and derivatives and progeny thereof. "Plant cells" include without limitation, for example, cells from seeds, from mature and immature embryos, meristematic tissues, seedlings, callus tissues in different differentiation states, leaves, flowers, roots, shoots, gametophytes, grains, kernels, sporophytes, pollen and microspores, protoplasts, macroalgae and microalgae. The different plant cells can either be haploid, diploid or multiploid. The term "plant organ" refers to plant tissue or a group of tissues that constitute a morphologically and functionally distinct part of a plant. Typically, the term "grain" is used to describe the mature kernel produced by a plant grower for purposes other than growing or reproducing the species, and "seed" means the mature kernel used for growing or reproducing the species. For the purposes of the present invention, "grain", "seed", and "kernel", will be used interchangeably.

[0061] A "plant material" as used herein refers to any material which can be obtained from a plant during any developmental stage. The plant material can be obtained either in planta or from an in vitro culture of the plant or a plant tissue or organ thereof. The term thus comprises plant cells, tissues and organs as well as developed plant structures as well as sub-cellular components like nucleic acids, polypeptides and all chemical plant substances or metabolites which can be found within a plant cell or compartment and/or which can be produced by the plant, or which can be obtained from an extract of any plant cell, tissue or a plant in any developmental stage. The term also comprises a derivative of the plant material, e.g., a protoplast, derived from at least one plant cell comprised by the plant material. The term therefore also comprises meristematic cells or a meristematic tissue of a plant.

[0062] A "control" or "control plant cell" or "control tissue" or "control organ" or "control plant" provides reference point for measuring changes in phenotype of a subject plant or plant part in which (genetic) modification, modulation and/or alteration, such as indicated in the various aspects of the present invention, has been affected to a gene, a protein or a substance or molecule of interest. A control plant or control plant part (e.g. control plant cell, control tissue, or control organ) may comprise, for example: (a) a wild-type plant or cell, i.e., of the same genotype as the starting material for the (genetic) modification, modulation and/or alteration which resulted in the subject plant or plant part; (b) a plant or plant part of the same genotype as the starting material but which has been transformed with a null construct (i.e., with a construct which has no known effect on the trait of interest, such as a construct comprising a marker gene); or (c) a plant or plant part which is a non-transformed segregant among progeny of a subject plant or plant part. In particular, a control plant or control plant cell may comprise a plant or plant part of the same genotype, but lacking the modification of the at least one gene encoding at least one wall-associated kinase or the modulation of the expression level of at least one wall-associated kinase and/or the transcription level, the expression level, or the function of at least one molecule within the signaling pathway from the at least one wall-associated kinase to the synthesis of at least one benzoxazinoid or within the synthesis pathway of at least one benzoxazinoid.

[0063] A "plasmid" refers to a circular autonomously replicating extrachromosomal element in the form of a double-stranded nucleic acid sequence. In the field of genetic engineering these plasmids are routinely subjected to targeted modifications by inserting, for example, genes encoding a resistance against an antibiotic or an herbicide, a gene encoding a target nucleic acid sequence, a localization sequence, a regulatory sequence, a tag sequence, a marker gene, including an antibiotic marker or a fluorescent marker, and the like. The structural components of the original plasmid, like the origin of replication, are maintained. According to certain embodiments of the present invention, the localization sequence can comprise a nuclear localization sequence, a plastid localization sequence, preferably a mitochondrion localization sequence or a chloroplast localization sequence, or a localization sequence for targeting a kinase of interest to the plasma membrane of a cell of interest. Said localization sequences are available to the skilled person in the field of plant biotechnology. A variety of plasmid vectors for use in different target cells of interest is commercially available and the modification thereof is known to the skilled person in the respective field.

[0064] The terms "protein", "amino acid" or "polypeptide" are used interchangeably herein and refer to an amino acid sequence having a catalytic enzymatic function or a structural or a functional effect. The term "amino acid" or "amino acid sequence" or "amino acid molecule" comprises any natural or chemically synthesized protein, peptide, polypeptide and enzyme or a modified protein, peptide, polypeptide and enzyme, wherein the term "modified" comprises any chemical or enzymatic modification of the protein, peptide, polypeptide and enzyme, including truncations of a wild-type sequence to a shorter, yet still active portion. In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook, Fritsch & Maniatis, *Molecular Cloning: A Laboratory Manual*, Second Edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (herein

"Sambrook et al., 1989"); *DNA Cloning: A Practical Approach*, Volumes I and II (D. N. Glover ed. 1985); Oligonucleotide Synthesis (M. J. Gait ed. 1984); *Nucleic Acid Hybridization* (B. D. Hames & S J. Higgins eds. (1985); *Transcription and Translation* (B. D. Hames & S. J. Higgins, eds. (1984); among others.

[0065] The term "regulatory sequence" or "regulatory region" as used herein refers to a nucleic acid or an amino acid sequence, which can direct and/or influence the transcription and/or translation and/or modification of a nucleic acid sequence of interest. A regulatory sequence may be a promoter sequence, an enhancer, a silencer, a transcription factor and the like.

[0066] The terms "resistance" or "tolerance" or "resistant" or "tolerant" as used herein refers to the capacity of a plant to resist to the phenotype as caused by infestation with a pathogen, in particular a fungal pathogen, disclosed herein to a certain degree, i.e., the prevention, reduction or delay of an infection caused by a (fungal) pathogen. "Resistance"/"Tolerance", therefore, does not exclusively refer to a "black or white" phenotype implying a phenotype where no symptoms occur at all after infestation for a resistant plant. The terms "resistance" or "tolerance" or "resistant" or "tolerant" rather imply a gradual improvement of infestation symptoms as observed for a plant having no resistance to a given pathogen. A classification score scheme for phenotyping experiments in field trials at various locations with natural and artificial *H. turcicum* inoculation (from the Deutsche Maiskomitee (DMK, German maize committee); AG variety 27.02.02; (DMK J. Rath; R P Freiburg H. J. Imgraben) shows a resistance level from 9 (low) to 1 (high) for maize as exemplary plant, wherein each score represents the following phenotype: 1: Plants exhibit no symptoms of disease, 0%; 2: Beginning of infestation, first small spots (less than 2 cm) visible. Less than 5% of leaf surface affected. 3: Some spots have developed on a leaf stage. Between 5-10% of leaf surface affected. 4: 10-20% of leaf surface affected. Clearly visible spots on several leaf stages. 5: 20-40% of leaf surface affected. Spots start to coalesce. 6: 40-60% of leaf surface affected. Systematic infestation visible on leaves. 7: 60-80% of leaf surface affected. Approximately half of leaves destroyed or dried out because of fungal infestation. 8: 80-90% of leaf surface affected. More than half of leaves destroyed or dried out because of fungal infestation. 9: 90-100% of leaf surface affected. The plants are almost completely dried out.

[0067] The term "TILLING" as used herein is an abbreviation for "Targeting Induced Local Lesions in Genomes" and describes a well-known reverse genetics technique designed to detect unknown SNPs (single nucleotide polymorphisms) in genes of interest using an enzymatic digestion and is widely employed in plant genomics. The technique allows for the high-throughput identification of an allelic series of mutants with a range of modified functions for a particular gene. TILLING combines mutagenesis (e.g., chemical or via UV-light) with a sensitive DNA screening-technique that identifies single base mutations.

[0068] The terms "transgene" or "transgenic" as used herein refer to at least one nucleic acid sequence that is taken from the genome of one organism, or produced synthetically, and which is then introduced into a host cell or organism or tissue of interest and which is subsequently integrated into the host's genome by means of "stable" transformation or transfection approaches. In contrast, the term "transient" transformation or transfection or introduction refers to a way of introducing molecular tools including at least one nucleic acid (comprising at least one of DNA, RNA, single-stranded or double-stranded or a mixture thereof) and/or at least one amino acid sequence, optionally comprising suitable chemical or biological agents, to achieve a transfer into at least one compartment of interest of a cell, including, but not restricted to, the cytoplasm, an organelle, including the nucleus, a mitochondrion, a vacuole, a chloroplast, or into a membrane, resulting in transcription and/or translation and/or association and/or activity of the at least one molecule introduced without achieving a stable integration or incorporation and thus inheritance of the respective at least one molecule introduced into the genome of a cell.

[0069] The term "transient introduction" as used herein thus refers to the transient introduction of at least one nucleic acid and/or amino acid sequence according to the present disclosure, preferably incorporated into a delivery vector or into a recombinant construct, with or without the help of a delivery vector, into a target structure, for example, a plant cell, wherein the at least one nucleic acid sequence is introduced under suitable reaction conditions so that no integration of the at least one nucleic acid sequence into the endogenous nucleic acid material of a target structure, the genome as a whole, occurs, so that the at least one nucleic acid sequence will not be integrated into the endogenous DNA of the target cell. As a consequence, in the case of transient introduction, the introduced genetic construct will not be inherited to a progeny of the target structure, for example a prokaryotic or a plant cell. The at least one nucleic acid and/or amino acid sequence or the products resulting from transcription, translation, processing, post-translational modifications or complex building thereof are only present temporarily, i.e., in a transient way, in constitutive or inducible form, and thus can only be active in the target cell for exerting their effect for a limited time. Therefore, the at least one sequence or effector introduced via transient introduction will not be heritable to the progeny of a cell. The effect mediated by at least one sequence or effector introduced in a transient way can, however, potentially be inherited to the progeny of the target cell.

[0070] A "variant" in the context of a nucleic acid or amino acid sequence protein means a nucleic acid or amino acid sequence derived from the native nucleic acid or amino acid sequence, or another starting sequence, by deletion (so-called truncation) or addition of one or more sequences to the 5'/N-terminal and/or 3'/C-terminal end of the native nucleic acid or amino acid sequence; deletion or addition of one or more nucleic acid or amino acid sequence at one or more sites in the native nucleic acid or amino acid sequence; or substitution of one or more nucleic acid or amino acid sequence at one or more sites in the native protein. Variant proteins encompassed by the present invention are biologically active, that is they continue to possess all or some of the activity of the native proteins of the invention as described herein. Such variants may result from, for example, genetic polymorphism or from human manipulation.

[0071] As used herein, a "homolog" means a protein in a group of proteins that perform the same biological function, e.g. proteins that belong to the same Pfam protein family. Homologs are expressed by homologous genes. With reference to homologous genes, homologs include orthologs,

e.g., genes expressed in different species that evolved from a common ancestral genes by speciation and encode proteins retain the same function, but do not include paralogs, e.g., genes that are related by duplication but have evolved to encode proteins with different functions. Homologous genes include naturally occurring alleles and artificially-created variants. Degeneracy of the genetic code provides the possibility to substitute at least one base of the protein encoding sequence of a gene with a different base without causing the amino acid sequence of the polypeptide produced from the gene to be changed. When optimally aligned, homolog proteins have typically at least about 60% identity, in some instances at least about 70%, for example about 80% or 85% and even at least about 90%, 92%, 94%, 96%, 97%, 98%, 99% or 99.5% identity, preferably over the full length of the protein; homologous genes have typically at least about 60% identity, in some instances at least about 70%, for example about 80% or 85% and even at least about 90%, 92%, 94%, 96%, 97%, 98%, 99% or 99.5% identity, preferably over the full length of the gene, in particular the coding regions of the gene. Homologs are identified by comparison of amino acid sequence, e.g. manually or by use of a computer-based tool using known homology-based search algorithms such as those commonly known and referred to as BLAST, FASTA, and Smith-Waterman. A local sequence alignment program, e.g. BLAST, can be used to search a database of sequences to find similar sequences, and the summary Expectation value (E-value) used to measure the sequence base similarity. Because a protein hit with the best E-value for a particular organism may not necessarily be an ortholog, e.g., have the same function, or be the only ortholog, a reciprocal query is used to filter hit sequences with significant E-values for ortholog identification. The reciprocal query entails search of the significant hits against a database of amino acid sequences from the base organism that are similar to the sequence of the query protein. A hit can be identified as an ortholog, when the reciprocal query's best hit is the query protein itself or a protein encoded by a duplicated gene after speciation. A further aspect of the homologs encoded by DNA useful in the transgenic plants of the invention are those proteins that differ from a disclosed protein as the result of deletion or insertion of one or more amino acids in a native sequence.

[0072] Other functional homolog proteins differ in one or more amino acids from those disclosed herein as the result of one or more of the well-known conservative amino acid substitutions, e.g., valine is a conservative substitute for alanine and threonine is a conservative substitute for serine. Conservative substitutions for an amino acid within the native sequence can be selected from other members of a class to which the naturally occurring amino acid belongs. Representative amino acids within these various classes include, but are not limited to: (1) acidic (negatively charged) amino acids such as aspartic acid and glutamic acid; (2) basic (positively charged) amino acids such as arginine, histidine, and lysine; (3) neutral polar amino acids such as glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; and (4) neutral nonpolar (hydrophobic) amino acids such as alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine. Conserved substitutes for an amino acid within a native amino acid sequence can be selected from other members of the group to which the naturally occurring amino acid belongs. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulfur-containing side chains is cysteine and methionine. Naturally conservative amino acids substitution groups are: valine-leucine, valine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, aspartic acid-glutamic acid, and asparagine-glutamine. A further aspect of the invention includes proteins that differ in one or more amino acids from those of a described protein sequence as the result of deletion or insertion of one or more amino acids in a native sequence.

[0073] Whenever the present disclosure relates to the percentage of the homology or identity of nucleic acid or amino acid sequences these values define those as obtained by using the EMBOSS Water Pairwise Sequence Alignments (nucleotide) programme (www.ebi.ac.uk/Tools/psa/emboss_water/nucleotide.html) nucleic acids or the EMBOSS Water Pairwise Sequence Alignments (protein) programme (www.ebi.ac.uk/Tools/psa/emboss_water/) for amino acid sequences, preferably over the entire length of the sequence, i.e., any percentage value provided means the % homology or % identity as measured over the whole length of a subject or starting sequence in comparison to an identical or variant further sequence. Those tools provided by the European Molecular Biology Laboratory (EMBL) European Bioinformatics Institute (EBI) for local sequence alignments use a modified Smith-Waterman algorithm (see www.ebi.ac.uk/Tools/psa/ and Smith, T. F. & Waterman, M. S. "Identification of common molecular subsequences" *Journal of Molecular Biology,* 1981 147 (1):195-197). When conducting an alignment, the default parameters defined by the EMBL-EBI are used. Those parameters are (i) for amino acid sequences: Matrix=BLOSUM62, gap open penalty=10 and gap extend penalty=0.5 or (ii) for nucleic acid sequences: Matrix=DNAfull, gap open penalty=10 and gap extend penalty=0.5.

BRIEF DESCRIPTION OF THE DRAWINGS

[0074] FIG. **1** (FIGS. **1** A and B) shows (FIG. **1** A) the structure of benzoxazinone (BXD) in the aglucone form. Derivatives from the BXD basic structure of FIG. **1** A are: HBOA (R^1=H, R^2=H, R^3=H), DHBOA (R^1=H, R^2=OH, R^3=H), HMBOA (R^1=H, R^2=OMe, R^3=H), HM_2BOA (R^1=H, R^2=OMe, R^3=OMe), DIBOA (R^1=OH, R^2=H, R^3=H), TRIBOA (R^1=OH, R^2=OH, R^3=H), DIMBOA (R^1=OH, R^2=OMe, R^3=H), DIM_2BOA (R^1=OH, R^2=OMe, R^3=OMe), HDMBOA (R^1=OMe, R^2=OMe, R^3=H), or HDM2BOA (R^1=OMe, R^2=OMe, R^3=OMe). FIG. **1** B shows the basic structure of a benzoxazolinone, a natural degradation product of BXDs and also comprised by the term BXD as used herein. Specific benzoxazolinones are BOA (R^2=H, R^3=H), MBOA (R^2=OMe, R^3=H), or M_2BOA (R^2=OMe, R^3=OMe).

[0075] FIG. **2** (FIGS. **2** A and B) shows the rate of successful penetration events for fungal penetrations as described in Example 9. (FIG. **2** A) Hyphae detected inside of host tissues. In the upper and lower panels the focus is on the epidermis and hyphae, respectively. The arrows in the

lower image of Fig. A indicate the hyphae inside the host tissues. (FIG. **2** B) RLK1b, RLK1d and RLK1f are ZmWAK-RLK1 mutants with compromised NCLB resistance that were produced in RP3Htn1, while RLK1b-wt, RLK1d-wt and RLK1f-wt are the corresponding sister lines, respectively. Statistics was conducted using Student's t test, based on three independent experiments. The asterisks represent a significant difference of **p<0.01 or *p<0.05. Error bars indicate ±standard error.

[0076] FIG. **3** (FIG. **3** A to C) shows the disease phenotype of Htn1-NILs and the corresponding parents as detailed in Example 10 below. (FIG. **3** A) The disease symptom of the second leaves at 16 dpi (from left to right for the lines w22, w22Htn1, B37 and B37Htn1). (FIG. **3** B) Rate of infected of tested plants B37, w22, w22Htn1, and B37Htn1. (FIG. **3** C) Area under the disease progress curve (AUDPC) for the isogenic lines detailed below the x-axis. *AUDPC in this panel was calculated as described in Hurni et al. 2015, based on calculating sum of rate of infected plants (%).

[0077] FIG. **4** shows the result of a transcriptome analysis in Htn1-NILs, which revealed a set of DEGs. FIG. **4** shows the number of DEGs in the two Htn1 NILs compared to the corresponding susceptible lines as further described in Example 10 below.

[0078] FIG. **5** (FIG. **5** A to F) shows the content of BXDs in w22 and w22Htn1. (FIG. **5** A) shows the proposed bio-synthesis pathway of secondary metabolites BXDs. The genes encoding the proteins catalyzing each step of enzymatic reactions are presented besides and below the arrows. The contents of BXDs compounds DIMBOA-Glc (FIG. **5** B), DIMBOA (FIG. **5** C), HMBOA-Glc (FIG. **5** D), DIM_2BOA-Glc (FIG. **5** E) and HDMBOA-Glc (FIG. **5** F) were determined at before inoculation, at 3 dpi and 10 dpi. The statistics were conducted using Tukey's HSD (P=0.05) in eight biological replicates. The ns stands for no significance. Error bars are ±SE. See also Example 10 below.

[0079] FIG. **6** (FIG. **6** A to D) shows that the presence of ZmWAK-RLK1 results in decreased DIM_2BOA-Glc content possibly by down-regulating Igl expression in the maize RP3 genetic background (see also Example 11). (A) Content of DIM_2BOA-Glc (n=8). Expression analysis of Bx1 (B), Igl (C) and ZmWAK-RLK1 (D) in Htn1 mutants and sister lines before infection at 21 days after sowing (n=5). Statistics was conducted using Student's t test. The asterisks represent a significant difference of **p<0.01 or *p<0.05. Error bars are ±SE.

[0080] FIG. **7** (FIG. **7** A to E) shows the content of BXDs compounds DIMBOA-Glc (A), DIMBOA (B), HMBOA-Glc (C), and DIM_2BOA-Glc (D), HDMBOA-Glc (E) in second leaves at 10 dpi. Further information is provided in Example 12 below. The statistics were conducted using Student's t test (P=0.05) in eight biological replicates. The asterisks represent a significant difference of **p<0.01 or *p<0.05. The ns stands for no significance. Error bars are ±SE.

[0081] FIG. **8** (FIG. **8** A to D) (see also Example 12) shows that compromising the biosynthesis of BXDs decreases the susceptibility of NCLB disease at the seedling stage. (FIGS. **8** A and B): Visual symptoms and quantified NCLB disease severity in bx mutants. (FIG. **8** C): The transcriptional level of ZmWAK-RLK1 at 10 dpi. (FIG. **8** D) shows the proposed model of ZmWAK-RLK1 underlying NCLB disease resistance. The resistance allele suppressed the biosynthesis of major BXDs compounds, which likely served as the susceptibility component for promoting NCLB disease. Statistic test was conducted using Student's t test. The asterisks represent a significant difference of **p<0.01 or *p<0.05. The ns stands for no significance. Error bars are ±SE.

[0082] FIG. **9** (FIG. **9** A to D) shows the content of BXDs compounds DIMBOA-Glc (A), DIMBOA (B), HMBOA-Glc (C), and HDMBOA-Glc (D) in second leaves of Htn1 NILs and mutants at 21 days after sowing. Further information is provided in Example 12 below. The statistics were conducted using Student's t test (P=0.05) in eight biological replicates. The asterisks represent a significant difference of **p<0.01 or *p<0.05. The ns stands for no significance. Error bars are ±SE.

[0083] FIG. **10** shows relative RLK1 expression in tissues. These samples were harvested from 21 days old seedlings without pathogen inoculation. Different lower case letters in the graphs indicate a difference which is statistically different. See also Example 13 below.

[0084] FIG. **11** (FIGS. **11** A and P) shows the transcription levels of genes in Htn1-NILs and the corresponding parental lines. The expression of genes (FIG. **11** A) ZmWAK-RLK1, (FIG. **11** B) Bx1, (FIG. **11** C) Igl, (FIG. **11** D) Bx2, (FIG. **11** E) Bx3, (FIG. **11** F) Bx4, (FIG. **11** G) Bx5, (FIG. **11** H) Bx6, (FIG. **11** I) Bx7, (FIG. **11** J) Bx8, (FIG. **11** K) Bx9, (FIG. **11** L) Bx10/11, (FIG. **11** M) Bx12, (FIG. **11** N) Bx13, (FIG. **11** O) Glu1 and (FIG. **11** P) Glu2 were quantified. The different colors and patterns of the bars indicate timepoints before and after infection as shown in the legend for FIG. **11** A which also applies for FIG. **11** B to P. The statistics were conducted separately in w22 and B37 genetic background using Tukey's HSD (P=0.05) in four biological replicates. Error bars are ±SE. Different lower case letters in the graphs indicate a difference which is statistically different. See also Example 10 below.

[0085] FIG. **12** (FIG. **12** A to C) shows ZmWAK-RLK1 localization to the plasma membrane. (FIG. **12** A-B) Fluorescent signals in onion epidermal cells after transient expression of ZmWAK-RKL1-eGFP (SEQ ID NO: 9) and the positive control PIP2A-mCherry that is known to localize to the plasma membrane (Kammerloher, Werner, et al. "Water channels in the plant plasma membrane cloned by immunoselection from a mammalian expression system." *The Plant Journal* 6.2 (1994): 187-199). Signals are shown before (FIG. **12** A) and after (FIG. **12** B) plasmolysis with 0.8 M mannitol. (FIG. **12** C) Fluorescent signals in *N. benthamiana* leaves two days after infiltration. Notably, the signals shown in white in the respective graphs correspond to the originally green (column 1 RLK_eGFP), red (column 2 PIP2A_mCherry), and yellow (merge, column 4) fluorescent signal. Column 3 (DIC) represents the differential interference contrast to visualize the cellular structures as control.

[0086] FIG. **13** is a table showing the log FC and annotation of 215 the differentially expressed genes (DEGs) detected in B37Htn1/B37 and w22Htn1/w22 in at least one of timepoints.

DETAILED DESCRIPTION

[0087] Based on the experiments and data underlying the present invention, it was found that the genes involved in the biosynthesis of benzoxazinoids (BXDs) and derivatives thereof, mainly hydrolysis based derivatives like hydroxamic acids, are not only involved in the defense mechanism against *E. turcicum* but rather can also effect and

mediate plant-defense, i.e., resistance mechanisms against various other fungal pathogens in a series of crop plants. The present invention thus implements both the link between a wall associated kinases (WAK), downstream signaling molecules, such as Bx1, Bx2, Bx6, Bx14 and Igl, or any other enzyme involved in the benzoxazinoid synthesis pathway, and the decrease of BXD secondary metabolites and further technically implements the finding that this decrease of BXD secondary metabolites is associated with increased fungal resistance.

[0088] The present invention thus provides in a first aspect a method for producing a plant having increased fungal resistance, wherein the fungal resistance is regulated by at least one wall-associated kinase, the method comprising: (i) (a) providing at least one plant cell, tissue, organ, or whole plant having a specific genotype with respect to the presence of at least one gene encoding a wall-associated kinase in the genome of said plant cell, tissue, organ, or whole plant; or (i) (b) introducing at least one gene encoding at least one wall-associated kinase into the genome of at least one cell of at least one of a plant cell, tissue, organ, or whole plant; and (ii) (a) modifying at least one gene encoding at least one wall-associated kinase in the at least one plant cell, tissue, organ, or whole plant; and/or (ii) (b) modulating the expression level of at least one wall-associated kinase and/or the transcription level, the expression level, or the function of at least one molecule within the signaling pathway from the at least one wall-associated kinase to the synthesis of at least one benzoxazinoid or within the synthesis pathway of at least one benzoxazinoid in the at least one plant cell, tissue, organ, or whole plant; and (iii) producing a population of plants from the at least one plant cell, tissue, organ, or whole plant; and (iv) selecting a plant having an increased fungal resistance, from the plant population, based on the determination of a reduced synthesis of a benzoxazinoid preferably in response to a fungal infection, wherein the selected plant have an increased fungal resistance based on the reduced synthesis of a benzoxazinoid, and/or wherein the synthesis of the benzoxazinoid is regulated by the at least one wall-associated kinase.

[0089] Wall associated kinases (WAKs) have recently been identified as major components of fungal and bacterial disease resistance in several cereal crop species. However, the molecular mechanisms of WAK-mediated resistance are presently largely unknown. According to this invention, the function of the maize gene ZmWAK-RLK1 (Htn1) that confers quantitative resistance to northern corn leaf blight (NCLB) caused by the hemibiotrophic fungal pathogen *Exserohilum turcicum* was investigated. ZmWAK-RLK1 (Htn1) was found to localize to the plasma membrane and its presence resulted in a modification of the infection process by specifically reducing pathogen penetration into host tissues. Furthermore, the ubiquitous expression of ZmWAK-RLK1 and the findings on the signaling pathway downstream of ZmWAK-RLK1 demonstrate the function of this and associated wall-associated kinases as master regulators and crucial signaling mediators in plant defense against fungal disease.

[0090] A transcriptome analysis of near-isogenic lines (NILs) differing for ZmWAK-RLK1 revealed that several genes involved in the biosynthesis of the secondary metabolites benzoxazinoids (BXDs) were differentially expressed in the presence of ZmWAK-RLK1. Particularly the content of BXD compounds DIMBOA-Glc, DIMBOA, HMBOA-Glc and DIM_2BOA-Glc were significantly lower in the NILs with ZmWAK-RLK1. Furthermore, DIM_2BOA-Glc, which is an inactive glucoside of BXDs, was significantly elevated in ZmWAK-RLK1 mutants with compromised NCLB resistance. In addition, maize mutants that were affected in BXDs biosynthesis showed reduced susceptibility to *E. turcicum* infection at the seedling stage. We thus conclude that BXD biosynthesis increases susceptibility to *E. turcicum* infection and that the ZmWAK-RLK1-mediated NCLB resistance results from a reduction of these compounds. These findings indicate a novel link between WAKs underlying quantitative disease resistance and the defense mechanism mediated by the secondary metabolites BXDs that have been known for their involvement in cereal insect resistance. The term "WAK" as used herein may comprise a plant receptor-like kinase associated with the signal transduction directly or indirectly effecting the biosynthesis of genes involved in the BXD synthesis, or interacting with signaling mechanism and/or protein-protein interactions being involved in the BXD synthesis.

[0091] The plant immune response "caused" by a WAK can thus be of direct or indirect nature. As it is known to the skilled person, receptor-like kinases usually comprise at least one extracellular signaling domain, e.g., for sensing PAMPs and/or DAMPs, a transmembrane domain, and an intracellular kinase domain. The kinase domain allows the WAK to transform the extracellular signal into an intracellular response transferred via a cascade of proteins involved in the downstream signal transduction. Usually, receptor kinases thus indirectly initiate the activation and transfer into the nucleus/organelle of a transcription factor which regulates the transcription of a target gene. Furthermore, plant WAKs can trigger defense responses such as reactive oxygen species (ROS) accumulation through the activation of a NADPH oxidase, nitric oxide production, callose deposition, besides a MAP kinase-mediated activation of defense gene expression. The terms "causing" or "caused" as used herein in the context of a WAK or another plant receptor kinase is thus to be construed broadly to comprise any direct or indirect effect the activity of the WAK can have on downstream signaling molecules, wherein the molecules can be selected from at least one amino acid sequence, preferably an enzyme in the signal transduction cascade downstream of the WAK or a peptide being able to stimulate or inhibit complex formation downstream of the WAK or signal transduction downstream of a WAK, a metabolite, such as any secondary metabolite produced by a plant, a ROS, or an indirect effect on the regulation of the transcription and/or translation of another downstream gene/protein. A physical interaction of the WAK in the form of a signaling complex may occur to cause an action. In another embodiment, the action caused by the WAK is mediated by a downstream molecule, e.g., a downstream kinase phosphorylating another molecule, in an indirect way. In the terminal part of the WAK signaling cascade, a transcription activator or repressor can be induced to regulate the transcription of a target gene of a WAK, preferably a target gene in the jasmonic acid and/or BXD biosynthesis pathway. Besides a protein-DNA interaction, WAK signaling can also imply protein-protein interactions influencing the BXD biosynthesis pathway.

[0092] In another aspect, the methods of the present invention further may comprise the step of introducing, modifying and/or modulating at least one further or other gene into at

least one plant cell, tissue, organ, or whole plant to provide a synergistic effect in increasing fungal disease by decreasing the synthesis of at least one BXD compound associated with fungal resistance. In preferred embodiments, the at least one further or other gene is selected from a bx1, bx2, igl, bx6, bx11, bx14, opr2, lox3 or aoc1 gene (SEQ ID NOs: 10, 12, 14, 16, 18, 20, 22, 24 or 26, respectively), or a homologous gene thereof, or the respective proteins encoded by said genes as set forth exemplary in SEQ ID NOs: 11, 13, 15, 17, 19, 21, 23, 25 or 27, respectively, or homologs thereof. As disclosed herein, certain genes involved in the jasmonic acid pathway, the ethylene pathway, the lignin synthesis pathway, a plant defense pathway, a further receptor-like kinase pathway, or a cell wall pathway, and preferably certain genes involved in the jasmonic acid pathway, contribute to the signaling pathway of at least one functional WAK, wherein there may be a synergistic effect provided by the presence of a specific functional WAK and a specific non-functional or less functional gene of the jasmonic acid pathway, as the presence of both will contribute to an even significantly reduced amount of a BXD compound of interest and thus a more than additive increase in fungal resistance.

[0093] The present invention thus provides specific target genes which can be modulated in addition or alternatively to the at least one WAK of interest to provide a significantly improved fungal defense strategy for a plant of interest. These results are based on different functional studies including comparative transcriptome analysis in defined specific WAK genotypes, namely in two pairs of near isogenic lines, w22 and W22Htn1 as well as B37 and B37Htn1 (see Example 10 below), after fungal specific stimuli by analyzing the RNA sequencing datasets. Furthermore, additional RT-qPCR experiments and systematic RNA sequencing were conducted to decipher the plant immune network as triggered by a WAK, e.g., Htn1, (Examples 6 and 10 and Tables 1 and 2). These data demonstrated a cross-talk between the WAK and the benzoxazinoid synthesis and jasmonic acid pathway and thus provided new candidates to provide new elite plant lines comprising both a specific WAK as well as a specific genotype with respect to enzymes involved in the benzoxazinoid synthesis and jasmonic acid pathway.

[0094] In one embodiment according to the present invention, the method for producing a plant having increased fungal resistance may comprise the modification of at least one gene encoding at least one wall-associated kinase, and optionally at least one further or other gene, for example a bx1, bx2, igl, bx6, bx11, bx14, opr2, lox3 or aoc1 gene, in the at least one plant cell, tissue, organ, or whole plant. The modification can be conducted by any means of plant breeding, including classical and modern methods of plant breeding, and/or techniques of molecular biology. Classical plant breeding methods may comprise the deliberate interbreeding (crossing) of closely or distantly related species to produce new crops with desirable properties. Plants are crossed to introduce traits/genes from a particular variety into a new genetic background to provide plants having modified and/or increased quality, yield, tolerance (against abiotic stress), resistance (against biotic stress), etc., characteristics. Breeding nowadays also includes methods like marker-assisted selection, reverse breeding and the targeted combination with molecular biology tools known and available to the skilled person.

[0095] In one embodiment according to the present invention, the modulation or modulating of at least one wall-associated kinase, and/or of at least one further or other gene, for example a bx1, bx2, igl, bx6, bx11, bx14, opr2, lox3 or aoc1 gene, can thus comprise at least one of modulating the expression level of at least one wall-associated kinase, preferably increasing the expression at least one wall-associated kinase, and/or modulating the function or activity of and/or activity of at least one wall-associated kinase, for example, by providing at least one molecule interacting with the extracellular signalling domain of at least one WAK, e.g., an activator, or by providing at least one molecule interacting with the intracellular signalling domain of at least one WAK, such as a molecule inducing or inhibiting kinase activity. In one embodiment, the modulation or modulating of at least one wall-associated kinase, and/or of at least one further or other gene, for example a bx1, bx2, igl, bx6, bx11, bx14, opr2, lox3 or aoc1 gene, can comprise the targeted introduction of at least one mutation into a WAK and/or a further or other gene of interest to modulate the activity of the WAK and the further or other protein encoded by the at least one further or other gene in a targeted way. Embodiments comprising the modulation of at least one wall-associated kinase thus aim at influencing the activity of the at least one WAK within without modifying the nucleic acid sequence and thus possibly the amino acid sequence of a WAK of interest. In certain embodiments, wherein at least one further or other gene, for example a bx1, bx2, igl, bx6, bx11, bx14, opr2, lox3 or aoc1 gene, is modulated, the modulation may aim at reducing the activity of a at least one allele of a bx1, bx2, igl, bx6, bx11, bx14, opr2, lox3 or aoc1 gene to decrease the amount of BXD compound synthesized. For example, BX1 and IGL enzymes are accountable for the bulk of BX biosynthesis. Therefore, inhibiting the presence of a functional BX enzyme, preferably a BX1, BX2 or BX6 enzyme, or an Igl enzyme can contribute to the provision of a reduced BXD synthesis and thus an increased fungal resistance in a plant of interest.

[0096] The modulation according to the present invention can comprise any direct or indirect interaction between two molecules, i.e., a receptor-ligand interaction, a transcription factor-transcription factor binding site interaction, an interaction of an enzyme, e.g., a kinase with its target site, an interaction of a peptide or nucleic acid modulator with a target site, an antibody-antigen interaction, an interaction with a DNA or histone binding protein and its cognate ligand (DNA or histone), a hybridization between two nucleic acid sequences/molecules and the like.

[0097] In one embodiment, the transcription level of at least one WAK within at least one cell of at least one of a plant cell, tissue, organ, or whole plant can be modified or modulated by specifically influencing a regulatory sequence of a WAK gene. In another embodiment, the modulation affects at least one gene, for example a bx1, bx2, igl, bx6, bx11, bx14, opr2, lox3 or aoc1 gene. This modulation or modification can comprise the introduction of at least one specific mutation, for example to activate a promoter of interest, or the modulation or modification can be in trans by providing a transcription factor modulating the transcription of at least one WAK gene, wherein the at least one WAK gene according to all embodiments of the present invention may comprise an endogenously occurring WAK gene, or a

WAK gene introduced into at least one cell of at least one of a plant cell, tissue, organ, or whole plant.

[0098] According to the various aspects and embodiments of the present invention, a signalling pathway from the at least one wall-associated kinase to the synthesis of at least one benzoxazinoid in at least one plant cell, tissue, organ, or whole plant thus implies the whole chain of molecular actions downstream of a WAK as sensing molecule triggering a signalling cascade involving various different effectors until the synthesis of a BXD compound.

[0099] In one embodiment according to the various aspects of the present invention, the at least one wall-associated kinase is WAK-RLK1, preferably selected from Htn1, Ht2, or Ht3, or an allelic variant thereof, a mutant or a functional fragment thereof, or a gene encoding the same, preferably wherein the at least one wall-associated kinase a) is encoded by a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 or 7, or a functional fragment thereof, b) is encoded by a nucleic acid molecule comprising the nucleotide sequence having at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to nucleotide sequence of SEQ ID NO: 1 or 7, preferably over the entire length of the sequence, c) is encoded a nucleic acid molecule hybridizing with a complementary sequence to a) or b) under stringent conditions, d) is encoded by a nucleic acid molecule comprising the nucleotide sequence coding for an amino acid sequence of SEQ ID NO: 2 or 8, or a functional fragment thereof, e) is encoded by a nucleic acid molecule comprising the nucleotide sequence coding for an amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to amino acid sequence of SEQ ID NO: 2 or 8, preferably over the entire length of the sequence, f) comprising the amino acid sequence of SEQ ID NO: 2 or 8, or g) comprising an amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to amino acid sequence of SEQ ID NO: 2 or 8, preferably over the entire length of the sequence, provided that any sequence of a) to g), optionally after expression, still encodes at least one functional Htn1, Ht2, or Ht3, or an allelic variant, a mutant, or a functional fragment thereof. In a preferred embodiment, the at least one wall-associated kinase is selected from Htn1 (RLK1) or an allelic variant, a mutant or a functional fragment thereof, or a gene encoding the same. Variants may further comprise any functional splice variant of a WAK gene. As it is known to the skilled person, eukaryotic mRNA comprising introns is spliced during processing from a precursor mRNA into a mature mRNA giving rise to a protein after translation (protein biosynthesis).

[0100] "Functional" or "functional fragment" or "variant" as used in connection with a WAK or any other receptor-like kinase or any at least one further gene/protein, for example a bx1, bx2, igl, bx6, bx11, bx14, opr2, lox3 or aoc1 gene, or the corresponding proteins Bx1, Bx2, Igl, Bx6, Bx11, Bx14, Opr2, Lox3 or Aoc1, according to the present disclosure means a fragment of an amino acid or nucleic acid sequence with reference to the respective (longer) sequence occurring in the natural environment of a plant genome of interest, whereas the functional fragment still comprises—optionally after transcription, processing and translation—at least one function of the respective parent sequence. The functional fragment may be less sterically demanding and thus more convenient for certain approaches. Furthermore, the functional fragment may be fused to another domain to create a fusion molecule for functional assays, e.g., a fusion with a gene encoding a protein having fluorescence activity. In another embodiment, the functional fragment may be fused to a tag and the like. Therefore, a functional fragment may also comprise a sequence comprising codon optimizations on the nucleic acid level, or comprising certain mutations, said mutations not influencing the activity or function of a WAK, or another receptor-like kinase of interest.

[0101] Preferably, any function variant at least comprises a truncated form of the extracellular signalling domain of a WAK and an active intracellular kinase domain, wherein the intracellular kinase domain is able to initiate downstream signalling. Notably, the extracellular domain, the transmembrane domain and/or the intracellular kinase domain of a WAK according to the present invention can comprise at least one mutation. Said mutation may lead to an increased signalling activity to represent a functional variation or functional mutation in the sense of the present invention.

[0102] In certain aspects according to the present invention, at least one further or other gene, for example a bx1, bx2, igl, bx6, bx11, bx14, opr2, lox3 or aoc1 gene, is introduced, and/or modified and/or modulated according to the methods of the present invention, variants or mutants representing "loss-of-function", or having reduced activity might be specifically preferred for the purpose of the present invention in case that the at least one variant or mutant results in a decreased BXD synthesis. Particularly, it was found according to the present invention that there is a cross-talk between the WAK signaling pathway and the BXD synthesis pathway, mainly the BXD synthesis pathway as mediated by Bx1, Bx2, Bx6, Bx11 and BX14 and/or Igl, wherein the targeted insertion, modulation or modification of at least one WAK, or the gene encoding the same, and a further effector, or the gene encoding the same, for example a bx1, bx2, igl, bx6, bx11, bx14, opr2, lox3 or aoc1 gene, contribute to a enhanced fungal resistance, in particular NCLB resistance, in a plant as the regulation of both pathways leads to a reduced BXD signature. Wherein the WAK pathway plays the role of the general "pacemaker" in this regulatory network which senses and forwards signals due to its recognition and kinase function, there is also a feedback regulation between the further effectors involved in the jasmonic acid and BXD synthesis pathway. The master regulator function of WAK is demonstrated by the fact that the combined expression of Bx1 and Igl was consistently lower in genotypes with ZmWAK-RLK1 (FIGS. **11** B and C and Example 10) demonstrating that ZmWAK-RLK1 and other WAKs have the capacity to induce a concerted action also regulating the benzoxazinoid pathway and the jasmonic acid pathway.

[0103] In one embodiment according to the various aspects of the present invention, the method for producing a plant having increased fungal resistance thus comprises the introduction and/or modification and/or modulation of at least one WAK, or a gene encoding the same, wherein the WAK at least comprises a functional intracellular kinase domain, for example a sequence selected from SEQ ID NOs: 1, 2, 7, or 8, or an allelic variant or mutant thereof, and wherein the method further comprises the introduction and/

or modification and/or modulation of at least one BX or Igl protein, or a gene encoding the same, wherein the corresponding bx gene, or the Igl gene comprises at least one mutation, or wherein the bx gene, or the Igl gene is of a specific genotype is knocked-out, so that the WAK activity and the decreased or deleted BX protein or Igl protein activity results in a decreased BXD biosynthesis.

[0104] Receptor-like kinases can be further divided into RD and non-RD kinases, depending on the presence or absence of an arginine residue at the catalytic site of the kinase domain. ZmWAK-RLK2 contains an RD kinase and ZmWAK-RLK1 has a non-RD kinase domain (cf. for instance positions 505 and 506 of SEQ ID NO: 2, amino acids F (phenylalanine) and D (aspartic acid), respectively). Most receptor-like kinases involved in plant immunity identified so far belong to the non-RD kinases, whereas RD kinases are thought to play a role in other processes such as development. Variants of SEQ ID NO: 2 have been constructed (cf. SEQ ID NOs: 3 to 6 and Hurni et al., 2015). It was found that mutations at positions M455, G497 and G548 (with reference to SEQ ID NO: 2) may result in a higher susceptibility to NCLB. All said positions reside in the serine threonine kinase domain of ZmWAK-RLK1. A functional variant according to the present invention will thus avoid any mutation or combination of mutations in the kinase domain of a WAK which results in decreased fungal resistance. Exemplary mutants of SEQ ID NO: 2 are presented with SEQ ID NOs: 3 and 4 (RLK1b, M455I) and SEQ ID NOs: 5 and 6 (RLK1d, G497E). A further mutant analyzed herein, RLK1f, comprises a mutation G548R in comparison to the wild-type sequence according to SEQ ID NO: 2. All mutants were tested in comparison to the respective sister lines as described herein. Based on these structural data the importance of a functional intracellular kinase domain of a WAK could be deduced. Therefore, a functional variant or a functional mutant of a WAK may comprise at least one mutation in comparison to the cognate wild-type sequence which at least one mutation does not disturb the downstream signaling of the WAK in that sense that a functional mutant or variant will decrease the level of a specific BXD compound to in turn increase fungal resistance of a plant, plant cell, tissue, or organ comprising such a functional variant of a WAK, or the sequence encoding the same.

[0105] According to certain embodiments of the present invention, more than one gene encoding a WAK, or a functional fragment thereof, or the sequence encoding the same, can be introduced into, or modulated or modified in at least one plant cell, tissue, organ, or whole plant. The introgression of several WAKs can have a synergistic effect in providing enhanced fungal resistance, particularly in case an elite line can be established based on the staggering of more than one WAK into the genome of a plant of interest according to the disclosure of the present invention. As described herein, WAKs represent the key signalling molecules initiating an immune cascade downstream of and mediated by the intracellular kinase domain of the WAKs. Therefore, more than one WAK may thus have a dosage effect positively downregulating BXD synthesis and thus increasing fungal resistance in a plant, in particular a crop plant, of interest. Furthermore, at least one further gene or protein, preferably being selected from any one of SEQ ID NOs: 10 to 27 or homologous genes or homologs thereof, can be additionally or alternatively modified as detailed above to provide a plant cell, tissue, organ or whole plant as material for producing a plant with improved fungal resistance properties, preferably resistance against NCLB. Further target sequences to be modified having an implication in the cross-talk between WAK signalling and BXD biosynthesis are disclosed in Tables 1 and 3 herein.

[0106] In a further embodiment according to the present invention, there is provided a method, wherein the reduced synthesis of at least one benzoxazinoid is achieved by providing at least one wall-associated kinase, an allelic variant, a mutant or a functional fragment thereof, or a gene encoding the same, wherein the at least one wall-associated kinase comprises a sequence which can directly or indirectly influence the benzoxazinoid pathway and at least one further plant metabolic pathway, preferably a disease resistance associated pathway, wherein the plant metabolic pathway is selected from the group consisting of the jasmonic acid pathway, the ethylene pathway, the lignin synthesis pathway, a defense pathway, a receptor-like kinase pathway, a cell wall associated pathway, preferably, wherein the at least one further plant metabolic pathway is the jasmonic acid pathway and wherein the reduced synthesis of at least one benzoxazinoid is achieved by an decreased or down-regulated Igl and/or Bx1 expression as induced by at least one WAK of interest.

[0107] Several differentially expressed genes (DEGs) identified by the inventors of the present invention belonged to several different immune networks and to different disease resistance associated pathways including benzoxazinoids (BXDs) biosynthesis, (phytohormone) jasmonic acids (JAs), ethylene, lignin, defense and receptor-like kinases as well as cell wall were found in Htn1 NILs (Example 10, FIG. **13**). Surprisingly, six genes of the BXDs biosynthesis pathway showed differential expression in at least one timepoint, including Bx1 (SEQ ID NOs: 10 and 11), Bx2 (SEQ ID NOs: 12 and 13), Igl-like (SEQ ID NOs: 14 and 15), Bx6 (SEQ ID NOs: 16 and 17), Bx11 (SEQ ID NOs: 18 and 19) and Bx14 (SEQ ID NOs: 20 and 21) (see also FIG. **13**, column 2 of table for further reference to publicly available data base entries for gene IDs and names) demonstrating a cross-regulatory network between the WAK and further pathways in fungal defense. This was particularly surprising because BXDs as secondary metabolites have so far not been associated with defense against fungi mediated by WAK kinases. More surprisingly, the transcriptome data and functional assays also, for the first time, revealed DEGs that are part of immune networks including the phytohormone jasmonic acids that plays a central role in regulating resistance against hemibiotrophic and necrotrophic diseases. JAs treatment can induce the accumulation of BXD compounds (Oikawa, Akira, Atsushi Ishihara, and Hajime Iwamura. "Induction of HDMBOA-Glc accumulation and DIMBOA-Glc 4-O-methyltransferase by jasmonic acid in poaceous plants." *Phytochemistry* 61.3 (2002): 331-337; Oikawa, Akira, et al. "Accumulation of HDMBOA-Glc is induced by biotic stresses prior to the release of MBOA in maize leaves." *Phytochemistry* 65.22 (2004): 2995-3001). The present invention thus provides evidence that there is an additional link between the WAK kinase signaling pathway and the jasmonic acid pathway which paves the way for a variety of new disease and particularly fungal resistance strategies as disclosed herein. The enzymatic properties of IGL are similar to BX1, but the transcriptional regulation of their corresponding genes is different. Like other Bx genes,

Bx1 is constitutively expressed during the early developmental stages of the plant, which correlates with endogenous BX levels. Plants carrying the mutant alleles of the Bx1 gene produce only a fraction of the BXs that are found in Bx1 wild-type plants. Therefore, a WAK according to the present invention may act as a master regulator bridging anti-fungal signalling with the effectors of the jasmonic acid pathway and other pathways, preferably an effector selected from the group consisting of SEQ ID NOs: 10 to 27 or homologous genes or homologs thereof.

[0108] In one embodiment, the introduction at least one additional gene encoding at least one wall-associated kinase into at least one cell of at least one of a plant cell, tissue, organ, or whole plant may comprise the introduction of a nucleic acid sequence, comprising DNA and/or RNA in a single stranded and/or double stranded form, or an amino acid sequence, by means of breeding techniques, or by means of molecular biology to transfer a functional WAK of interest, or an additional functional WAK of interest, or the sequence encoding the same, into at least one cell of interest. Said at least one additional gene can be also any gene, wherein the resulting protein/enzyme is involved in the BXD biosynthesis pathway or in a jasmonic acid pathway, such as Bx1, Bx2, Bx3, Bx4, Bx5, Bx7, Bx8, Bx9, Bx10, Bx11, Bx12, Bx13, Bx14, Igl, Glu1, Glu2, OPR2, LOX3 or AOC1 (see FIG. **13** and SEQ ID NOs: 10 to 27), or any variant thereof, or a combination of the aforementioned genes/proteins. Preferably, the at least one additional gene comprises at least one mutation which changes the function of the naturally occurring respective additional gene, wherein the mutation, in the coding or within a regulatory region, causes decreased synthesis of the respective BXD compound, or wherein the mutation, in a regulatory region, such as a promoter region, or in a coding region, causes a reduced signal transduction from a WAK kinase located upstream in the signalling cascade so the said mutation results in a decreased synthesis of a BXD compound. In another embodiment, the gene encoding at least one of Bx1, Bx2, Bx3, Bx4, Bx5, Bx7, Bx8, Bx9, Bx10, Bx11, Bx12, Bx13, Bx14, Igl, Glu1, Glu2, OPR2, LOX3 or AOC1 may be deleted or partially deleted within the genome of a plant cell of interest, or the gene may be modified in a targeted way.

[0109] Further enzymes involved in the regulation of the BXD synthesis which can be modulated, introduced or modified according to the methods of the present invention to achieve an increased fungal resistance in a plant cell, plant or plant material pathway are selected from the group of jasmonic synthesis pathway enzymes, including 12-oxo-phytodienoic acid reductase 2 (OPR2), Lipoxygenase 3 (LOX3) or Allene oxide cyclase 1 (AOC1), ethylene pathway enzymes, such as S-adenosylmethionine synthase, lignin pathway enzymes, such as, for example, Caffeoyl-CoA O-methyltransferase 1 (OMT1) or OMT2, enzymes and proteins involved in plant defense mechanisms, such as, for example SAF1—Safener induced 1; Glu2athione S-transferase, and any combination thereof.

[0110] Presently, WAKs are the only known proteins that can physically link the cell wall to the plasma membrane (Brutus, Alexandre, et al. "A domain swap approach reveals a role of the plant wall-associated kinase 1 (WAK1) as a receptor of oligogalacturonides." *Proceedings of the National Academy of Sciences* 107.20 (2010): 9452-9457). Therefore, further structurally and functionally related cell wall spanning or associated kinases are suitable as WAKs according to the present invention, e.g., maize qHSR1 (Zuo, Weiliang, et al. "A maize wall-associated kinase confers quantitative resistance to head smut." *Nature genetics* 47.2 (2015): 151-157), or rice OsWAK/Xa4 gene conferring quantitative rice blight resistance by strengthening the cell wall (Hu et al. 2017).

[0111] In another embodiment according to the present invention, the step of introducing at least one gene into at least one cell of at least one of a plant cell, tissue, organ, or whole plant may comprise the introduction of a gene, wherein the amino acid sequence or enzyme encoded by said gene is involved in the catalytic pathway downstream of a WAK kinase, wherein the additional gene is introduced alone, or together with at least one gene encoding a WAK kinase or a variant thereof.

[0112] "Benzoxazinoids" or "BXDs" are a class of indole-derived plant chemical defenses comprising compounds with a 2-hydroxy-2H-1,4-benzoxazin-3(4H)-one skeleton and their derivatives. BXDs have been described as phytochemicals in monocots, including grasses, including important cereal crops such as maize, wheat and rye, as well as a certain dicot species. The term "BXDs" as used herein refers to both benzoxazinones (glucosides and corresponding aglucones containing a 2-hydroxy-2H-1,4-benzoxazin-3(4H)-one skeleton) and their downstream derivative products during metabolic pathways, benzoxazolinones, as well as any intermediates. The term BXD may thus also comprise a derivative being the result of the activity of hydrolyzing glucosidases found in plastids, cytoplasm, and cell walls, or derivatives and intermediated being the result of degradation to benzoxazolinones via oxo-cyclo/ring-chain tautomerism. Further comprised are downstream metabolites directly being derivable from any benzoxazolinone. The term "BXDs" shall further comprise any open form, nitrenium form or complex, e.g., a metal complex from a BXD. BXD basic structures are represented in FIG. **1**.

[0113] By the term "reduced/decreased synthesis of a benzoxazinoid" or "reduced synthesis of at least one benzoxazinoid" or "reducing the amount of at least one benzoxazinoid" or "reduction on BXDs content" or "reduced amount of a BXD compound" or the like, is meant that the plant cell, tissue, organ, or whole plant according to the present invention exhibit an amount of a benzoxazinoid, at least one benzoxazinoid or the benzoxazinoid of interest which is reduced by at least 10%, 15%, 20% or 25%, preferably by at least 30%, 35%, 40% or 45%, more preferably by at least 50%, 60% or 70% as compared to a corresponding control plant cell, control tissue, control organ, or control whole plant of the same genotype, but lacking the modification of the at least one gene encoding at least one wall-associated kinase or the modulation of the expression level of at least one wall-associated kinase and/or the transcription level, the expression level, or the function of at least one molecule within the signaling pathway from the at least one wall-associated kinase to the synthesis of at least one benzoxazinoid or within the synthesis pathway of at least one benzoxazinoid. In one embodiment according to the various aspects of the present invention, the benzoxazinoid whose synthesis is regulated by the at least one wall-associated kinase and optionally regulated by the at least one further enzyme of the jasmonic acid and/or benzoxazionoid pathway is selected from at least one of DIM_2BOA, DIMBOA, HMBOA, HM_2BOA, HDMBOA, HDM_2BOA,

HBOA, DHBOA, DIBOA or TRIBOA, the aforementioned benzoxazinoid being in the glucoside or aglucone form, or a benzoxazolinone, or any combination of the aforementioned benzoxazinoids, preferably wherein the benzoxazinoid whose synthesis is regulated by the at least one wall-associated kinase is selected from at least one of DIM_2BOA, DIMBOA, HMBOA or HDMBOA, the aforementioned benzoxazinoid being in the glucoside or aglucone form, or any combination of the aforementioned benzoxazinoids.

[0114] In one embodiment according to the various aspects of the present invention, a reduced content of BXDs can be achieved by introducing at least one gene encoding at least one wall-associated kinase into at least one cell of at least one of a plant cell, tissue, organ, or whole plant, wherein the at least one wall-associated kinase causes a reduced synthesis of at least one BXD. More than one WAK encoding gene and different allelic variants of a WAK gene may be introduced into a cell of interest in addition to a WAK gene potentially already being present in the genome of a plant cell of interest. The presence of several WAKs or receptor-like kinases involved in the BXD synthesis may thus be favourable in order to increase the copy number and thus the dosage effect of a gene of interest.

[0115] In one embodiment, quantitative NCLB disease resistance is based on a decrease of the biosynthesis of at least one secondary metabolite BXDs, preferably DIM_2BOA-Glc, DIMBOA, HMBOA, DIMBOA-Glc or HMBOA-Glc, and the methods according to the various aspects of the present invention comprise the addition of a scavenger molecules interacting with and this neutralizing the activity of at least one secondary metabolite BXD to reduce the amount of the of at least one secondary metabolite BXD susceptibility component to decrease fungal infection at least one plant cell, tissue, organ, or whole plant.

[0116] According to the present invention, there are thus provided methods for producing a plant having increased fungal resistance, wherein the fungal resistance is regulated by at least one wall-associated kinase. "Regulated" in this context thus implies a direct or indirect regulation mediated by at least one wall-associated kinase. This regulation may imply a signalling cascade initiated by the at least one wall-associated kinase and proceeding through further molecules involved in the signalling cascade. The regulation can be on a protein, RNA or nucleic acid level. Furthermore, the regulation may imply a cross-talk or feedback regulation, for example implying a Bx1, Bx2, Bx3, Bx4, Bx5, Bx7, Bx8, Bx9, Bx10, Bx11, Bx12, Bx13, Bx14, Igl, Glu1, Glu2, OPR2, LOX3 or AOC1 enzyme, or the gene encoding the same, or the transcriptional regulation of such a further gene encoding Bx1, Bx2, Bx3, Bx4, Bx5, Bx7, Bx8, Bx9, Bx10, Bx11, Bx12, Bx13, Bx14, Igl, Glu1, Glu2, OPR2, LOX3 or AOC1, or a modulation or modification of a gene encoding Bx1, Bx2, Bx3, Bx4, Bx5, Bx7, Bx8, Bx9, Bx10, Bx11, Bx12, Bx13, Bx14, Igl, Glu1, Glu2, OPR2, LOX3 or AOC1.

[0117] In one embodiment, the pathogen according to the present disclosure is a fungal pathogen infesting a plant. The disease caused by a fungal pathogen and the respective fungus may be selected from Plume blotch *Septoria* (*Stagonospora*) *nodorum*, Leaf blotch (*Septoria tritici*), Ear fusarioses (*Fusarium* spp.), Late blight (*Phytophthora infestans*), Anthrocnose leaf blight or Anthracnose stalk rot (*Colletotrichum graminicola* (teleomorph: *Glomerella graminicola* Politis) *Glomerella tucumanensis*), *Curvularia* leaf spot (*Curvularia clavata, C. eragrostidis, =C. maculans* (teleomorph: *Cochliobolus eragrostidis*), *Curvularia inaequalis, C. intermedia* (teleomorph: *Cochliobolus intermedius*), *Curvularia lunata* (teleomorph: *Cochliobolus lunatus*), *Curvularia pallescens* (teleomorph: *Cochliobolus pallescens*), *Curvularia senegalensis, C. tuberculata* (teleomorph: *Cochliobolus tuberculatus*), *Didymella* leaf spot (*Didymella exitalis*), *Diplodia* leaf spot or streak (*Stenocarpella macrospora=Diplodialeaf macrospora*), Brown stripe downy mildew (*Sclerophthora rayssiae* var. *zeae*), Crazy top downy mildew (*Sclerophthora macrospora=Sclerospora macrospora*), Green ear downy mildew (*Sclerospora graminicola*), Leaf spots (various minor leaf spots) (*Alternaria alternata, Ascochyta maydis, A. tritici, A. zeicola, Bipolaris victoriae=Helminthosporium victoriae* (teleomorph: *Cochliobolus victoriae*), *C. sativus* (anamorph: *Bipolaris sorokiniana=H. sorokinianum=H. sativum*), *Epicoccum nigrum, Exserohilum prolatum=Drechslera prolata* (teleomorph: *Setosphaeria prolata*) *Graphium penicillioides, Leptosphaeria maydis, Leptothyrium zeae, Ophiosphaerella herpotricha*, (anamorph: *Scolecosporiella* sp.), *Paraphaeosphaeria michotii, Phoma* sp., *Septoria zeae, S. zeicola, S. zeina*, Northern corn leaf blight (*Setosphaeria turcica* (anamorph: *Exserohilum turcicum=Helminthosporium turcicum*), Northern corn leaf spot (*Cochliobolus carbonum* (anamorph: *Bipolaris zeicola=Helminthosporium carbonum*)), *Phaeosphaeria* leaf spot (*Phaeosphaeria maydis=Sphaerulina maydis*), *Rostratum* leaf spot (*Setosphaeria rostrata*, (anamorph: *Helminthosporium rostratum*)), Java downy mildew (*Peronosclerospora maydis=Sclerospora maydis*), Philippine downy mildew (*Peronosclerospora philippinensis=Sclerospora philippinensis*), Sorghum downy mildew (*Peronosclerospora sorghi=Sclerospora sorghi*), *Spontaneum* downy mildew (*Peronosclerospora spontanea=Sclerospora spontanea*), Sugarcane downy mildew (*Peronosclerospora sacchari=Sclerospora sacchari*), *Sclerotium* ear rot (southern blight) (*Sclerotium rolfsii* Sacc. (teleomorph: *Athelia rolfsii*)), Seed rot-seedling blight (*Bipolaris sorokiniana, B. zeicola=Helminthosporium carbonum, Diplodia maydis, Exserohilum pedicillatum, Exserohilum turcicum=Helminthosporium turcicum, Fusarium avenaceum, F. culmorum, F. moniliforme, Gibberella zeae* (anamorph: *F. graminearum*), *Macrophomina phaseolina, Penicillium* spp., *Phomopsis* sp., *Pythium* spp., *Rhizoctonia solani, R. zeae, Sclerotium rolfsii, Spicaria* sp.), *Selenophoma* leaf spot (*Selenophoma* sp.), Yellow leaf blight (*Ascochyta ischaemi, Phyllosticta maydis* (teleomorph: *Mycosphaerella zeae-maydis*), Zonate leaf spot (*Gloeocercospora sorghi*).

[0118] Further plant pathogenic fungi include Plasmodiophoromycota, such as *Plasmodiophora brassicae* (clubroot of crucifers), *Spongospora subterranea, Polymyxa graminis*, Oomycota, such as *Bremia lactucae* (downy mildew of lettuce), *Peronospora* (downy mildew) in snapdragon (*P. antirrhini*), onion (*P. destructor*), spinach (*P. effusa*), soybean (*P. manchurica*), tobacco ("blue mold"; *P. tabacina*) alfalfa and clover (*P. trifolium*), *Pseudoperonospora humuli* (downy mildew of hops), Plasmopara (downy mildew in grapevines) (*P. viticola*) and sunflower (*P. halstedii*), *Sclerophthora macrospora* (downy mildew in cereals and grasses), *Pythium* (for example damping-off of *Beta* beet caused by *P. debaryanum*), *Phytophthora infestans* (late blight in potato and in tomato and the like), Albugo spec., Ascomycota, such as *Microdochium nivale* (snow mold of

rye and wheat), *Fusarium, Fusarium graminearum, Fusarium culmorum* (partial ear sterility mainly in wheat), *Fusarium oxysporum* (*Fusarium* wilt of tomato), *Blumeria graminis* (powdery mildew of barley (sp. *hordei*) and wheat (f. sp. *tritici*)), *Erysiphe pisi* (powdery mildew of pea), *Nectria galligena* (*Nectria* canker of fruit trees), *Uncinula necator* (powdery mildew of grapevine), *Pseudopeziza tracheiphila* (red fire disease of grapevine), *Claviceps purpurea* (for example, rye and grasses), *Gaeumannomyces graminis* (take-all on wheat, rye and other grasses), *Magnaporthe grisea, Pyrenophora graminea* (leaf stripe of barley), *Pyrenophora teres* (net blotch of barley), *Pyrenophora tritici-repentis* (leaf blight of wheat), *Venturia inaequalis* (apple scab), *Sclerotinia sclerotium* (stalk break, stem rot), *Pseudopeziza medicaginis* (leaf spot of alfalfa, white and red clover), Basidiomycetes, such as *Typhula incarnata* (typhula blight on barley, rye, wheat), *Ustilago maydis* (blister smut on maize), *Ustilago nuda* (loose smut on barley), *Ustilago tritici* (loose smut on wheat, spelt), *Ustilago avenae* (loose smut on oats), *Rhizoctonia solani* (*rhizoctonia* root rot of potato), *Sphacelotheca* spp. (head smut of sorghum), *Melampsora lini* (rust of flax), *Puccinia graminis* (stem rust of wheat, barley, rye, oats), *Puccinia recondita* (leaf rust on wheat), *Puccinia dispersa* (brown rust on rye), *Puccinia hordei* (leaf rust of barley), *Puccinia coronata* (crown rust of oats), *Puccinia striiformis* (yellow rust of wheat, barley, rye and a large number of grasses), *Uromyces appendiculatus* (brown rust of bean), *Sclerotium rolfsii* (root and stem rots of many plants), Deuteromycetes (Fungi imperfecti), such as *Septoria* (*Stagonospora*) *nodorum* (glume blotch) of wheat (*Septoria tritici*), *Pseudocercosporella herpotrichoides* (eyespot of wheat, barley, rye), *Rynchosporium secalis* (leaf spot on rye and barley), *Alternaria solani* (early blight of potato, tomato), *Phoma betae* (blackleg on *Beta* beet), *Cercospora beticola* (leaf spot on *Beta* beet), *Alternaria brassicae* (black spot on oilseed rape, cabbage and other crucifers), *Verticillium dahliae* (*verticillium* wilt), *Colletotrichum*, such as *Colletotrichum lindemuthianum* (bean anthracnose), *Phoma lingam* (blackleg of cabbage and oilseed rape), *Botrytis cinerea* (grey mold of grapevine, strawberry, tomato, hops and the like).

[0119] Preferred fungal diseases to be prevented and the corresponding causative pathogens which can be combated based on the disclosure of the present invention in a crop plant of interest are selected from a fungus from the order of Pleosporales, comprising *E. turcicum/H. turcicum* causing northern corn leaf blight (NCLB), particularly affecting maize and wheat plants, or comprising *Bipolaris maydis* causing southern corn leaf blight, the order of Pucciniales causing rust disease, comprising *Puccinia sorghi* causing common rust, or *Diploida macrospora* causing *Diploida* leaf streak/blight, or *Colletotrichum graminicola* causing Anthracnose, or *Fusarium* spp., preferably *Fusarium* verticilioides causing *Fusarium* stalk rot, or *Gibberella* spp., e.g., *Gibberella zeae* causing *Giberella* stalk rot, or *Sphacelotheca reiliana* causing maize head smut are thus plant diseases caused by pathogenic fungi which can be prevented in the plants and by the methods of the present invention.

[0120] In one embodiment according to the various aspects of the present invention the at least one gene encoding at least one wall-associated kinase may be stably integrated into the genome of the at least one plant cell, tissue, organ, or whole plant, or the at least one gene encoding at least one wall-associated kinase may transiently introduced into a plant cell, tissue, organ, or whole plant.

[0121] In another embodiment according to the various aspects of the present invention at least one further gene encoding at least one enzyme within the signalling cascade downstream of a wall-associated kinase may be stably integrated into the genome of the at least one plant cell, tissue, organ, or whole plant, or the at least one further gene encoding at least one enzyme within the signalling cascade downstream of a wall-associated kinase may transiently introduced into a plant cell, tissue, organ, or whole plant.

[0122] Methods for introducing a gene of interest into a plant cell of interest by means of molecular biology or conventional and modern breeding and associated tools and methodologies are disclosed herein and are known to the skilled person.

[0123] In one embodiment, the transient introduction may comprise the direct introduction of an amino acid effector instead of the introduction of a gene of interest.

[0124] In one embodiment according to the various aspects of the present invention the at least one gene encoding at least one wall-associated kinase may be stably integrated into the genome of the at least one plant cell, tissue, organ, or whole plant, wherein the introduction of the at least one gene encoding at least one wall-associated kinase comprises the introgression of the at least one gene during plant breeding.

[0125] Any of a number of standard breeding techniques can be used, depending upon the species to be crossed. Since expression of the genes or nucleic acids of the invention may lead to phenotypic changes, plants comprising the recombinant nucleic acids of the invention can be sexually crossed with a second plant to obtain a final product. Thus, the seed of the invention can be derived from a cross between two transgenic plants of the invention, or a cross between a transgenic or mutant plant of the invention and another plant. The desired effects, e.g., expression of the at least one WAK gene or a mutant allele of the invention to produce a plant having a modified BXD synthesis profile, or a modulated Bx1, Bx2, Bx3, Bx4, Bx5, Bx7, Bx8, Bx9, Bx10, Bx11, Bx12, Bx13, Bx14, Igl, Glu1, Glu2, OPR2, LOX3 or AOC1 profile, can be enhanced when both parental plants express the genes or mutant alleles of the invention, or if both allels are modified or even deleted, depending on the target to be modified in accordance with the disclosure of the present invention. The desired effects can be passed to future plant generations by standard propagation means. "Introgressing", as also detailed above, thus means the integration of a gene or allele in a plant's genome by natural means, i.e. by crossing a plant comprising the gene or allele of interest described herein with a plant not comprising said gene or allele. The offspring can be selected for those comprising the gene or allele of interest.

[0126] Furthermore, the methods of the present invention can result in the creation or provision of a plant material, comprising grains or seeds, relating to any means known in the art to produce further plants, plant parts or seeds and includes inter alia vegetative reproduction methods, such as, for example, air or ground layering, division, (bud) grafting, micropropagation, stolons or runners, storage organs such as bulbs, corms, tubers and rhizomes, striking or cutting, or twin-scaling, sexual reproduction, comprising crossing with another plant, and asexual reproduction, such as e.g. apomixis, somatic hybridization and the like.

[0127] In one embodiment according to the various aspects of the present invention, the modification of the at least one gene encoding at least one wall-associated kinase within step (ii) (a) or (ii) (b) of the method for producing a plant having increased fungal resistance, or a modification of a gene encoding Bx1, Bx2, Bx3, Bx4, Bx5, Bx7, Bx8, Bx9, Bx10, Bx11, Bx12, Bx13, Bx14, Igl, Glu1, Glu2, OPR2, LOX3 or AOC1, may be performed by at least one of a site-specific nuclease (SSN) or a catalytically active fragment thereof, or a nucleic acid sequence encoding the same, oligonucleotide directed (ODM) mutagenesis (ODM), chemical mutagenesis, or TILLING.

[0128] TILLING, initially a functional genomics tool in model plants, has been extended to many plant species and become of paramount importance to reverse genetics in crops species. A major recent change to TILLING has been the application of next-generation sequencing (NGS) to the process, which permits multiplexing of gene targets and genomes. NGS will ultimately lead to TILLING becoming an in silico procedure. Because it is readily applicable to most plants, it remains a dominant non-transgenic method for obtaining mutations in known genes and thus represents a readily available method for non-transgenic approaches according to the methods of the present invention. As it is known to the skilled person, TILLING usually comprises the chemical mutagenesis, e.g., using ethyl methanesulfonate (EMS), or UV light induced modification of a genome of interest, together with a sensitive DNA screening-technique that identifies single base mutations in a target gene, wherein the target gene may encode a protein being selected from the group of a receptor-like kinase, such as a WAK, an enzyme involved in benzoxazinoid synthesis or metabolism, defense, the lignin pathway, the jasmonic acid synthesis pathway, or a transcription factor involved in one of the aforementioned metabolic and/or signalling pathways.

[0129] SSNs and ODM mutagenesis both are suitable techniques for precision genome engineering in plant cells. As it is known to the skilled person, ODM offers a rapid, precise and non-transgenic breeding alternative for trait improvement in agriculture to address this urgent need. ODM is a precision genome editing technology, which uses oligonucleotides to make targeted edits in plasmid, episomal and chromosomal DNA of plant systems.

[0130] In one embodiment according to the various aspects and embodiments of the present invention, the at least one site-specific nuclease (SSN), or the nucleic acid sequence encoding the same, may be selected from at least one of a CRISPR nuclease, including Cas or Cpf1 nucleases, a TALEN, a ZFN, a meganuclease, a base editor complex, a restriction endonuclease, including Fold or a variant thereof, or two site-specific nicking endonucleases, or a variant or a catalytically active fragment thereof. Said targeted genome engineering SSNs can be suitable for both, the introduction of a gene of interest not yet present in a specific genotype, as well as the targeted mutagenesis of a gene of a given specific genotype to modulate (up- or downregulate) the activity of an enzyme encoded by a gene of interest to be modified in a highly precise way.

[0131] SSNs meanwhile emerged as indispensable prerequisite for site-directed genome engineering. SSNs are (programmable) nucleases, which can be used to break a nucleic acid of interest at a defined position to induce either a double-strand break (DSB) or one or more single-strand breaks. Alternatively, said nucleases can be chimeric or mutated variants, no longer comprising a nuclease function, but rather operating as recognition molecules in combination with another enzyme. Those nucleases or variants thereof are thus key to any gene editing or genome engineering approach. In recent years, many suitable nucleases, especially tailored endonucleases have been developed comprising meganucleases, a base editor complex, zinc finger nucleases, TALE nucleases, and CRISPR nucleases, comprising, for example, Cas, Cpf1, CasX or CasY nucleases as part of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system. The use of those SSNs and the necessary accessory molecules, for example crRNAs, tracrRNAs, or gRNAs, and delivery systems are thus envisaged for performing the methods according to the present invention.

[0132] A "base editor" as used herein refers to a protein or a fragment thereof having the same catalytical activity as the protein it is derived from, which protein or fragment thereof, alone or when provided as molecular complex, referred to as base editing or base editor complex herein, has the capacity to mediate a targeted base modification, i.e., the conversion of a nucleotide base of interest resulting in a point mutation of interest which in turn can result in a targeted mutation, if the base conversion does not cause a silent mutation, but rather a conversion of an amino acid encoded by the codon comprising the position to be converted with the base editor. Preferably, the base editor is temporarily or permanently linked to at least one site-specific effector, or optionally to a component of at least one site-specific effector complex. The linkage can be covalent and/or non-covalent. Multiple publications have shown targeted base conversion, primarily cytidine (C) to thymine (T), using a CRISPR/Cas9 nickase or non-functional nuclease linked to a cytidine deaminase domain, Apolipoprotein B mRNA-editing catalytic polypeptide (APOBEC1), e.g., APOBEC derived from rat. The deamination of cytosine (C) is catalyzed by cytidine deaminases and results in uracil (U), which has the base-pairing properties of thymine (T). Most known cytidine deaminases operate on RNA, and the few examples that are known to accept DNA require single-stranded (ss) DNA.

[0133] A "CRISPR nuclease" according to the present invention can be a CRISPR-based nuclease, or the nucleic acid sequence encoding the same, which is selected from the group consisting of (a) Cas9, including SpCas9, SaCas9, SaKKH-Cas9, VQR-Cas9, St1Cas9, or (b) Cpf1, including AsCpf1, LbCpf1, FnCpf1, (c) CasX, or (d) CasY, or any variant or derivative of the aforementioned CRISPR-based nucleases, or a CRISPR-based nuclease comprising a mutation in comparison to the respective wild-type sequence so that the resulting CRISPR-based nuclease is converted to a single-strand specific DNA nickase, or to a DNA binding effector lacking all DNA cleavage ability. A "CRISPR(-based) nuclease", as used herein, is thus any nuclease which has been identified in a naturally occurring CRISPR system, which has subsequently been isolated from its natural context, and which preferably has been modified or combined into a recombinant construct of interest to be suitable as tool for targeted genome engineering. Any CRISPR-based nuclease can be used and optionally reprogrammed or additionally mutated to be suitable for the various embodiments according to the present invention as long as the original wild-type CRISPR-based nuclease provides for DNA recognition, i.e., binding properties. Said DNA recognition can be PAM dependent. CRISPR nucleases having optimized

and engineered PAM recognition patterns can be used and created for a specific application. The expansion of the PAM recognition code can be suitable to target the site-specific effector complexes to a target site of interest, independent of the original PAM specificity of the wild-type CRISPR-based nuclease. Cpf1 variants can comprise at least one of a S542R, K548V, N552R, or K607R mutation, preferably mutation S542R/K607R or S542R/K548V/N552R in AsCpf1 from *Acidaminococcus* (cf. SEQ ID NO: 24). Furthermore, modified Cas variant, e.g., Cas9 variants, can be used according to the methods of the present invention as part of a base editing complex, e.g. BE3, VQR-BE3, EQR-BE3, VRER-BE3, SaBE3, SaKKH-BE3 (see Kim et al., Nat. Biotech., 2017, doi:10.1038/nbt.3803). Therefore, according to the present invention, artificially modified CRISPR nucleases are envisaged, which might indeed not be any "nucleases" in the sense of double-strand cleaving enzymes, but which are nickases or nuclease-dead variants, which still have inherent DNA recognition and thus binding ability. Other suitable Cpf1-based effectors for use in the methods of the present invention are derived from *Lachnospiraceae bacterium* (LbCpf1, e.g., NCBI Reference Sequence: WP_051666128.1), or from *Francisella tularensis* (FnCpf1, e.g., UniProtKB/Swiss-Prot: A0Q7Q2.1). Variants of Cpf1 are known (cf. Gao et al., BioRxiv, http://dx.doi.org/10.1101/091611). Variants of AsCpf1 with the mutations S542R/K607R and S542R/K548V/N552R that can cleave target sites with TYCV/CCCC and TATV PAMs, respectively, with enhanced activities in vitro and in vivo are thus envisaged as site-specific effectors according to the present invention. Genome-wide assessment of off-target activity indicated that these variants retain a high level of DNA targeting specificity, which can be further improved by introducing mutations in non-PAM-interacting domains. Together, these variants increase the targeting range of AsCpf1 and thus provide a useful addition to the CRISPR/Cas genome engineering toolbox.

[0134] Due to the fact that receptor-like kinases and BX enzymes (cf. SEQ ID NOs: 10 to 13 and 16 to 21), Igl (SEQ ID NOs: 14 and 15), OPR2 (SEQ ID NOs:22 and 23), LOX3 (SEQ ID NOs:24 and 25), and AOC1 (SEQ ID NOs:26 and 27) are ubiquitously found in a variety of plants, particularly monocotyledonous plants (monocots) and dicotyledonous plants (dicots) of agronomic interest, the methods according to the present invention can be used for the targeted optimization of several important monoct and dicot crop plants.

[0135] In one embodiment according to the various aspects of the present invention, the at least one plant cell, tissue, organ, or whole plant provided in step (i) (a) may be selected from the group consisting of *Hordeum vulgare, Hordeum bulbusom, Sorghum bicolor, Saccharum officinarium, Zea* spp., including *Zea mays, Setaria italica, Oryza minuta, Oryza sativa, Oryza australiensis, Oryza alta, Triticum aestivum, Triticum durum, Secale cereale*, Triticale, *Malus domestica, Brachypodium distachyon, Hordeum marinum, Aegilops tauschii, Daucus glochidiatus, Beta* spp., including *Beta vulgaris, Daucus pusillus, Daucus muricatus, Daucus carota, Eucalyptus grandis, Nicotiana sylvestris, Nicotiana tomentosiformis, Nicotiana tabacum, Nicotiana benthamiana, Solanum lycopersicum, Solanum tuberosum, Coffea canephora, Vitis vinifera, Erythrante guttata, Genlisea aurea, Cucumis sativus, Marus notabilis, Arabidopsis arenosa, Arabidopsis lyrata, Arabidopsis thaliana, Crucihimalaya himalaica, Crucihimalaya wallichii, Cardamine nexuosa, Lepidium virginicum, Capsella bursa pastoris, Olmarabidopsis pumila, Arabis hirsute, Brassica napus, Brassica oleracea, Brassica rapa, Raphanus sativus, Brassica juncacea, Brassica nigra, Eruca vesicaria* subsp. *sativa, Citrus sinensis, Jatropha curcas, Populus trichocarpa, Medicago truncatula, Cicer yamashitae, Cicer bijugum, Cicer arietinum, Cicer reticulatum, Cicer judaicum, Cajanus cajanifolius, Cajanus scarabaeoides, Phaseolus vulgaris, Glycine max, Gossypium* sp., *Astragalus sinicus, Lotus japonicas, Torenia fournieri, Allium cepa, Allium fistulosum, Allium sativum, Helianthus annuus, Helianthus tuberosus* and *Allium tuberosum*, or any variety or subspecies belonging to one of the aforementioned plants, preferably wherein the plant cell, tissue, organ, or whole plant in step (i) is selected from *Zea mays* or *Triticum* spp., or any variety or subspecies belonging to one of the aforementioned plants.

[0136] In one aspect of the present invention, there is thus disclosed a plant cell, tissue, organ, whole plant or plant material, or a derivative or a progeny thereof, obtainable by any one of the methods according to the various aspects disclosed herein. The plant cell, tissue, organ, whole plant or plant material, or a derivative or a progeny thereof obtained according to the present invention will have at least one optimized agronomic trait, wherein this trait is disease resistance or tolerance, preferably fungus resistance or tolerance, more preferably resistance or tolerance against NCLB caused by *E. turcicum* or a related fungal diseases caused by any one of the related fungal pathogens disclosed herein. Based on the disclosure provided herein demonstrating the functional mechanism of a WAK induced quantitative NCLB resistance, said resistance being associated with a reduction of the BXD biosynthesis, which in turn inhibits the hemibiotrophic fungus *E. turcicum* and related fungi, the teachings provided herein can be used to provide a plant cell, tissue, organ, whole plant or plant material, or a derivative or a progeny thereof having a favourable BXD content, preferably a reduced BXD content, so that the plant cell, tissue, organ, whole plant or plant material, or a derivative or a progeny thereof has an increased resistance against fungal infection, i.e., fungal infestation and persistence.

[0137] In yet a further embodiment according to the present invention, more than one agronomic property of a plant cell or plant of interest can be modified in addition to the introduction, modulation and/or modification of a WAK or WAK gene of interest. Said agronomic properties are selected from seed emergence, vegetative vitality, stress tolerance, disease resistance or tolerance against a further fungus, or against another pathogen, comprising a virus, bacterium, a nematode, an insect etc., herbicide resistance, branching tendency, flowering time, seed clusters, seed density, stability and storability, threshing capability (uniform ripening), lodging resistance, increased yield (seed size, yield etc.), or a modified composition of a molecule of agronomic importance (e.g. starch, carbohydrate, protein etc.) of interest, and the like.

[0138] In another aspect according to the present invention there is provided method for identifying at least one gene involved in increased pathogen resistance, preferably increased fungal resistance, in a plant cell, tissue, organ, whole plant, or plant material the method comprising: (i) determining the genotype of at least one plant cell, tissue, organ, whole plant, or plant material with respect to the presence of at least one gene encoding a wall-associated

kinase in the genome of said plant cell, tissue, organ, whole plant or plant material; (ii) optionally: determining the benzoxazinoid signature of the at least one plant cell, tissue, organ, whole plant, or plant material of step (i); (iii) exposing the at least one plant cell, tissue, organ, whole plant, or plant material of step (i) or (ii) to a stimulus, optionally wherein the stimulus is correlated with the benzoxazinoid signature in the at least one plant cell, tissue, organ, whole plant, or plant material, preferably wherein the stimulus is associated with a fungal pathogen infection; (iv) performing an analysis of at least one analyte obtained from the at least one plant cell, tissue, organ, whole plant, or plant material of step (i) or (ii) after exposition to the stimulus; (v) determining at least one gene being regulated upon exposition to a stimulus according to step (iii) in at least one cell of the at least one plant cell, tissue, organ, whole plant, or plant material as derivable from the analysis of at least one analyte as defined in step (iv), (vi) subjecting the at least one gene as determined in step (v) to a functional characterization; and (vii) providing at least one gene involved in increased pathogen resistance, preferably increased fungal resistance, in a plant cell, tissue, organ, whole plant, or plant material.

[0139] In one embodiment, the determination of the genotype of at least one plant cell, tissue, organ, whole plant, or plant material with respect to the presence of at least one gene encoding a wall-associated kinase may be performed by determining in the genome of a plant cell, tissue, organ, whole plant, or plant material of interest the presence and/or transcript level of a WAK gene of interest, preferably a WAK gene comprising a nucleotide sequence according to SEQ ID NO: 1 or 7, or comprising a nucleotide sequence having at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to one of the nucleotide sequence according to SEQ ID NO: 1 or 7, preferably over the entire length of the sequence, or comprising a nucleotide sequence hybridizing with a nucleotide sequence complementary to the nucleotide sequence according to SEQ ID NO: 1 or 7 preferably under stringent conditions, or comprising a nucleotide sequence encoding for an amino acid sequence of SED ID NO: 2 or 8 or for an amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to one of SEQ ID NO: 2 or 8.

[0140] In another embodiment, determining the benzoxazinoid signature comprises a step of determination of the presence and/or the transcript level of at least one gene from the BXD biosynthesis pathway and/or the jasmonic acid pathway, The gene may be selected from any one of SEQ ID NOs: 10, 12, 14, 16, 18, 20, 22, 24 or 26, or a variant, homologous gene, allel or mutant or a fragment thereof. Bioinformatic tools for the determination and/or alignment of sequences of interest are disclosed herein, or are readily available to the skilled person.

[0141] In one embodiment, the determination of the genotype of at least one plant cell, tissue, organ, whole plant, or plant material with respect to the presence of at least one gene encoding a wall-associated kinase may also comprise the sequencing of a gene having a certain sequence identity, e.g., 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to one of the gene sequences disclosed herein, which gene has not yet been annotated in a publicly available genome database to determine the precise sequence of said gene by means of molecular biology, e.g., PCR techniques.

[0142] In one embodiment, the method for identifying at least one gene involved in increased pathogen resistance, preferably increased fungal resistance, in a plant cell, tissue, organ, whole plant, or plant material may comprise the determination of the benzoxazinoid (BXD) signature of the at least one plant cell, tissue, organ, whole plant, or plant material of step (i). The benzoxazinoid signature means the qualitative and/or quantitative determination of at least one BXD secondary metabolite of interest as disclosed herein. This determination can provide a reference value for any subsequent analysis. The benzoxazinoid signature determination, which can be performed at different timepoints and with or without the addition of a stimulus, thus can provide information on the background level of a specific BXD present before and after addition of a stimulus. Furthermore, the BXD signature may provide data on the total amount of mixed BXD compounds synthesized in a plant, plant cell, tissue, organ or whole plant under suitable and defined conditions. The BXD signature may thus serve as reference value to have a benchmark for any subsequent modifications and/or modulations performed in accordance with the methods of the present invention. Due to the fact that BXD synthesis depends on the action of different enzymes in the terminal branch of the synthesis pathway, more than one different BXD compound may be analyzed to provide a BXD signature of a plant cell, tissue, organ, or whole plant of interest representing a full picture of the different BXD compounds synthesized by the plant under defined conditions (timepoint, stimulus, stimulus amount and environmental factors, for example, biotic or abiotic stress).

[0143] In one embodiment, the method for identifying at least one gene involved in increased pathogen resistance, preferably increased fungal resistance, in a plant cell, tissue, organ, whole plant, or plant material may comprise the exposition of the at least one plant cell, tissue, organ, whole plant, or plant material of step (i) or (ii) to a stimulus, optionally wherein the stimulus is correlated with the benzoxazinoid signature in the at least one plant cell, tissue, organ, whole plant, or plant material, preferably wherein the stimulus is associated with a fungal pathogen infection.

[0144] A "stimulus" in this context refers to any naturally occurring, endogenous or exogenous, or non-naturally occurring substance chemical substance stimulating a plant cell, tissue, organ, whole plant. Preferably, the stimulus is a stimulus derived from or associated with a pathogen, preferably a fungal pathogen. The stimulus may be a known PAMP or DAMP triggering an immune response mediated by a receptor-like kinase in a plant cell, tissue, organ, whole plant. The correlation may be of direct or indirect nature. The "stimulus" may also be an endogenous substance, e.g., a BXD or jasmonic acid, or a synthetic variant thereof, as BXD compounds and jasmonic acids may induce feedback regulation mechanisms in a plant cell. The "stimulus" may the pathogen by itself causing the desired response in a plant.

[0145] The stimulus may thus be any environmental stimulus which will cause a response in a plant, wherein the response is effected by a signal cascade, or reaction within a plant cell, tissue, organ, whole plant, e.g., resulting in a different transcriptome profile in comparison to the transcriptome profile of a non-stimulated plant. Preferably, the

stimulus is correlated with a benzoxazinoid signature in at least one plant cell, tissue, organ, whole plant, or plant material. In one embodiment, where the correlation between a stimulus and the BXD signature is not known, a correlation between a stimulus of interest and the BXD signature can be easily determined by measuring the up- or down-regulation of genes within the BXD signalling pathway upon addition of a stimulus of interest to determine a direct or indirect correlation.

[0146] In a preferred embodiment, the stimulus is associated with a fungal pathogen, but is not restricted thereto. As it is known in the field of plant pathophysiology, plants evolved sophisticated strategies to respond to a stimulus as provided by a variety of different plant pathogens to initiate defense responses. Certain response may be highly specific for a pathogen, or one specific molecule associated or produced by said pathogen, whereas other defense strategies are part of a global regulatory network as associated by a stimulus of interest. According to the methods of the present invention it is thus possible to analyze the effect of a stimulus of interest a pathway of interest to identify any implication in the BXD or jasmonic acid biosynthesis pathway having a favourable effect on plant fungal response as disclosed herein in a highly targeted way to identify new target genes contributing to a favourable fungal defense response in a plant cell, tissue, organ, or whole plant of interest.

[0147] In one embodiment, the method for identifying at least one gene involved in increased pathogen resistance, preferably increased fungal resistance, in a plant cell, tissue, organ, whole plant, or plant material according to the present invention can comprise an additional step of electronically transmitting and/or electronically storing data on a computer readable medium.

[0148] An "analyte" obtained from the at least one plant cell, tissue, organ, or whole plant may comprises a nucleic acid, including DNA and RNA, an amino acid sequence, or a plant metabolite.

[0149] In one embodiment, a transcriptome analysis, i.e., an analysis of the sum total of all the messenger RNA molecules expressed from the genes of an organism, using RNA obtained from the at least one plant cell, tissue, organ, or whole plant of step (ii) after exposition to the stimulus is performed to obtain data on any changes in the transcription profile of certain genes in a plant cell, tissue, organ, whole plant treated with a stimulus of interest in comparison to plant cell, tissue, organ, or whole plant not treated with the respective stimulus. A variety of different tools to perform a transcriptome analysis of genome-wide differentially expressed RNA and to analyze altered gene expression/transcription is available to the skilled person. In one embodiment, the determination of at least one gene being regulated upon exposition to a stimulus according to step (iii) of the above method for identifying at least one gene involved in increased pathogen resistance in at least one cell of the at least one plant cell, tissue, organ, whole plant thus comprises the determination of the transcription level of a gene. Preferably, differentially regulated, or highly regulated genes, e.g., genes being significantly up- or down-regulated in comparison to a non-treated plant or plant cell, may be further analyzed.

[0150] In another embodiment, a proteome analysis, i.e., an analysis of the entire complement of proteins that is or can be expressed by a plant cell, tissue, or organism, using amino acids obtained from the at least one plant cell, tissue, organ, or whole plant of step (ii) after exposition to the stimulus is performed to obtain data on any changes in the transcription profile in a plant cell, tissue, organ, whole plant treated with a stimulus of interest in comparison to plant cell, tissue, organ, or whole plant not treated with the respective stimulus. Several methods for quantitative and qualitative proteome analysis, of the whole proteome or parts thereof, are available to the skilled person.

[0151] In yet another embodiment, an analysis of a metabolite, e.g., a substance produced by the at least one plant cell, tissue, organ or whole plant and representing an intermediate or product of its metabolism, is performed to identify the effect of a stimulus has on the overall constitution and production level with respect to said metabolite of interest.

[0152] In one embodiment of the method for identifying at least one gene involved in increased pathogen resistance, preferably increased fungal resistance, in a plant cell, tissue, organ, whole plant, or plant material, a gene of interest determined, said gene being regulated upon exposition to a stimulus, preferably a stimulus influencing a BXD signature, may be subjected to a functional characterization. The functional characterization may comprise an in silico analysis, an in vitro analysis, an in vivo analysis, or a combination of the aforementioned analyses. The in silico analysis may comprise the determination of any known function of said gene in different plant, or information on available allelic variants of said gene in different plants or different germplasm. Furthermore, the in silico analysis may comprise the determination of the locus of a gene such determined in the genome of a plant of interest, or the determination of regulatory sequences associated with the gene of interest. An in vitro analysis or manipulation may comprise the cloning, sequencing and characterization of the gene of interest and/or the creation of an expression construct, or vector, or a fusion construct, or the creation of mutants of a gene of interest. An in vitro analysis or manipulation may further comprise the introduction of a gene of interest, comprised by a suitable construct, into a target plant, tissue, organ or whole plant of interest by a suitable delivery vector. The in vivo analysis may comprise the analysis of different plants or plant cells, tissues or organs from different species, cultivars or varieties comprising or not comprising the gene of interest in their genome to provide a functional characterization of the phenotype the gene of interest may participate in, optionally by subjecting the different plants or plant cells, tissues or organs from different species, cultivars or varieties to different stimuli and controlled conditions to be able to compare the respective results.

[0153] In one embodiment, at least one gene involved in increased pathogen resistance as identified according to the methods of the present invention can be further subjected to directed mutagenesis studies and subsequent functional analyses to identify mutations positively or negatively effecting a phenotype of interest, wherein the phenotype is a change in the BXD signature, or a change of fungal resistance in comparison to the respective wild-type. Methods to introduce (multiple) site-directed mutations into a given gene of interest are available to the skilled person.

[0154] In one aspect of the present invention there is provided a plant cell, tissue, organ, whole plant or plant material, or a derivative or a progeny thereof, obtainable by introducing at least one gene as provided by the method for

identifying at least one gene involved in increased pathogen resistance, preferably increased fungal resistance into the genome of at least one cell of at least one of a plant cell, tissue, organ, or whole plant.

[0155] In one embodiment, the plant cell, tissue, organ, whole plant or plant material, or a derivative or a progeny thereof, comprises at least one wall-associated kinase (WAK) selected from Htn1, Ht2, or Ht3, or an allelic variant, a mutant or a functional fragment thereof, or a gene encoding the same, preferably wherein the at least one wall-associated kinase a) is encoded by a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 or 7, or a functional fragment thereof, b) is encoded by a nucleic acid molecule comprising the nucleotide sequence having at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to nucleotide sequence of SEQ ID NO: 1 or 7, preferably over the entire length of the sequence, c) is encoded a nucleic acid molecule hybridizing with a complementary sequence to a) or b) under stringent conditions, d) is encoded by a nucleic acid molecule comprising the nucleotide sequence coding for an amino acid sequence of SEQ ID NO: 2 or 8, or a functional fragment thereof, e) is encoded by a nucleic acid molecule comprising the nucleotide sequence coding for an amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to amino acid sequence of SEQ ID NO: 2 or 8, preferably over the entire length of the sequence, f) comprising the amino acid sequence of SEQ ID NO: 2 or 8, or g) comprising an amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to amino acid sequence of SEQ ID NO: 2 or 8, preferably over the entire length of the sequence, provided that the sequence, optionally after expression, still encodes at least one functional Htn1, Ht2, or Ht3, or an allelic variant, a mutant, or a functional fragment thereof. In a preferred embodiment, the plant cell, tissue, organ, whole plant or plant material, or a derivative or a progeny thereof, comprises at least one wall-associated kinase or an allelic variant, a mutant or a functional fragment thereof, or a gene encoding the same, which has been introduced or introgressed, or which at least one wall-associated kinase or an allelic variant, a mutant or a functional fragment thereof, or a gene encoding the same, comprises at least one mutation enhancing the kinase activity of the at least one WAK.

[0156] In another preferred embodiment, the plant cell, tissue, organ, whole plant or plant material, or a derivative or a progeny thereof, comprises at least one further introduced or introgressed enzyme, or the gene encoding the same, wherein the at least one further gene or enzyme is selected from a bx1, bx2, igl, bx6, bx11, bx14, opr2, lox3 or aoc1 gene (SEQ ID NOs: 10, 12, 14, 16, 18, 20, 22, 24 or 26, respectively), or a homologous gene thereof, or the respective proteins encoded by said genes (SEQ ID NOs: 11, 13, 15, 17, 19, 21, 23, 25 or 27, respectively), or a homolog thereof or an allelic variant or mutant thereof, preferably a mutant resulting in decreased transcription and/or translation of the bx1, bx2, igl, bx6, bx11, bx14, opr2, lox3 or aoc1 gene or protein, respectively. The at least one mutation may thus reside in a regulatory region of such a gene leading to a reduced transcription, or the mutation may result in at least one point mutation affecting the catalytic activity of the translated protein so that said protein or enzyme has a decreased capability to synthesize a BXD compound.

[0157] In one embodiment, the introduction of at least one gene into plant cell, tissue, organ, whole plant or plant material, obtainable by introducing at least one gene as provided by the method for identifying at least one gene involved in increased pathogen resistance, preferably increased fungal resistance, is a stable introduction, preferably a stable introduction mediated by plant breeding, or a stable introduction mediated by means of molecular biology, comprising *Agrobacterium*-mediated transformation, genome editing, or a combination thereof.

[0158] In one embodiment, the introduction may be effected by introgression of the at least one gene identified, and/or the introduction may be effected may any means of molecular biology. In one embodiment the introduction of a gene or allele determined can take place by recombination between two donor genomes, e.g., in a fused protoplast, wherein at least the donor protoplast carries the gene allele of interest in its genome. In any case, any progeny or derivatives comprising the gene allele of interest can then be subjected to repeated back-crossing steps with a plant line carrying a genetic background of interest to select for the gene allele of interest in the resulting derivatives or progeny. The result may be the fixation of the gene allele of interest such introgressed in a selected genetic background. The whole process of introgression can, for example, take place by a mixture of breeding strategies and techniques of molecular biology to achieve at a genotype/phenotype of interest for a given germplasm, plant, plant cell or plant material.

[0159] In one embodiment, there is thus provided an improved donor source of germplasm having, e.g. by introgression, enhanced resistance to a fungus of interest, preferably wherein the fungus resistance against which resistance is increased, or the disease caused by said fungus is selected from a fungus of the order of Pleosporales, comprising *E. turcicum/H. turcicum* causing northern corn leaf blight (NCLB), particularly affecting maize and wheat plants, southern corn leaf blight (*Bipolaris maydis*), the order of Pucciniales causing rust disease, comprising common rust (*Puccinia sorghi*), or *Diploida* leaf streak/blight (*Diploida macrospora/Stenocarpella macrospora*), or *Colletotrichum graminicola*, or *Fusarium* spp., preferably *Fusarium verticilioides* causing *Fusarium* stalk rot, or *Gibberella* spp., e.g., *Gibberella zeae* causing *Giberella* stalk rot, rust, stalk rot, maize head smut (*Sphacelotheca reiliana*), and *Diploida* leaf streak/blight. This germplasm can then serve as basis for further breeding steps.

[0160] In another embodiment, the introduction of at least one gene as identified and provided by the method for identifying at least one gene involved in increased pathogen resistance into at least one plant cell, tissue, organ, whole plant may be effected by at least one means of molecular biology, comprising the use of a delivery vehicle or vector. Optionally, the method can further comprise the modification or modulation of a gene of interest using at least one of a site-specific nuclease (SSN) or a catalytically active fragment thereof, or a nucleic acid sequence encoding the same, oligonucleotide directed mutagenesis, chemical mutagenesis, or TILLING, wherein the at least one site-specific nuclease (SSN), or the nucleic acid sequence encoding the

same, is selected from at least one of a CRISPR nuclease, including Cas or Cpf1 nucleases, a TALEN, a ZFN, a meganuclease, a base editor complex, a restriction endonuclease, including Fok1 or a variant thereof, or two site-specific nicking endonucleases, or a variant or a catalytically active fragment thereof.

[0161] In yet a further aspect according to the present invention, there is provided a method of increasing pathogen resistance, preferably fungal resistance, in a plant cell, tissue, organ, whole plant, or plant material, the method comprising: (i) providing at least one plant cell, tissue, organ, whole plant or plant material; (ii) (a) treating the at least one plant cell, tissue, organ, whole plant or plant material according to step (i) with a substance neutralizing the effect of at least one benzoxazinoid, and/or (ii) (b) treating the at least one plant cell, tissue, organ, whole plant or plant material according to step (i) with a substance activating the signalling pathway downstream of at least one wall-associated kinase; and/or (ii) (c) treating the at least one plant cell, tissue, organ, whole plant or plant material according to step (i) with a substance modulating or modifying the activity of at least promoter or at least one regulatory sequence of at least one gene of the at least one plant cell, tissue, organ, whole plant or plant material of step (i), wherein said at least promoter or at least one regulatory sequence is involved in the regulation of transcription of at least on gene involved in the signalling pathway of, or downstream of at least one wall-associated kinase or involved in the synthesis pathway of at least one benzoxazinoid; (ii) (d) treating the at least one plant cell, tissue, organ, whole plant or plant material according to step (i) with a substance inhibiting the synthesis of at least one benzoxazinoid; (iii) reducing the amount of at least one benzoxazinoid and thereby increasing pathogen resistance, preferably fungal resistance, in at least one plant cell, tissue, organ, whole plant, or plant material.

[0162] A "a substance neutralizing the effect of at least one benzoxazinoid" as used herein is to be construed broadly and comprises any naturally occurring or synthetic molecule, which can interact with a BXD compound to decrease the natural effect of the BXD compound said BXD compound would exert (endogenously and/or exogenously) on a plant or the plant environment. Preferably, the substance neutralizing the effect of at least one benzoxazinoid can be added to a plant cell, tissue, organ, or whole plant, optionally coated or together with a suitable delivery vehicle, so that the substance can be transferred into a plant cell of interest. Alternatively, the substance can be added to a plant cell, tissue, organ or whole plant to neutralize the effect of a volatile compound released by a plant cell, tissue, organ or whole plant. Preferably, the substance neutralizing the effect of at least one benzoxazinoid is not toxic to the plant cell, tissue, organ, or whole plant, or to the environment. As it is known that jasmonic acid (JA) treatment can induce the accumulation of BXD compounds (Oikawa et al, 2002 and 2004), a substance according to the present invention may also be a substance scavenging or reducing the amount of jasmonic acid to decrease the accumulation of a BXD compound, which in turn leads to the increased fungal resistance of a plant cell, tissue, organ or whole plant of interest. The substance may also interfere with the transcription of at least one Bx, Igl or a further gene involved in the BXD or jasmonic acid biosynthesis pathway.

[0163] In a further embodiment, alone or in combination with the use of a neutralizing substance, at least one plant cell, tissue, organ, whole plant or plant material can be treated with a substance activating the signalling pathway downstream of at least one wall-associated kinase. As used in the context of molecular biology, the terms "upstream" and "downstream" can refer to the temporal and mechanistic order of cellular and molecular events. For example, in signal transduction cascade, the second messenger or an intracellular kinase acts downstream to—that is to say, temporally after—activation of cell membrane receptors, for example a WAK. The other way around, activation of cell membrane receptors occurs upstream of—that is to say, prior to—the production of second messengers or the activation or inhibition of further enzymes acting later and intracellularly in the signalling cascade. Such an activating substance can be selected from a substance acting from the exterior of a plant or plant cell, for example, a substance being a PAMP or DAMP for a receptor-like kinase, e.g., a WAK, so that a stronger signal is received and the receptor mediated response is enhanced. Furthermore, the substance may activate the kinase function of a WAK, or any kinase downstream of the WAK. Finally, the substance may act at the interface between the WAK and a further BXD or jasmonic acid biosynthesis pathway.

[0164] For the wheat WAK gene TaWAK/Snn1 it was shown that it is hijacked by the necrotrophic effector SnTox1 that triggers programmed cell death allowing a pathogen to feed and grown on the dead tissue (Shi et al. 2016). Furthermore, these data show that elicitors recognized by WAKs can both be cell wall derived degraded polysaccharides (e.g. OGs) or pathogenic short peptides (SnTox1) (Brutus et al. 2010; Shi et al. 2016). Thus, there is increasing evidence for a complex nature and functional divergence of WAKs in perception of types of ligands and in their role of interacting with biotic diseases in a direct as well as an indirect way. Preferably, an activating substance according to the present invention is a substance directly activating a WAK of interest which in turn, directly or indirectly, leads to a decreased synthesis of at least one BXD compound, which in turn increases the fungal resistance of a plant cell, tissue, organ or whole plant.

[0165] The inventors of the present invention demonstrated that the WAK ZmWAK-RLK1 functions upstream of the BXDs biosynthesis pathway and decreases the content of secondary metabolites BXDs compounds, e.g., DIM_2BOA-Glc. As the BXD class of secondary metabolites has been found in many of cereal species such maize, wheat and rice, which are the most important food crops worldwide, the methods according to the various aspects of the present invention can thus be used to effect the WAK signaling cascade intrinsically linked to the BXD synthesis, which in turn was found to be key to provide new strategies in fungal defense in plants, preferably for reducing susceptibility to northern corn leaf blight already at the seeding stage. For example, the storage glucoside DIM_2BOA-Glc was found to be constantly lower in susceptible ZmWAK-RLK1 mutants, which suggested DIM_2BOA-Glc severed as a candidate susceptibility compound for promoting *E. turcicum* infection. Knock-out of this compound has been shown to slightly increase the performance of corn leaf aphids *Rhopalosiphum maidis* (Handrick, Vinzenz, et al. "Biosynthesis of 8-O-methylated benzoxazinoid defense compounds in maize." *The Plant Cell* 28.7 (2016): 1682-1700), a completely

different class of plant pathogens, not infecting, yet feeding on a plant, whereas the functional mechanism of DIM_2BOA-Glc in interaction with phloem-feeding insects as presently known is possibly different and antagonistic. The methods and findings according to the present invention and mainly the new insights in gap bridge of WAKs and the secondary defense metabolite BXDs can also be used to provide new defense mechanisms against aphids and other phloem feeding insects to a plant, preferably a crop plant.

[0166] In one preferred embodiment, the methods comprise the modulation or modification of at least one further gene from a BXD and/or jasmonic acid biosynthesis pathway as disclosed herein to further decrease the content of secondary metabolites BXDs compounds, e.g., DIM_2BOA-Glc and thus to enhance fungal resistance in a plant.

[0167] In yet a further embodiment of the method of increasing pathogen resistance, preferably fungal resistance, in a plant cell, tissue, organ, whole plant, or plant material according to the present invention, the method comprises treating the at least one plant cell, tissue, organ, whole plant or plant material according to step (i) with a substance modulating the activity of at least promoter or at least one regulatory sequence of at least one gene of the at least one plant cell, tissue, organ, whole plant or plant material of step (i), wherein said at least promoter or at least one regulatory sequence is involved in the regulation of transcription of at least on gene involved in the signalling pathway of, or downstream of at least one wall-associated kinase, or involved in the synthesis pathway of at least one benzoxazinoid. By modulating or modifying the activity of a promoter or regulatory sequence, the transcription level of a gene of interest and in turn the expression level of a protein of interest can be influenced in a targeted way on a molecular level, or by introducing a transcription factor, preferably a synthetic transcription factor like TAL efector activator/repressor or CRISPR-dCas9 activator/repressor, for a given promoter/gene into a cell. In embodiments, where a promoter is modified in a targeted way, the modification is performed by at least one of a site-specific nuclease (SSN) or a catalytically active fragment thereof, or a nucleic acid sequence encoding the same, oligonucleotide directed mutagenesis, chemical mutagenesis, or TILLING.

[0168] In one embodiment, the at least one site-specific nuclease (SSN), or the nucleic acid sequence encoding the same, is selected from at least one of a CRISPR nuclease, including Cas or Cpf1 nucleases, a TALEN, a ZFN, a meganuclease, a base editor complex, a restriction endonuclease, including Fold or a variant thereof, a recombinase, or two site-specific nicking endonucleases, or a variant or a catalytically active fragment thereof. Preferably, a targeted point mutation is introduced modifying the promoter region, wherein the modification can make use of a transient introduction of the site-specific nuclease tools to obtain a non-transgenic plant cell, tissue, organ or whole plant.

[0169] In yet another or further embodiment of the method of increasing pathogen resistance, preferably fungal resistance, in a plant cell, tissue, organ, whole plant, or plant material according to the present invention, the method comprises treating the at least one plant cell, tissue, organ, whole plant or plant material according to step (i) with a substance inhibiting the synthesis of at least one benzoxazinoid. A substance inhibiting the synthesis of at least one benzoxazinoid can be a double stranded RNA (dsRNA) which is suitable to reduce the expression level of at least on gene involved in the signalling pathway of, or downstream of at least one wall-associated kinase, or involved in the synthesis pathway of at least one benzoxazinoid, wherein by said reduction of the expression level the synthesis or the amount of at least one benzoxazinoid and thereby increasing pathogen resistance, preferably fungal resistance, in at least one plant cell, tissue, organ, whole plant, or plant material. This down regulating of gene expression is well-known to a person skilled in art as RNAi approach or miRNA interference approach (Fire, A, Xu, S, Montgomery, M, Kostas, S, Driver, S, Mello, C. (1998). Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*, Nature 391 (6669): 806-811). Preferably the substance inhibiting the synthesis of at least one benzoxazinoid is at least one siRNA or an siRNA library directed to at least on gene involved in the signalling pathway of, or downstream of at least one wall-associated kinase, or involved in the synthesis pathway of at least one benzoxazinoid. The siRNA or siRNA library can be part of one or more expression cassettes. The siRNA may comprise a first strand of RNA of 15 to 30 nucleotides in length having a 5' end and a 3' end, wherein the first strand is complementary to at least 15 nucleotides of the at least on gene involved in the signalling pathway of, or downstream of at least one wall-associated kinase, or involved in the synthesis pathway of at least one benzoxazinoid, and an second strand of RNA of 15 to 30 nucleotides in length having a 5' end and a 3' end, and wherein at least 12 nucleotides of the first and second strands are complementary to each other and form a small interfering RNA (siRNA) duplex under physiological conditions, and wherein the siRNA silences the at least on gene involved in the signalling pathway of, or downstream of at least one wall-associated kinase, or involved in the synthesis pathway of at least one benzoxazinoid.

[0170] The various embodiments of the aspect of the present invention being directed to a method of increasing pathogen resistance, preferably fungal resistance, in a plant cell, tissue, organ, whole plant, or plant material, alone or in combination, may result in the targeted reduction of the amount of at least one benzoxazinoid and thereby may lead to an increased pathogen resistance, preferably fungal resistance, in at least one plant cell, tissue, organ, whole plant, or plant material. In yet a further aspect according to the present invention there is thus provided use of a substance a for increasing pathogen resistance, preferably fungal resistance, in at least one plant cell, tissue, organ, whole plant, or plant material. The substance may act as a plant protective agent and may be applied to a plant exogenously, or the substance may be a scavenger of any plant molecule or material, or the substance may act as a modulator of WAK, of the downstream signalling cascade, or of a cellular pathway disclosed herein being related to the WAK signalling pathway, preferably a BXD and/or jasmonic acid biosynthesis pathway, or the substance may act on the transcription of any gene involved in the WAK or an associated pathway as disclosed herein, wherein the substance can thus directly or indirectly influence, preferably decrease, the amount of a BXD compound produced and stored in a plant cell. Thereby the use of the substance according to the present invention will lead to a decreased BXD level and thus an increased fungal resistance in a plant cell, tissue, organ, or whole plant of interest.

Delivery Methods:

[0171] A variety of suitable delivery techniques suitable according to the methods of the present invention for introducing genetic material into a plant cell are known to the skilled person., e.g. by choosing direct delivery techniques ranging from polyethylene glycol (PEG) treatment of protoplasts (Potrykus, Ingo, et al. "Direct gene transfer to cells of a graminaceous monocot." *Molecular and General Genetics MGG* 199.2 (1985): 183-188), procedures like electroporation (D'Halluin, Kathleen, et al. "Transgenic maize plants by tissue electroporation." *The plant cell* 4.12 (1992): 1495-1505), microinjection (Neuhaus, G., et al. "Transgenic rapeseed plants obtained by the microinjection of DNA into microspore-derived embryoids." *Theoretical and Applied Genetics* 75.1 (1987): 30-36), silicon carbide fiber whisker technology (Kaeppler, H. F., et al. "Silicon carbide fiber-mediated stable transformation of plant cells." *Tag Theoretical and Applied Genetics* 84.5 (1992): 560-566), viral vector mediated approaches (Gelvin, Nature Biotechnology 23, "Viral-mediated plant transformation gets a boost", 684-685 (2005)) and particle bombardment (see e.g. Sood et al., 2011, Biologia Plantarum, 55, 1-15).

[0172] Despite transformation methods based on biological approaches, like *Agrobacterium* transformation or viral vector mediated plant transformation, and methods based on physical delivery methods, like particle bombardment or microinjection, have evolved as prominent techniques for introducing genetic material into a plant cell or tissue of interest. Helenius et al. ("Gene delivery into intact plants using the Helios™ Gene Gun", Plant Molecular Biology Reporter, 2000, 18 (3):287-288) discloses a particle bombardment as physical method for introducing material into a plant cell. Currently, there thus exists a variety of plant transformation methods to introduce genetic material in the form of a genetic construct into a plant cell of interest, comprising biological and physical means known to the skilled person on the field of plant biotechnology and which can be applied to introduce at least one gene encoding at least one wall-associated kinase into at least one cell of at least one of a plant cell, tissue, organ, or whole plant. Notably, said delivery methods for transformation and transfection can be applied to introduce the tools of the present invention simultaneously. A common biological means is transformation with *Agrobacterium* spp. which has been used for decades for a variety of different plant materials. Viral vector mediated plant transformation represents a further strategy for introducing genetic material into a cell of interest. Physical means finding application in plant biology are particle bombardment, also named biolistic transfection or microparticle-mediated gene transfer, which refers to a physical delivery method for transferring a coated microparticle or nanoparticle comprising a nucleic acid or a genetic construct of interest into a target cell or tissue. Physical introduction means are suitable to introduce nucleic acids, i.e., RNA and/or DNA, and proteins. Likewise, specific transformation or transfection methods exist for specifically introducing a nucleic acid or an amino acid construct of interest into a plant cell, including electroporation, microinjection, nanoparticles, and cell-penetrating peptides (CPPs). Furthermore, chemical-based transfection methods exist to introduce genetic constructs and/or nucleic acids and/or proteins, comprising inter alia transfection with calcium phosphate, transfection using liposomes, e.g., cationic liposomes, or transfection with cationic polymers, including DEAD-dextran or polyethylenimine, or combinations thereof. Said delivery methods and delivery vehicles or cargos thus inherently differ from delivery tools as used for other eukaryotic cells, including animal and mammalian cells and every delivery method has to be specifically fine-tuned and optimized so that a construct of interest for introducing and/or modifying at least one gene encoding at least one wall-associated kinase in the at least one plant cell, tissue, organ, or whole plant; and/or can be introduced into a specific compartment of a target cell of interest in a fully functional and active way. The above delivery techniques, alone or in combination, can be used for in vivo (in planta) or in vitro approaches.

[0173] In one embodiment, a regulatory sequence according to the present invention may be a promoter sequence, wherein the editing or mutation or modulation of the promoter comprises replacing the promoter, or promoter fragment with a different promoter (also referred to as replacement promoter) or promoter fragment (also referred to as replacement promoter fragment), wherein the promoter replacement results in any one of the following or any one combination of the following: an increased promoter activity, an increased promoter tissue specificity, a decreased promoter activity, a decreased promoter tissue specificity, a new promoter activity, an inducible promoter activity, an extended window of gene expression, a modification of the timing or developmental progress of gene expression in the same cell layer or other cell layer, for example, extending the timing of gene expression in the tapetum of anthers, a mutation of DNA binding elements and/or a deletion or addition of DNA binding elements. The promoter (or promoter fragment) to be modified can be a promoter (or promoter fragment) that is endogenous, artificial, pre-existing, or transgenic to the cell that is being edited. The replacement promoter or fragment thereof can be a promoter or fragment thereof that is endogenous, artificial, pre-existing, or transgenic to the cell that is being edited.

[0174] The present invention will now be illustrated by the following Examples, which are not construed to limit the scope of the present invention.

EXAMPLES

Example 1: Plant Material and Growth Conditions

[0175] Seventeen maize inbred lines were used, including: (1) historical cultivars B37 and w22, and the NILs B37Htn1 and w22Htn1 that contain the NCLB resistance gene Htn1; (2) Breeding line RP3 and its NIL line RP3Htn1 carrying Htn1 from KWS (see US 2016/0201080 A1); (3) three pairs of mutants RLK1b (S, compromising Htn1 resistance), RLK1d and RLK1f, and corresponding sister lines RLK1b-wt (R, carrying functional Htn1), RLK1d-wt, and RLK1f-wt, which were produced by EMS-mutagenesis in RP3Htn1 (Hurni et al. 2015); (4) three maize mutants (bx1, bx2 and bx6) and parental line w22, which were provided by Prof. Georg Jander (Cornell University, Ithaca, US). Two or three maize seeds were sown in each Jiffy pot (ø8 cm), and fifteen pots were placed in one tray. Seedling plants were grown in a greenhouse condition of 16 h at 20° C. in the day, 8 h at 18° C. in the night and approximately 60% relative humidity.

Example 2: NCLB Infection Tests in the Greenhouse

[0176] Testing for NCLB resistance using *E. turcicum* isolate Passau-1 was performed as previously described with minor modification (Hurni et al. 2015). After the second leaves had fully emerged, the newly emerged leaves were cut and removed until the end of each test experiment. Single spore inoculation and culture on PDA medium plate, harvest and quantification of progeny spores were described (Hurni et al. 2015). Instead of infection by dropping 80 µl spore suspension into the leaf sheath of the second leaf twice, here maize seedlings were infected once by spray (sprayer: ø28 mm, Semadeni, Ostermundigen, Switzerland). Each 4 trays (ca. 60-80 seedlings) were sprayed with 4 ml of spore suspension (4.5×10^4 spores/ml). A very high humidity mic-condition was produced by placing plastic hoods on top of each tray after infection. Each plant was scored for disease symptom between 11 and 25 days and the severity was evaluated by calculating the area under the disease progress curve (AUDPC) or by quantifying the diseased leaf area of the inoculated second leaves (PrimDLA). About 15 seedling plants were scored in each genotype in each experiment.

Example 3: Vector Construction and Subcellular Localization

[0177] The coding sequence of ZmWAK-RLK1 was amplified using a cDNA clone as template, which was initially amplified in NCLB resistance genotype RP1Htn1 (Hurni et al. 2015). The PCR fragment was introduced into the Gateway donor vector pDONR207 using the Gateway® BP Clonase® II Enzyme mix (Thermo Fisher Scientific, Wilmington, USA). The generated entry vector carrying the target ZmWAK-RLK1 sequence was inserted by recombination with the destination vector pUBC-GFP-DEST, to produce an in-frame ZmWAK-RLK1+c'-eGFP fusion protein construct driven by *Arabidopsis* ubiqutin-10 (UBQ10) gene promotor (Grefen, Christopher, et al. "A ubiquitin-10 promoter-based vector set for fluorescent protein tagging facilitates temporal stability and native protein distribution in transient and stable expression studies." *The Plant Journal* 64.2 (2010): 355-365). The UBQ10::ZmWAK-RLK1-c'-eGFP construct (SEQ ID NO:9) together with a reference plasmid PIP2A-mCherry (Cutler et al., Random GFP:: cDNA fusions enable visualization of subcellular structures in cells of *Arabidopsis* at a high frequency. Proc. Natl Acad. Sci. USA 97, 3718-3723, (2000) contains 35S::PIP2A_c'_RFP construct, which is localized to the plasma membrane) were mixed with nanograde gold particles and co-bombarded into onion epidermal cells, which were subsequently incubated at 20° C. in the dark for 2-3 days until being ready for observation using a confocal microscope. Plasmolysis was induced by adding a 0.8 M mannitol solution. Furthermore, both plasmids were transformed into *Agrobacterium* GV3101 and co-infiltrated into 4-week-old *N. bentaniana* leaves, which were ready for observation 2 days post infiltration. The primers used for vector construct are provided in the below Table 1.

TABLE 1

Forward (F) and reverse (R) primers used for vector constructs

Order	Target genes	Primers (5' to 3')	PCR efficiency (E) R^2 of Calibration curve slope	Description
1	Actin	F - SEQ ID NO: 28 R - SEQ ID NO: 29	E = 109.8%, R^2 = 0.995, Slope = −3.107	Reference gene
2	FPGS	F - SEQ ID NO: 30 R - SEQ ID NO: 31	E = 104.9%, R^2 = 0.990, Slope = −3.209	Reference gene
3	ZmWAK-RLK1	F - SEQ ID NO: 32 R - SEQ ID NO: 33	E = 104.0%, R^2 = 0.996, Slope = −3.229	WAK-RLK1 = ZmWAK-RLK1 herein.
4	BX1 (BX: benzoxazinless)	F - SEQ ID NO: 34 R - SEQ ID NO: 35	E = 100.1%, R^2 = 0.994, Slope = −3.319	Benzoxazinoid pathway
5	BX2	F - SEQ ID NO: 36 R - SEQ ID NO: 37	E = 126.1%, R^2 = 0.989, Slope = −2.821	Benzoxazinoid pathway
6	BX3	F - SEQ ID NO: 38 R - SEQ ID NO: 39	E = 107.4%, R^2 = 0.994, Slope = −3.156	Benzoxazinoid pathway
7	BX4	F - SEQ ID NO: 40 R - SEQ ID NO: 41	E = 100.9%, R^2 = 0.994, Slope = −3.300	Benzoxazinoid pathway
8	Bx5	F - SEQ ID NO: 42 R - SEQ ID NO: 43	E = 109.7%, R^2 = 0.992, Slope = −3.109	Benzoxazinoid pathway
9	BX6	F - SEQ ID NO: 44 R - SEQ ID NO: 45	E = 107.6%, R^2 = 0.961, Slope = −3.153	Benzoxazinoid pathway
10	BX7	F - SEQ ID NO: 46 R - SEQ ID NO: 47	E = 106.9%, R^2 = 0.989, Slope = −3.167	Benzoxazinoid pathway
11	BX8	F - SEQ ID NO: 48 R - SEQ ID NO: 49	E = 114.0%, R^2 = 0.987, Slope = −3.027	Benzoxazinoid pathway
12	BX9	F - SEQ ID NO: 50 R - SEQ ID NO: 51	E = 117.3%, R^2 = 0.999, Slope = −2.967	Benzoxazinoid pathway
13	BX10 + BX11	F - SEQ ID NO: 52 R - SEQ ID NO: 53	E = 109.5%, R^2 = 0.999, Slope = −3.114	Benzoxazinoid pathway
14	BX12	F - SEQ ID NO: 54 R - SEQ ID NO: 55	E = 95.4%, R^2 = 0.996, Slope = −3.437	Benzoxazinoid pathway
15	BX13	F - SEQ ID NO: 56 R - SEQ ID NO: 57	E = 99.3%, R^2 = 0.961, Slope = −3.340	Benzoxazinoid pathway
16	IGL (Indole Glycerol Phosphate Lyase)	F - SEQ ID NO: 58 R - SEQ ID NO: 59	E = 111.8%, R^2 = 0.996, Slope = −3.069	Benzoxazinoid pathway
17	GLU1 (GLU: beta glucosidase)	F - SEQ ID NO: 60 R - SEQ ID NO: 61	E = 110.6%, R^2 = 0.998, Slope = −3.092	Benzoxazinoid pathway

TABLE 1-continued

Forward (F) and reverse (R) primers used for vector constructs

Order	Target genes	Primers (5' to 3')	PCR efficiency (E) R^2 of Calibration curve slope	Description
18	GLU2	F - SEQ ID NO: 62 R - SEQ ID NO: 63	E = 110.4%, R^2 = 0.985, Slope = −3.095	Benzoxazinoid pathway
19	ZmWAK-RLK1 plasmid construction	F - SEQ ID NO: 64 R - SEQ ID NO: 65		

Example 4: Mycelium Development

[0178] The second leaves of 21-day seedling plants were harvested and cut into 2×2 cm^2 leaf segments, which were placed and incubated on the phytoagar plates. A spore suspension (4.5×10^4 spores/ml) was painted using swabs on the leaf surface. The petri dishes carrying samples were sealed using PARAFILM and incubated 24 hours at room temperature until harvest. Trypan blue straining was conducted as previously described (Chung, Chia-Lin, et al. "Resistance loci affecting distinct stages of fungal pathogenesis: use of introgression lines for QTL mapping and characterization in the maize-*Setosphaeria turcica* pathosystem." *BMC plant biology* 10.1 (2010): 103). The infected segments at 1 dpi were incubated overnight in an acetic acid:ethanol (1:3, v/v) solution, and then in a mixed solution of acetic acid:ethanol:glycerol (1:5:1, v/v/v) for 4 hours. The samples were stained overnight in 0.01% (w/v) trypan blue lactophenol solution, and then washed once using ddH_2O and stored in 60% glycerol ready for use. Specimens were placed on slides and examined under the ZEISS Axio Imager 2 microscope system (CARL ZEISS, Jena, Germany). The numbers of germinated spores, germ tubes, appressoria and successful penetrations (hyphae inside of cell or between cell walls) were counted. Three independent experiments were performed.

Example 5: RNA Extraction, RNA Sequencing and Data Analysis

[0179] The second leaves of seedling plants were harvested with four biological replicates at 0, 9-hpi, 3-dpi and 10-dpi, which corresponded to before inoculation, the germination/penetration, biotrophic growth and necrotrophic growth, respectively (Jennings, P. R., and A. J. Ullstrup. "A HISTOLOGICAL STUDY OF 3 *HELMINTHOSPORIUM* LEAF BLIGHTS OF CORN." *Phytopathology* 47.12 (1957): 707-714; Hilu, H. M., and A. L. Hooker. "Host-pathogen relationship of *Helminthosporium turcicum* in resistant and susceptible corn seedlings." (1964): 570-5). Forty-eight samples (4 genotypes, 4 time points, 3 biological replicates) were subjected for total RNA extraction using SV Total RNA Isolation Kits (Promega, Dübendorf, Switzerland). 1 μl of total RNA was checked by Nanodrop 1000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, USA) to estimate the RNA concentration. Meanwhile, 15 plants in each genotype were evaluated for the AUDPC value to control if the infection worked.

[0180] The quantity and quality in RNA sequencing were determined using Qubit® 1.0 Fluorometer (Thermo Fisher Scientific, Wilmington, USA) and Bioanalyzer 2100 (Agilent, Waldbronn, Germany). The TruSeq Stranded mRNA Sample Prep Kit (Illumina, Inc., Hayward, USA) was used for library preparation. 1 μg of total RNA per sample was ribosome depleted and then subjected for synthesizing double-strand cDNA. Each cDNA sample was fragmented, end-repaired, polyadenylated and then ligated with TruSeq adaptor that contains the index for multiplexing. The cDNA fragments containing TruSeq adapters at the both ends were enriched with PCR reaction. The enriched libraries were quantified and qualified, and then normalized to 10 nM. The TruSeq SR Cluster Kit v4 cBot (Illumina, Inc., Hayward, USA) was used for cluster generation using 8 pM of pooled normalized libraries. Sequencing was performed on the Illumina HiSeq2500 at single end 125 bp using the TruSeq SBS Kit v4 (Illumina, Inc., Hayward, USA).

[0181] The maize reference genome Zea_mays.AGPv3.27 and the corresponding annotation were downloaded (http://www.maizegdb.org/). The RNA sequencing reads were mapped on the reference genome with STAR (Dobin, Alexander, et al. "STAR: ultrafast universal RNA-seq aligner." *Bioinformatics* 29.1 (2013): 15-21) allowing one mismatch per 100 bp and no multimapper with the following command: STAR—outFilterMultiMapNmax 1—outFilterMismatchNoverLmax 0.01-alignIntronMax 10000. Read counts were determined from the mapping files with featureCounts 1.4.6 (Liao, Yang, Gordon K. Smyth, and Wei Shi. "featureCounts: an efficient general purpose program for assigning sequence reads to genomic features." *Bioinformatics* 30.7 (2013): 923-930). Statistical analyses were done with the R package edgeR and genes were tested for differential expression with pairwise comparisons and tagwise estimation of dispersion. A gene was considered to be expressed when at least 10 reads were mapped on it and a gene was considered to be differentially expressed with log $2FC\geq|2|$ and $FDR<0.01$. First, pairwise comparisons were performed between Htn1 and no Htn1 plants for each genotype and each time points separately. The results were then compared between time points and then between the two genotypes. The Gene Ontology analysis for differentially expressed genes (DEGs) was conducted by using online software agriGO (Du, Zhou, et al. "agriGO: a GO analysis toolkit for the agricultural community." *Nucleic acids research* 38.suppl_2 (2010): W64-W70). The significant terms were colored if adjusted $p\leq0.05$.

Example 6: RT-qPCR Assay

[0182] 1 μg total RNA was subjected for first strand cDNA synthesis using the iScript Advanced cDNA kit (172-5038, Rio-Rad). 1:20 diluted cDNA was applied for quantifying expression using a Real-Time System C1000TM Thermal cycler (96 or 384 wells, Bio-Rad). The expression of targets was normalized by the reference genes FPGS and Actin as described (Hurni et al. 2015). The primers for expression analysis are shown in Table 1 above.

Example 7: Benzoxazinoids (BXDs) Extraction and Measurement

[0183] 60-100 mg leaves (without veins) of the seedling plants were harvested and freezing immediately in liquid nitrogen, grinded and added the extraction buffer (1 mg

sample+10 µl extraction buffer). The samples were mixed thoroughly and centrifuged at 13,000 rpm under 4° C. The supernatant was transferred into new tube and centrifuged once more under same condition, to remove the possible leaf particles. The supernatant was collected being ready for BXDs measurement.

[0184] Benzoxazinoid contents were analyzed by an Acquity UPLC equipment (Waters) coupled to a UV detector and coupled to a mass spectrometer (Waters) (Meihls, Lisa N., et al. "Natural variation in maize aphid resistance is associated with 2, 4-dihydroxy-7-methoxy-1, 4-benzoxazin-3-one glucoside methyltransferase activity." *The Plant Cell Online* 25.6 (2013): 2341-2355). An Acquity BEH C18 column (Waters) was used. The temperatures of the autosampler and column were 15° C. and 40° C., respectively. The mobile phase consisted of 99% water, 1% acetonitrile, and 0.1% Formic acid (A) and acetonitrile and 0.1% Formic acid (B). Flow rate was set to 0.4 ml min^{-1} with 3% A and 97% B followed by column reconditioning. The injection volume was 5 µl. The extracted trace at 275 nm was used for benzoxazinoids quantification. The following extracted ion chromatograms were used for quantification with a mass window of ±0.01 D: mass-to-charge ratio (m/z) for DIMBOA (retention time [RT] 5.62 min) and DIMBOA-Glc (RT 5.64 min), m/z for HDMBOA-Glc (RT 8.19 min), m/z for HMBOA-Glc (RT 5.34 min) and DIM_2BOA-Glc (RT 5.825 min). Benzoxazinoids absolute concentrations were determined by external calibration curves obtained from purified DIMBOA-Glc, DIMBOA and HDMBOA-Glc standards.

Example 8: ZmWAK-RLK1 Encodes a Plasma Membrane Localized Protein

[0185] To determine the subcellular localization of the ZmWAK-RLK1 protein, a fusion construct consisting of a full-length coding sequence fused to the sequence of an enhanced green fluorescence protein (eGFP) at the C terminus was generated (cf. SEQ ID NO:9 for the nucleic acid plasmid construct). The ZmWAK-RLK1 fusion protein localized to the plasma membrane before and after plasmolysis when transiently expressed in onion epidermal cells. Furthermore, infiltration into leaves of *Nicotiana benthamiana* confirmed the localization of ZmWAK-RLK1 to the plasma membrane two days after infiltration (cf. FIG. **12**). These data, particularly confocal analysis of onion epidermal cells after transient expression of ZmWAK-RKL1-eGFP and the positive control PIP2A-mCherry that is known to localize to the plasma membrane demonstrate that ZmWAK-RLK1 is a plasma membrane protein.

Example 9: ZmWAK-RLK1 Reduced Fungal Penetrations

[0186] Spores of the hemibiotrophic fungus *E. turcicum* penetrate the maize epidermis mostly between 6-18 hours after inoculation (hpi) (Jennings and Ullstrup, 1957). To investigate if ZmWAK-RLK1 changes the outcome of fungal penetration attempts, we investigated the infection process at one day post inoculation (dpi) using trypan blue staining (data not shown). The number of successful penetration events were evaluated in three EMS-induced ZmWAK-RLK1 loss-of-function mutant lines (RLK1b, RLK1d and RLK1f) and their corresponding sister lines that were generated in the near isogenic line (NIL) RP3Htn1 (Hurni et al. 2015). No significant difference in the establishment of germ tubes and appressoria was observed in genotypes with/without ZmWAK-RLK1 (data not shown). In contrast, the number of successful penetration events was significantly lower if ZmWAK-RLK1 was functional compared to loss-of-function mutants as demonstrated in FIGS. **2** A and B. This indicates that ZmWAK-RLK1 leads to a reduction of pathogen penetrations into host tissues.

Example 10: Transcriptome and Metabolism Analysis Identified Alterations to the BXDs Biosynthesis Pathway in the Presence of ZmWAK-RLK1

[0187] To decipher the immune network specifically influenced by ZmWAK-RLK1, we performed a transcriptome analysis by RNA sequencing in two pairs of near isogenic lines, w22 and W22Htn1 as well as B37 and B37Htn1. NCLB development was significantly reduced in the presence of ZmWAK-RLK1 in both NILs (FIG. **3** A-C). Leaf samples were collected at 0 and 9 hpi (penetration stage) as well as 3 (biotrophic growth) and 10 dpi (necrotrophic growth) (Jennings and Ullstrup, 1957). Forty-eight samples were sequenced and 1.159 billion reads were obtained (Table 2). More than 820 million reads were uniquely mapped with an average of 17.08 million reads per sample (70.7%) (Table 2). No obvious difference of percentage of mapped reads in genotypes with or without Htn1 was observed. A total of 15,345 genes were expressed and they were used for further analysis. By conducting a multidimensional scaling (MDS) analysis using expression normalized by edgeR, the biological replicates for the same genotype-timepoints combination were mostly grouped together, suggesting the repeatability of replicates (data not shown). More differentially expressed gene (DEGs) were detected in B37Htn1/B37 compared to w22Htn1/w22 (FIG. **4**). These genes differently expressed in both NILs in at least one of timepoints. To identify DEGs associated with ZmWAK-RLK1 and to rule out genetic background effects, only genes that were differentially expressed in both NIL pairs were considered.

TABLE 2

Statistics of RNA-seq reads sequenced and mapped

Sample number	Samples	Raw reads	Uniquely mapped reads[a]	Percentage of uniquely mapped reads (%)	Percentage of multi-mapped reads (%)	Percentage of unmapped reads (%)
1	0-w22-1	19,894,158	12,757,882	64.13	12.66	20.34
2	0-w22-2	25,718,662	18,612,570	72.37	6.63	20.18
3	0-w22-3	24,937,383	17,948,211	71.97	6.41	20.85
4	0-w22Htn1-1	27,452,042	20,264,307	73.82	5.96	19.70

TABLE 2-continued

Statistics of RNA-seq reads sequenced and mapped

Sample number	Samples	Raw reads	Uniquely mapped reads[a]	Percentage of uniquely mapped reads (%)	Percentage of multi-mapped reads (%)	Percentage of unmapped reads (%)
5	0-w22Htn1-2	19,815,200	14,439,891	72.87	6.07	20.44
6	0-w22Htn1-3	24,173,527	16,215,534	67.08	9.55	21.40
7	0-B37-1	22,180,888	14,689,311	66.23	5.27	28.03
8	0-B37-2	24,474,895	18,273,286	74.66	5.96	18.75
9	0-B37-3	27,774,311	20,221,020	72.80	6.73	19.74
10	0-B37Htn1-1	26,728,646	19,277,404	72.12	6.16	21.04
11	0-B37Htn1-2	24,336,731	17,640,859	72.49	6.06	20.82
12	0-B37Htn1-3	22,625,055	16,499,617	72.93	6.15	20.30
13	9h-w22-1	25,343,283	17,460,901	68.90	7.81	22.49
14	9h-w22-2	26,384,078	18,489,206	70.08	7.16	22.03
15	9h-w22-3	21,846,924	13,680,343	62.62	5.97	30.81
16	9h-w22Htn1-1	34,074,874	23,121,230	67.85	6.27	25.40
17	9h-w22Htn1-2	24,276,403	17,299,847	71.26	7.02	21.07
18	9h-w22Htn1-3	30,200,422	20,381,456	67.49	7.23	24.62
19	9h-B37-1	17,962,777	12,878,288	71.69	7.38	20.10
20	9h-B37-2	23,815,810	16,892,476	70.93	7.12	21.22
21	9h-B37-3	24,188,988	17,604,433	72.78	7.15	19.38
22	9h-B37Htn1-1	25,195,971	17,304,100	68.68	7.80	22.65
23	9h-B37Htn1-2	23,902,398	16,173,755	67.67	7.27	24.40
24	9h-B37Htn1-3	24,731,481	17,279,056	69.87	7.13	22.39
25	3d-w22-1	22,399,000	15,772,632	70.42	6.44	22.49
26	3d-w22-2	26,175,191	18,816,409	71.89	6.43	21.06
27	3d-w22-3	23,253,678	16,758,123	72.07	6.45	20.82
28	3d-w22Htn1-1	23,878,639	17,185,076	71.97	5.92	21.58
29	3d-w22Htn1-2	20,568,384	14,338,420	69.71	7.09	22.35
30	3d-w22Htn1-3	34,008,596	24,127,141	70.94	5.81	22.76
31	3d-B37-1	23,690,913	14,726,042	62.16	4.70	32.71
32	3d-B37-2	27,646,350	19,934,380	72.10	5.89	21.42
33	3d-B37-3	21,114,803	16,208,484	76.76	5.69	16.98
34	3d-B37Htn1-l	21,758,134	15,409,264	70.82	5.68	23.03
35	3d-B37Htn1-2	27,236,811	19,866,766	72.94	5.84	20.68
36	3d-B37Htn1-3	22,700,637	16,553,064	72.92	5.60	21.00
37	10d-w22-1	23,051,842	16,566,330	71.87	6.16	21.24
38	10d-w22-2	28,611,557	20,463,896	71.52	6.01	21.77
39	10d-w22-3	12,829,435	7,480,564	58.31	4.82	36.38
40	10d-w22Htn1-1	24,435,356	17,199,294	70.39	5.45	23.76
41	10d-w22Htn1-2	28,996,022	21,442,119	73.95	5.78	19.69
42	10d-w22Htn1-3	18,649,090	13,429,238	72.01	5.78	21.71
43	10d-B37-1	13,275,463	9,658,508	72.75	5.46	21.16
44	10d-B37-2	15,217,491	11,288,257	74.18	5.38	19.89
45	10d-B37-3	23,349,541	17,096,788	73.22	5.36	20.83
46	10d-B37Htn1-1	20,104,137	14,270,397	70.98	4.94	23.79
47	10d-B37Htn1-2	38,203,107	27,216,898	71.24	5.40	22.82
48	10d-B37Htn1-3	26,094,601	18,820,165	72.12	5.61	21.68
	Total	1,159,283,685	820,033,238	70.74	NA[b]	NA[b]
	Mean	24,151,743	17,084,026	70.74	NA[b]	NA[b]

[a]Parameters for mapping: less than 1% mismatch, 1 locus mapped, intron size is less than 10 kb;
[b]NA = not analyzed.

[0188] Two-hundred and fifteen common DEGs were identified across all time points (FIG. **13**). 132 and 83 genes were induced and repressed in the ZmWAK-RLK1-containing NIL compared to the respective susceptible line (heat map and Venn diagram not shown). 108 DEGs were only found at timepoint 0 before inoculation, while 107 of DEGs were found only after infection. Twenty-nine DEGs were differently expressed at all time points including timepoint. An overrepresentation analysis using agriGO was performed to identify enriched Gene Ontology (GO) terms associated with ZmWAK-RLK1. The functional groups were enriched for terms of defense response (e.g. GO:0009814) and metabolic/biosynthetic process (e.g. GO:0006725) (data not shown).

[0189] To further analyze if the presence of ZmWAK-RLK1 is associated with BXDs biosynthesis (FIG. **5** A), the content of major BXDs in second leaves of w22Htn1 and w22 before and after infection was quantified (FIG. **5** B to F). The content of four BXDs DIMBOA-Glc, DIMBOA, HMBOA-Glc and DIM_2BOA-Glc was significantly lower in w22Htn1 compared to w22 at all timepoints (data not shown), which indicated a constitutive reduction on BXDs content when ZmWAK-RLK1 is present. Furthermore, via RT-qPCR the transcriptional levels of ZmWAK-RLK1 and specifically the genes in the BXDs biosynthesis pathway before and after pathogen inoculation were determined. The expression of genes (A) ZmWAK-RLK1, (B) Bx1, (C) Igl, (D) Bx2, (E) Bx3, (F) Bx4, (G) Bx5, (H) Bx6, (I) Bx7, (J) Bx8, (K) Bx9, (L) Bx10/11, (M) Bx12, (N) Bx13, (0) Glu1 and (P) Glu2 at different timepoints before and after infection was measured and statistics were conducted separately

in w22 and B37 genetic background using Tukey's HSD (P=0.05) in four biological replicates.

[0190] The ZmWAK-RLK1 expression in NILs showed no significant difference (FIG. **11** A). Overall, the transcriptional levels of BXD biosynthesis genes were genotype-specific (FIG. **11** A to P)). For instance, BX1 and its protein homolog IGL can convert indole-3-glycerol phosphate into indole, which is the first step of BXD metabolism (Frey et al. 2000). Their coding genes Bx1 and Igl showed opposite contributions of gene expression in B37 and w22, but the combined expression of Bx1 and Igl was consistently lower in genotypes with ZmWAK-RLK1 (FIGS. **11** B and C).

Example 11: Mutations in ZmWAK-RLK1 is Associated with the Reduction of Secondary Metabolite DIM_2BOA-Glc

[0191] To further analyze the role of different BXD biosynthesis genes as well as the metabolites of this pathway in NCLB resistance, the ZmWAK-RLK1 mutants (RLK1b, d and f, SEQ ID NOs: 3 to 6 and G548R mutant of SEQ ID NO:2) and their sister lines were used (Hurni et al. 2015). The transcript levels of several BXD genes were quantified and the content of major BXD compounds in mutants which lost the resistance caused by ZmWAK-RLK1 (FIG. **6** A to D). In contrast to the NILs, there was no difference in the DIMBOA-Glc, DIMBOA and HMBOA, HDMBOA-Glc concentration. However, the content of DIM_2BOA-Glc was significantly lower when ZmWAK-RLK1 was intact (FIG. **6** A, and further data not shown). The reduced DIM_2BOA-Glc correlated with a reduction in Igl transcript levels, while no obvious difference in the transcriptional levels of ZmWAK-RLK1 and Bx1 was detected in ZmWAK-RLK1 (FIG. **6** B to D) Bx6, Bx7 and Bx13 are key genes of the BXDs pathway to produce DIM_2BOA-Glc, and these genes were slightly but not significantly upregulated in mutants (data not shown). This phenomenon can be explained by a feedback regulation. Therefore, ZmWAK-RLK1 clearly seems to be associated with the reduction of secondary metabolite DIM_2BOA-Glc, possibly by reducing the expression levels of Bx1 and/or Igl.

Example 12: Mutations in BXDs Biosynthesis Genes Decreased NCLB Susceptibility

[0192] To further analyze the role of BXDs in NCLB resistance/susceptibility, mutants in the three BXD biosynthesis genes Bx1, Bx2 and Bx6 were tested upon inoculation with *E. turcicum*. These mutants showed strong reduction in several BXDs compounds, including DIM_2BOA-Glc (FIG. **7**). Interestingly, all three mutants showed a strong reduction of NCLB susceptibility at the seedling stage (FIGS. **8** A and B). This confirmed a negative correlation of BXDs content and NCLB disease resistance. Furthermore, the ZmWAK-RLK1 expression at 10 dpi was checked (FIG. **8** C). No significant difference was detected in the bx mutants if compared to the wild-type. Considering the reduction of Bx1 and Igl co-expression in ZmWAK-RLK1 genotypes (FIG. **6** A, FIGS. **5** B and C, FIG. **9** B), the data suggest that the BXDs synthesis pathway likely acts downstream of ZmWAK-RLK1.

[0193] Therefore, ZmWAK-RLK1 underlying quantitative NCLB disease resistance is based on a decrease of the biosynthesis of secondary metabolite BXDs, and DIM_2BOA-Glc served as a candidate susceptibility component for promoting fungal infection (FIG. **8** D).

Example 13: Tissue-Specific Expression of WAK-RLK1

[0194] Further to the determination of the subcellular localization of a ZmWAK-RLK1 protein (see Example 8 above), the tissue specific expression of RLK1 in different genotypes, and different time points was determined. The genes FPGS and ACTIN served as reference to normalize the expression. The results shown in FIG. **10** demonstrate that RLK1 is a ubiquitously and steadily expressed gene which further underpins the function of said kinase as master regulator in relevant plant metabolic pathways.

Example 14: Tilling

[0195] To develop and screen a mutant population of plant material, TILLING was performed. A TILLING mutant population can be created, e.g., starting from KWS line RP3Htn1 according to Kato (2000, The maize handbook, pp. 212-219)). Pollen is harvested from field-grown RP3Htn1 plants and treated with 0.1% EMS solution for 45 min. Silks of individual plants are then pollinated and emerging ears bagged. From 436 pollinated MO plants seeds were harvested, in one experiment. An additional propagation and selfing led to 10,084 individual M1 plants. Leaf material from these M1 plants was collected for DNA isolation. DNA of dried leaf samples (10 leaf discs bunches/sample) was isolated from 10,000 M1 individuals with the CTAB extraction method (Traitgenetics, Gatersleben, Germany). DNA is then aliquoted to 100 µl with 20 ng/µl. Primer development for mutant screening is performed. The amplification assay consisted of 20 ng/µl DNA, 5×GoTaq-Buffer, 25 µM dNTPs, 10 µM forward Primer, 10 µM reverse Primer, 5 Units/µl GoTaq. After denaturation for 300 s at 94° C. the amplification cycles were performed with 35 cycles of 60 s at 94° C., 60 s at 60° C. and 60 s at 72° C. followed by a final elongation time for 600 s at 72° C. Next, Sanger-sequencing of PCR products is performed according to established protocols. Sequences are then assembled, for example with the help of the software Lasergene Seqman NGen (DNASTAR) and heterozygote SNPs called with the software default settings. Positive mutant plants were sequenced again with the Sanger-method in order to confirm the polymorphism.

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gctgttgccg ttgcattcct gtgtctagtc attctcacat gcttcttggc ttgtagacat   1020

ggttcgctgc ccttcaaatc ggagaacaaa ccagggacaa ggattgagtc cttcctacag   1080

aagaacgaga gtatacatcc gaaaagatac acctacgcgg acgtgaaaag aatgacaaaa   1140

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gatggccgac aggtagcagt caagatgctg aaggacaccc aaggtgacgg cgaggaattc   1260

atgaacgagg tggctagcat cagcaggact tctcatgtca acgtcgtgac acttctaggg   1320

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gaaaggtatg ccttcaccgg tgacatgaac agtgagaatt tgctaacctg ggaaaggcta   1440

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cggatcgtgc attttgacat caagccacac aacatcctgt tagaccagga tttctgtcct   1560

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ctttatgaac atttggacga ctattgtgtt ggtgcttccg agattaatgg tgagaccaca   1860

gagctcgtga ggaagatgat agttgtaggt ctgtggtgca tacaagtgat tccgactgat   1920

cgaccaacaa tgacgagagt cgtcgagatg ttggaaggga gcacaagtaa tctagagttg   1980

ccacccagag ttctcttgag ctga                                          2004
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<210> SEQ ID NO 6
<211> LENGTH: 667
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: RLK1d - mutant Htn1

<400> SEQUENCE: 6

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Ala Ala His Val Val Ser Thr Ser Ala His Gly Glu Pro Pro Leu Pro
            20                  25                  30

Ser Pro Tyr Asn Thr Ser Ala His Gly Glu Pro Pro Leu Pro Ser Thr
        35                  40                  45

Tyr Asn Ala Ser Met Cys Ser Ser Phe Trp Cys Gly Gly Val Glu Ile
    50                  55                  60

Arg Tyr Pro Phe Tyr Leu Ala Asn Ala Ile Ala Asp Tyr Ser Gly Ser
65                  70                  75                  80

Tyr Tyr Ser Cys Gly Tyr Thr Asp Leu Ser Val Ser Cys Glu Leu Glu
                85                  90                  95

Val Glu Gly Ser Pro Thr Thr Trp Thr Pro Thr Ile Arg Leu Gly Gly
            100                 105                 110

Gly Asp Tyr Thr Val Lys Asn Ile Ser Tyr Leu Tyr Asp Gln Gln Thr
        115                 120                 125

Ile Ser Leu Ala Asp Arg Asp Val Leu Gly Gly Gly Gly Cys Pro Val
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    130                 135                 140

Val Arg His Asn Val Ser Phe Asp Glu Thr Trp Leu His Leu His Asn
145                 150                 155                 160

Ala Ser Ala Phe Asp Asn Leu Thr Phe Phe Phe Gly Cys His Trp Gly
                165                 170                 175

Pro Arg Asn Thr Pro Pro Glu Phe Ala Asp Tyr Asn Ile Ser Cys Ala
            180                 185                 190

Gly Phe Asn Thr Pro Thr Ile Ser Gly Gly Arg Ser Phe Val Phe Lys
        195                 200                 205

Thr Gly Asp Leu Asp Glu Gln Glu Glu Gln Glu Leu Ala Leu His Cys
    210                 215                 220

Asp Glu Val Phe Ser Val Pro Val Arg Arg Asp Ala Leu Gln Ala Ile
225                 230                 235                 240

Val Ser Asn Phe Ser Leu Thr Arg Asp Gly Tyr Gly Glu Val Leu Arg
                245                 250                 255

Gln Gly Phe Glu Leu Glu Trp Asn Arg Thr Ser Glu Asp Gln Cys Gly
            260                 265                 270

Arg Cys Glu Gly Ser Gly Ser Gly Gly Trp Cys Ala Tyr Ser Gln Lys
        275                 280                 285

Arg Glu Phe Leu Gly Cys Leu Cys Ser Gly Gly Lys Val Gly Ser Pro
    290                 295                 300

Phe Cys Lys Pro Ser Arg Ser Lys Arg Lys Glu Gly Pro Ile Val Gly
305                 310                 315                 320

Ala Val Ala Val Ala Phe Leu Cys Leu Val Ile Leu Thr Cys Phe Leu
                325                 330                 335

Ala Cys Arg His Gly Ser Leu Pro Phe Lys Ser Glu Asn Lys Pro Gly
            340                 345                 350

Thr Arg Ile Glu Ser Phe Leu Gln Lys Asn Glu Ser Ile His Pro Lys
        355                 360                 365

Arg Tyr Thr Tyr Ala Asp Val Lys Arg Met Thr Lys Ser Phe Ala Val
    370                 375                 380

Lys Leu Gly Gln Gly Gly Phe Gly Ala Val Tyr Lys Gly Ser Leu His
385                 390                 395                 400

Asp Gly Arg Gln Val Ala Val Lys Met Leu Lys Asp Thr Gln Gly Asp
                405                 410                 415

Gly Glu Glu Phe Met Asn Glu Val Ala Ser Ile Ser Arg Thr Ser His
            420                 425                 430

Val Asn Val Val Thr Leu Leu Gly Phe Cys Leu Gln Gly Ser Lys Arg
        435                 440                 445

Ala Leu Ile Tyr Glu Tyr Met Pro Asn Gly Ser Leu Glu Arg Tyr Ala
    450                 455                 460

Phe Thr Gly Asp Met Asn Ser Glu Asn Leu Leu Thr Trp Glu Arg Leu
465                 470                 475                 480

Phe Asp Ile Ala Ile Gly Thr Ala Arg Gly Leu Glu Tyr Leu His Arg
                485                 490                 495

Glu Cys Asn Thr Arg Ile Val His Phe Asp Ile Lys Pro His Asn Ile
            500                 505                 510

Leu Leu Asp Gln Asp Phe Cys Pro Lys Ile Ser Asp Phe Gly Leu Ala
        515                 520                 525

Lys Leu Cys Leu Asn Lys Glu Ser Ala Ile Ser Ile Ala Gly Ala Arg
    530                 535                 540
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Gly Thr Ile Gly Tyr Ile Ala Pro Glu Val Tyr Ser Lys Gln Phe Gly
545                 550                 555                 560

Ile Ile Ser Ser Lys Ser Asp Val Tyr Ser Tyr Gly Met Met Val Leu
                565                 570                 575

Glu Met Val Gly Ala Arg Asp Arg Asn Thr Ser Ala Asp Ser Asp His
            580                 585                 590

Ser Ser Gln Tyr Phe Pro Gln Trp Leu Tyr Glu His Leu Asp Asp Tyr
        595                 600                 605

Cys Val Gly Ala Ser Glu Ile Asn Gly Glu Thr Thr Glu Leu Val Arg
    610                 615                 620

Lys Met Ile Val Val Gly Leu Trp Cys Ile Gln Val Ile Pro Thr Asp
625                 630                 635                 640

Arg Pro Thr Met Thr Arg Val Val Glu Met Leu Glu Gly Ser Thr Ser
                645                 650                 655

Asn Leu Glu Leu Pro Pro Arg Val Leu Leu Ser
            660                 665


<210> SEQ ID NO 7
<211> LENGTH: 2004
<212> TYPE: DNA
<213> ORGANISM: Zea mays

<400> SEQUENCE: 7

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ggcgagcctc ctcttccgag cacttacaac gtctccatgt gctcggaatc gttctggtgc    180

ggcggcgtcg aaatccgcta cccgttctat cttgccaacg caaccgccga ctacagcggg    240

agctactact cctgcggcta caccgacttg agcgtttcct gcaaactcga ggtcgagggg    300

ccgacgacga catggacccc taccatccgt ctcggcggcg acaactacac cgtcaagaac    360

atcttgtacg actatcatac catctcactg gcggacagcg atgtgctcgg aggcggcgag    420

tgccccgtcg tccaccacaa cgtcagcttc gacgagacgt ggctgcacaa ccccagcgcc    480

ttcgacaacc tcaccttctt cttcggatgc cactgggggc cacgcgatac actgcctgaa    540

tttgccggca acaacatcag ctgcgccggg ttcagtactc cagctatcag cggtggaggc    600

tccttcgtgt tcaagcctga agatcttgac gaacatgcgg agcaggagtt ggcttcacac    660

tgcgacgagg ttttctccgt gccagtgaga agcgaggctc tgcagcaggc gatcgtcagc    720

aacctcagcc tcggggacgg gtacggcgag ctgcttaggc aggggatcga gttggaatgg    780

aaacggacat cggaggatca gtgtggccag tgcgaggaat cgggctccgg cggacggtgc    840

gcctacagcc agaagagaga attccttggc tgcttctgca gcggagggaa ggcgggcaac    900

ccgttctgca aaccatcaag atcaacaaaa aggagagaag cagcatctat tgttggtgct    960

gttgccgttg cattcctgtg tctagtcatt ctcacatgct tcttggcttg tagacattgt   1020

tcgctgccct tcaaatcgaa gaacaaacca gggacaagga ttgagtcctt cctacagaag   1080

aacgagagta gtatacatcc gaaaagatac acctacgcgg acgtgaaaag aatgacaaaa   1140

tccttcgctg tgaagctagg ccaaggtggg tttggtgctg tatacaaagg cagcctccac   1200

gatggccgac aggtagcagt caagatgctg aaggacaccc aaggtgacgg cgaggaattc   1260

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cggatcgtgc attttgacat caagccacac aacatcctgt tagaccagga tttctgccct 1560

aagatctctg actttggact ggccaagcta tgtctgaaca aagagagcgc tatctccatt 1620

gctggcgcaa gagggacgat agggtatatc gccccggagg tctactcaaa gcaatttgga 1680

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gcaagggaca ggaatacaag cgcagatagt gaccatagca gccaatattt ccctcagtgg 1800

ctttatgaac atttggacga ctattgtgtt ggtgcttccg agattaatgg tgagaccaca 1860

gagctcgtga ggaagatgat agttgtaggt ctgtggtgca tacaagtgat tccgactgat 1920

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ccacccagag ttctcttgag ctga 2004

<210> SEQ ID NO 8
<211> LENGTH: 667
<212> TYPE: PRT
<213> ORGANISM: Zea mays

<400> SEQUENCE: 8

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Ala Ala His Val Val Ser Thr Ser Ala His Ala Glu Pro Pro Leu Pro
20 25 30

Ser Pro Tyr Ser Thr Ser Ala His Gly Glu Pro Pro Leu Pro Ser Thr
35 40 45

Tyr Asn Val Ser Met Cys Ser Glu Ser Phe Trp Cys Gly Gly Val Glu
50 55 60

Ile Arg Tyr Pro Phe Tyr Leu Ala Asn Ala Thr Ala Asp Tyr Ser Gly
65 70 75 80

Ser Tyr Tyr Ser Cys Gly Tyr Thr Asp Leu Ser Val Ser Cys Lys Leu
85 90 95

Glu Val Glu Gly Pro Thr Thr Thr Trp Thr Pro Thr Ile Arg Leu Gly
100 105 110

Gly Asp Asn Tyr Thr Val Lys Asn Ile Leu Tyr Asp Tyr His Thr Ile
115 120 125

Ser Leu Ala Asp Ser Asp Val Leu Gly Gly Gly Glu Cys Pro Val Val
130 135 140

His His Asn Val Ser Phe Asp Glu Thr Trp Leu His Asn Pro Ser Ala
145 150 155 160

Phe Asp Asn Leu Thr Phe Phe Phe Gly Cys His Trp Gly Pro Arg Asp
165 170 175

Thr Leu Pro Glu Phe Ala Gly Asn Asn Ile Ser Cys Ala Gly Phe Ser
180 185 190

Thr Pro Ala Ile Ser Gly Gly Gly Ser Phe Val Phe Lys Pro Glu Asp
195 200 205

Leu Asp Glu His Ala Glu Gln Glu Leu Ala Ser His Cys Asp Glu Val
210 215 220

Phe Ser Val Pro Val Arg Ser Glu Ala Leu Gln Gln Ala Ile Val Ser

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225                 230                 235                 240

Asn Leu Ser Leu Gly Asp Gly Tyr Gly Glu Leu Leu Arg Gln Gly Ile
                245                 250                 255

Glu Leu Glu Trp Lys Arg Thr Ser Glu Asp Gln Cys Gly Gln Cys Glu
            260                 265                 270

Glu Ser Gly Ser Gly Gly Arg Cys Ala Tyr Ser Gln Lys Arg Glu Phe
        275                 280                 285

Leu Gly Cys Phe Cys Ser Gly Gly Lys Ala Gly Asn Pro Phe Cys Lys
    290                 295                 300

Pro Ser Arg Ser Thr Lys Arg Arg Glu Ala Ala Ser Ile Val Gly Ala
305                 310                 315                 320

Val Ala Val Ala Phe Leu Cys Leu Val Ile Leu Thr Cys Phe Leu Ala
                325                 330                 335

Cys Arg His Cys Ser Leu Pro Phe Lys Ser Lys Asn Lys Pro Gly Thr
            340                 345                 350

Arg Ile Glu Ser Phe Leu Gln Lys Asn Glu Ser Ser Ile His Pro Lys
        355                 360                 365

Arg Tyr Thr Tyr Ala Asp Val Lys Arg Met Thr Lys Ser Phe Ala Val
    370                 375                 380

Lys Leu Gly Gln Gly Gly Phe Gly Ala Val Tyr Lys Gly Ser Leu His
385                 390                 395                 400

Asp Gly Arg Gln Val Ala Val Lys Met Leu Lys Asp Thr Gln Gly Asp
                405                 410                 415

Gly Glu Glu Phe Met Asn Glu Val Ala Ser Ile Ser Arg Thr Ser His
            420                 425                 430

Val Asn Val Val Thr Leu Leu Gly Phe Cys Leu Gln Gly Ser Lys Arg
        435                 440                 445

Ala Leu Ile Tyr Glu Tyr Met Pro Asn Gly Ser Leu Glu Arg Tyr Ala
    450                 455                 460

Phe Thr Gly Asp Met Asn Ser Glu Asn Leu Leu Thr Trp Glu Arg Leu
465                 470                 475                 480

Phe Asp Ile Ala Ile Gly Thr Ala Arg Gly Leu Glu Tyr Leu His Arg
                485                 490                 495

Gly Cys Asn Thr Arg Ile Val His Phe Asp Ile Lys Pro His Asn Ile
            500                 505                 510

Leu Leu Asp Gln Asp Phe Cys Pro Lys Ile Ser Asp Phe Gly Leu Ala
        515                 520                 525

Lys Leu Cys Leu Asn Lys Glu Ser Ala Ile Ser Ile Ala Gly Ala Arg
    530                 535                 540

Gly Thr Ile Gly Tyr Ile Ala Pro Glu Val Tyr Ser Lys Gln Phe Gly
545                 550                 555                 560

Thr Ile Ser Ser Lys Ser Asp Val Tyr Ser Tyr Gly Met Met Val Leu
                565                 570                 575

Glu Met Val Gly Ala Arg Asp Arg Asn Thr Ser Ala Asp Ser Asp His
            580                 585                 590

Ser Ser Gln Tyr Phe Pro Gln Trp Leu Tyr Glu His Leu Asp Asp Tyr
        595                 600                 605

Cys Val Gly Ala Ser Glu Ile Asn Gly Glu Thr Thr Glu Leu Val Arg
    610                 615                 620

Lys Met Ile Val Val Gly Leu Trp Cys Ile Gln Val Ile Pro Thr Asp
625                 630                 635                 640
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Arg Pro Thr Met Thr Arg Val Val Glu Met Leu Glu Gly Ser Thr Ser
645 650 655

Asn Leu Glu Leu Pro Pro Arg Val Leu Leu Ser
660 665

<210> SEQ ID NO 9
<211> LENGTH: 11884
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: ZmWAK-RLK1 plasmid

<400> SEQUENCE: 9

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aacaactttg cgtgtaaaca acgctcaata cacgtgtcat tttattatta gctattgctt 180

caccgcctta gctttctcgt gacctagtcg tcctcgtctt ttcttcttct tcttctataa 240

aacaataccc aaagagctct tcttcttcac aattcagatt tcaatttctc aaaatcttaa 300

aaactttctc tcaattctct ctaccgtgat caaggtaaat ttctgtgttc cttattctct 360

caaaatcttc gattttgttt tcgttcgatc ccaatttcgt atatgttctt tggtttagat 420

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cgaatttgta gattaatctg agtttttctg attaacactc gagtgcggga tcctctaagg 660

gcccatcaca agtttgtaca aaaaagcagg cttcatggct gctcaccagc ctcacctctc 720

cgtcctcctc ctcgtcctcc tcgctgccca tgtcgtctcc acctccgccc atggcgagcc 780

tcctcttccg agcccttaca acacctccgc ccatggcgag cctcctcttc cgagcactta 840

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gctgaaggac acccaaggtg acggcgagga attcatgaac gaggtggcta gcatcagcag 1980

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gcgtcagcgg gagagccgtt gtaaggcggc agactttgct catgttaccg atgctattcg 5160

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gccgtgggtc gatgtttgat gttatggagc agcaacgatg ttacgcagca gggcagtcgc 5520

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cgcaatgaca ttcttgcagg tatcttcgag ccagccacga tcgacattga tctggctatc 5940

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caagaagaag atcgcttggc ctcgcgcgca gatcagttgg aagaatttgt ccactacgtg 6300

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acacaaattg ggagatatat catgcatgac caaaatccct taacgtgagt tttcgttcca 6540

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tcgg 11884

<210> SEQ ID NO 10
<211> LENGTH: 1044
<212> TYPE: DNA
<213> ORGANISM: Zea mays

<400> SEQUENCE: 10

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gacgcggccg tcgtcgtcac taccaccacc actgctagag ctgctgcggc tgctgtcacg 180

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tggagtgaag ccaagaacaa caacctggag ctggtgctgc tgacaacacc agccatacca 720

gaagacagga tgaaggagat caccaaggct tcagaaggct tcgtctacct ggtgagcgtg 780

aacggagtga caggtcctcg cgcaaacgtg aacccacgag tggagtcact catccaggag 840

gttaagaagg tgactaacaa gcccgttgct gttggcttcg gcatatccaa gcccgagcac 900

gtgaagcaga ttgcgcagtg gggcgctgac ggggtgatca tcggcagcgc catggtgagg 960

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ggcatgaaga acgcgctgcc atga 1044

<210> SEQ ID NO 11
<211> LENGTH: 347
<212> TYPE: PRT
<213> ORGANISM: Zea mays

<400> SEQUENCE: 11

Met Ala Phe Ala Pro Lys Thr Ser Ser Ser Ser Ser Leu Ser Ser Ala
1 5 10 15

Leu Gln Ala Ala Gln Ser Pro Pro Leu Leu Leu Arg Arg Met Ser Ser
20 25 30

Thr Ala Thr Pro Arg Arg Arg Tyr Asp Ala Ala Val Val Val Thr Thr
35 40 45

Thr Thr Thr Ala Arg Ala Ala Ala Ala Ala Val Thr Val Pro Ala Ala
50 55 60

Pro Pro Gln Ala Pro Ala Pro Ala Pro Val Pro Pro Lys Gln Ala Ala
65 70 75 80

Ala Pro Ala Glu Arg Arg Ser Arg Pro Val Ser Asp Thr Met Ala Ala
85 90 95

Leu Met Ala Lys Gly Lys Thr Ala Phe Ile Pro Tyr Ile Thr Ala Gly
100 105 110

Asp Pro Asp Leu Ala Thr Thr Ala Glu Ala Leu Arg Leu Leu Asp Gly
115 120 125

Cys Gly Ala Asp Val Ile Glu Leu Gly Val Pro Cys Ser Asp Pro Tyr
130 135 140

Ile Asp Gly Pro Ile Ile Gln Ala Ser Val Ala Arg Ala Leu Ala Ser
145 150 155 160

Gly Thr Thr Met Asp Ala Val Leu Glu Met Leu Arg Glu Val Thr Pro
165 170 175

Glu Leu Ser Cys Pro Val Val Leu Leu Ser Tyr Tyr Lys Pro Ile Met
180 185 190

Ser Arg Ser Leu Ala Glu Met Lys Glu Ala Gly Val His Gly Leu Ile
195 200 205

Val Pro Asp Leu Pro Tyr Val Ala Ala His Ser Leu Trp Ser Glu Ala
210 215 220

Lys Asn Asn Asn Leu Glu Leu Val Leu Leu Thr Thr Pro Ala Ile Pro
225 230 235 240

Glu Asp Arg Met Lys Glu Ile Thr Lys Ala Ser Glu Gly Phe Val Tyr
245 250 255

Leu Val Ser Val Asn Gly Val Thr Gly Pro Arg Ala Asn Val Asn Pro
260 265 270

Arg Val Glu Ser Leu Ile Gln Glu Val Lys Lys Val Thr Asn Lys Pro
275 280 285

Val Ala Val Gly Phe Gly Ile Ser Lys Pro Glu His Val Lys Gln Ile
290 295 300

Ala Gln Trp Gly Ala Asp Gly Val Ile Ile Gly Ser Ala Met Val Arg
305 310 315 320

Gln Leu Gly Glu Ala Ala Ser Pro Lys Gln Gly Leu Arg Arg Leu Glu
325 330 335

Glu Tyr Ala Arg Gly Met Lys Asn Ala Leu Pro
340 345

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<210> SEQ ID NO 12
<211> LENGTH: 1182
<212> TYPE: DNA
<213> ORGANISM: Zea mays

<400> SEQUENCE: 12

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gaggaggagg tgcgcggcgc gctcgacagg gtgcgccgcg ccgccgccgc gcgggaggcc 180

ctggacatga gcgagctcct gcactccttc gtcaacaacc tcgtctgccg cgccgtctcg 240

ggcaagttct ccatggagga aggccgcaac aggctgttcc gggagctcac cgacatcaac 300

gcgggactcc tcggagggtt ccacatccag gactacttcc ctagcctggg ccggatcgag 360

ctcgtcagga aggtggcgtg cgccaagacc aggcgcgtca ggaagcgctg ggacgacctc 420

ctcgacaagc tcatcgacga ccatgcggcc aggatggcaa cccatcagga cgaggacgac 480

gataaggact tcatatacgt cttgctgtct cttcagaagg agtatggcct caccagagac 540

catatcaagg ctatcttgat agacatgttc gaggccggaa ccgacacctc gtacatgacg 600

ttggagttcg ccatgacgga gctcatacgg aagccacacc tgatgaagaa gctgcaggaa 660

gaagtgcggc ggaacgtgcc ggcggggcag gagatggtca ccgaggacaa cctccccggc 720

atgacctacc tcaaggccgt catcaaggag acgctccgcc tgcacccgcc ggtcccgctc 780

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ctgccggcgg ggatgaaggc ggaggacgtg gacatgaccg aggtgtttgg gatcacggtc 1140

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<210> SEQ ID NO 13
<211> LENGTH: 393
<212> TYPE: PRT
<213> ORGANISM: Zea mays

<400> SEQUENCE: 13

Met Tyr Gly Ala Thr Asp Ser Cys Phe Ala Pro Tyr Gly Asp His Phe
1 5 10 15

Arg Lys Ala Arg Lys Leu Val Thr Val His Leu Leu Asn Ala Ser Lys
20 25 30

Val Arg Ser Gln Arg Pro Ala Arg Glu Glu Glu Val Arg Gly Ala Leu
35 40 45

Asp Arg Val Arg Arg Ala Ala Ala Ala Arg Glu Ala Leu Asp Met Ser
50 55 60

Glu Leu Leu His Ser Phe Val Asn Asn Leu Val Cys Arg Ala Val Ser
65 70 75 80

Gly Lys Phe Ser Met Glu Glu Gly Arg Asn Arg Leu Phe Arg Glu Leu
85 90 95

Thr Asp Ile Asn Ala Gly Leu Leu Gly Gly Phe His Ile Gln Asp Tyr

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            100                 105                 110

Phe Pro Ser Leu Gly Arg Ile Glu Leu Val Arg Lys Val Ala Cys Ala
        115                 120                 125

Lys Thr Arg Arg Val Arg Lys Arg Trp Asp Asp Leu Leu Asp Lys Leu
    130                 135                 140

Ile Asp Asp His Ala Ala Arg Met Ala Thr His Gln Asp Glu Asp Asp
145                 150                 155                 160

Asp Lys Asp Phe Ile Tyr Val Leu Leu Ser Leu Gln Lys Glu Tyr Gly
                165                 170                 175

Leu Thr Arg Asp His Ile Lys Ala Ile Leu Ile Asp Met Phe Glu Ala
            180                 185                 190

Gly Thr Asp Thr Ser Tyr Met Thr Leu Glu Phe Ala Met Thr Glu Leu
        195                 200                 205

Ile Arg Lys Pro His Leu Met Lys Lys Leu Gln Glu Glu Val Arg Arg
    210                 215                 220

Asn Val Pro Ala Gly Gln Glu Met Val Thr Glu Asp Asn Leu Pro Gly
225                 230                 235                 240

Met Thr Tyr Leu Lys Ala Val Ile Lys Glu Thr Leu Arg Leu His Pro
                245                 250                 255

Pro Val Pro Leu Leu Leu Pro His Tyr Ser Leu Asp Ala Cys Glu Val
            260                 265                 270

Ala Gly Tyr Thr Ile Pro Ala Asn Thr Arg Val Val Val Asn Ala Trp
        275                 280                 285

Ala Leu Gly Arg His Ser Gly Tyr Trp Glu Arg Glu Asn Glu Phe Val
    290                 295                 300

Pro Glu Arg Phe Leu Ser Gly Asp Val Ala Gly Gly Val Asp Leu Lys
305                 310                 315                 320

Pro Asn Glu Phe Gln Phe Leu Ala Phe Gly Ser Gly Arg Arg Met Cys
                325                 330                 335

Pro Gly Val His Ser Ala Ser Ala Thr Ile Glu Ala Met Leu Ser Asn
            340                 345                 350

Leu Met Tyr Arg Phe Asp Trp Gln Leu Pro Ala Gly Met Lys Ala Glu
        355                 360                 365

Asp Val Asp Met Thr Glu Val Phe Gly Ile Thr Val Ser Arg Lys Glu
    370                 375                 380

Lys Leu Leu Leu Val Pro Gln Ala Ala
385                 390


<210> SEQ ID NO 14
<211> LENGTH: 1020
<212> TYPE: DNA
<213> ORGANISM: Zea mays

<400> SEQUENCE: 14

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aggtctgtgg ccactgtaag ggcggtcgcc gcggtagctc cggctgcccc ggcggcgccg    180

gccaagctca ccgccggtgc cggcggccga tgcctgccgg tgtcgcagac catgtccagg    240

ctcagggcgc agggcaagac ggcgttcatc ccgtacatca ccgccggcga cccggacctg    300

gcgacgacgg cggaggcgct gcggctcctg gacgcctgcg gcgccgacgt catcgagctc    360
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ggcgttccct tctcggaccc ctacgccgac gggccggtca tccaggcgtc ggcggcgcgg 420

gcactggcga gcggcacgac gccggacggc gtgctggcga tgctgaagga ggtgacgccg 480

gagctgtcct gccccgtggt gctcttctcc tacttcaacc ccatcgtgcg gtggggcctg 540

gccgacttcg ccgccgccgt caaggaagcc ggcgtgcacg gcctcatagt tcccgacctc 600

ccgtatggga actcgtgcgc tctcactctc aggaccgaag ccatcaagaa caacctcgag 660

ctggtgctgc tcacaacacc atctacgcca gcggacagga tggaggagat aacacgagct 720

tcacgaggat tcgtctatct cgcgagcgtc aatggagtta caggtccacg cgcaaacgtg 780

aacacacgtg tccagtctct cattcaggag gttaaacagg tcactgacat acccgtggct 840

gtcgggtttg gcatatcgaa acctgagcat gtaaagcaga ttgcagagtg gggtgcagat 900

ggtgtgatca ttggtagcgc aatggtgaga cagttaggcg aagcagcctc tcccaaagaa 960

ggattgaaga ggctagagaa atacgccagg atcatgaaga acgcgctacc atgccagtga 1020

<210> SEQ ID NO 15
<211> LENGTH: 339
<212> TYPE: PRT
<213> ORGANISM: Zea mays

<400> SEQUENCE: 15

Met Ala Ser Ala Ile Lys Ala Ala Ser Thr Ser Ser Gln Trp Ser Ser
1 5 10 15

Ser Pro Ala Ala Val His Ser Ser Pro Leu Ser Lys Arg Leu Pro Ala
20 25 30

Ala Val Ala Met Pro Gly Arg Arg Arg Ser Val Ala Thr Val Arg Ala
35 40 45

Val Ala Ala Val Ala Pro Ala Ala Pro Ala Ala Pro Ala Lys Leu Thr
50 55 60

Ala Gly Ala Gly Gly Arg Cys Leu Pro Val Ser Gln Thr Met Ser Arg
65 70 75 80

Leu Arg Ala Gln Gly Lys Thr Ala Phe Ile Pro Tyr Ile Thr Ala Gly
85 90 95

Asp Pro Asp Leu Ala Thr Thr Ala Glu Ala Leu Arg Leu Leu Asp Ala
100 105 110

Cys Gly Ala Asp Val Ile Glu Leu Gly Val Pro Phe Ser Asp Pro Tyr
115 120 125

Ala Asp Gly Pro Val Ile Gln Ala Ser Ala Ala Arg Ala Leu Ala Ser
130 135 140

Gly Thr Thr Pro Asp Gly Val Leu Ala Met Leu Lys Glu Val Thr Pro
145 150 155 160

Glu Leu Ser Cys Pro Val Val Leu Phe Ser Tyr Phe Asn Pro Ile Val
165 170 175

Arg Trp Gly Leu Ala Asp Phe Ala Ala Ala Val Lys Glu Ala Gly Val
180 185 190

His Gly Leu Ile Val Pro Asp Leu Pro Tyr Gly Asn Ser Cys Ala Leu
195 200 205

Thr Leu Arg Thr Glu Ala Ile Lys Asn Asn Leu Glu Leu Val Leu Leu
210 215 220

Thr Thr Pro Ser Thr Pro Ala Asp Arg Met Glu Glu Ile Thr Arg Ala
225 230 235 240

Ser Arg Gly Phe Val Tyr Leu Ala Ser Val Asn Gly Val Thr Gly Pro

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               245                 250                 255

Arg Ala Asn Val Asn Thr Arg Val Gln Ser Leu Ile Gln Glu Val Lys
            260                 265                 270

Gln Val Thr Asp Ile Pro Val Ala Val Gly Phe Gly Ile Ser Lys Pro
        275                 280                 285

Glu His Val Lys Gln Ile Ala Glu Trp Gly Ala Asp Gly Val Ile Ile
    290                 295                 300

Gly Ser Ala Met Val Arg Gln Leu Gly Glu Ala Ala Ser Pro Lys Glu
305                 310                 315                 320

Gly Leu Lys Arg Leu Glu Lys Tyr Ala Arg Ile Met Lys Asn Ala Leu
               325                 330                 335

Pro Cys Gln
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<210> SEQ ID NO 16
<211> LENGTH: 1125
<212> TYPE: DNA
<213> ORGANISM: Zea mays

<400> SEQUENCE: 16

```
atggctccaa cgaccgccac caaggacgac agtggctacg gcgacgagcg ccggcgcgag     60

ctgcaggcgt tcgacgacac gaagctgggg gtgaaaggtc tggtggactc cggcgtcaag    120

tccatcccgt ccatcttcca ccacccgccg gaagccctga gcgacataat ttctccggcg    180

cctctgccgt cgtcgccacc ctccggcgcc gccatcccgg tggtggacct ctcggtgacg    240

cggcgcgagg acctagtcga gcaggtgagg cacgcggccg ggacggtggg cttcttctgg    300

ctggtgaacc acggcgtggc ggaggagctg atgggcggca tgctccgcgg ggtgcgccag    360

ttcaacgagg gccccgtgga ggcgaagcag gcgctctact ccagggacct ggctcgcaac    420

ctgcgcttcg cctccaactt cgacctcttc aaggccgctg cggccgactg gcgcgacacg    480

ctcttctgcg aggtggctcc gaacccgccg ccgcgggagg agctgccgga gccactgagg    540

aacgtgatgc tggagtacgg cgcggcggtg acgaagctgg cgcggttcgt gttcgagctg    600

ctgtccgagt ccctcggcat gcccagcgac cacctgtacg agatggagtg catgcagaac    660

ctgaacgtgg tgtgccagta ctacccgccg tgcccggagc cgcaccgcac cgtgggcgtc    720

aagaggcaca cggaccctgg cttcttcacc atcctcctgc aggacggcat gggcggcctg    780

caggtgcgcc tcggcaacaa cgggcagagc ggcggctgct gggtggacat cgctccccgg    840

cctggggcgc tcatggtcaa catcggtgat cttctgcagc tcgtgaccaa tgaccggttc    900

aggagcgtgg agcaccgggt gccagccaac aagagcagcg acacggcgag ggtctccgtg    960

gcgtccttct tcaacacgga cgtgaggagg tccgagagga tgtacggccc cattccagac   1020

cccagcaagc ccccgctgta caggagcgtc agggcccgag acttcatcgc aaagttcaac   1080

accataggac tcgatggccg cgcgctcgac cacttcaggc tctag                   1125
```

<210> SEQ ID NO 17
<211> LENGTH: 374
<212> TYPE: PRT
<213> ORGANISM: Zea mays

<400> SEQUENCE: 17

```
Met Ala Pro Thr Thr Ala Thr Lys Asp Asp Ser Gly Tyr Gly Asp Glu
1               5                   10                  15
```

-continued

```
Arg Arg Arg Glu Leu Gln Ala Phe Asp Asp Thr Lys Leu Gly Val Lys
            20                  25                  30

Gly Leu Val Asp Ser Gly Val Lys Ser Ile Pro Ser Ile Phe His His
        35                  40                  45

Pro Pro Glu Ala Leu Ser Asp Ile Ile Ser Pro Ala Pro Leu Pro Ser
    50                  55                  60

Ser Pro Pro Ser Gly Ala Ala Ile Pro Val Val Asp Leu Ser Val Thr
65                  70                  75                  80

Arg Arg Glu Asp Leu Val Glu Gln Val Arg His Ala Ala Gly Thr Val
                85                  90                  95

Gly Phe Phe Trp Leu Val Asn His Gly Val Ala Glu Glu Leu Met Gly
            100                 105                 110

Gly Met Leu Arg Gly Val Arg Gln Phe Asn Glu Gly Pro Val Glu Ala
        115                 120                 125

Lys Gln Ala Leu Tyr Ser Arg Asp Leu Ala Arg Asn Leu Arg Phe Ala
    130                 135                 140

Ser Asn Phe Asp Leu Phe Lys Ala Ala Ala Ala Asp Trp Arg Asp Thr
145                 150                 155                 160

Leu Phe Cys Glu Val Ala Pro Asn Pro Pro Pro Arg Glu Glu Leu Pro
                165                 170                 175

Glu Pro Leu Arg Asn Val Met Leu Glu Tyr Gly Ala Ala Val Thr Lys
            180                 185                 190

Leu Ala Arg Phe Val Phe Glu Leu Leu Ser Glu Ser Leu Gly Met Pro
        195                 200                 205

Ser Asp His Leu Tyr Glu Met Glu Cys Met Gln Asn Leu Asn Val Val
    210                 215                 220

Cys Gln Tyr Tyr Pro Pro Cys Pro Glu Pro His Arg Thr Val Gly Val
225                 230                 235                 240

Lys Arg His Thr Asp Pro Gly Phe Phe Thr Ile Leu Leu Gln Asp Gly
                245                 250                 255

Met Gly Gly Leu Gln Val Arg Leu Gly Asn Asn Gly Gln Ser Gly Gly
            260                 265                 270

Cys Trp Val Asp Ile Ala Pro Arg Pro Gly Ala Leu Met Val Asn Ile
        275                 280                 285

Gly Asp Leu Leu Gln Leu Val Thr Asn Asp Arg Phe Arg Ser Val Glu
    290                 295                 300

His Arg Val Pro Ala Asn Lys Ser Ser Asp Thr Ala Arg Val Ser Val
305                 310                 315                 320

Ala Ser Phe Phe Asn Thr Asp Val Arg Arg Ser Glu Arg Met Tyr Gly
                325                 330                 335

Pro Ile Pro Asp Pro Ser Lys Pro Pro Leu Tyr Arg Ser Val Arg Ala
            340                 345                 350

Arg Asp Phe Ile Ala Lys Phe Asn Thr Ile Gly Leu Asp Gly Arg Ala
        355                 360                 365

Leu Asp His Phe Arg Leu
    370


<210> SEQ ID NO 18
<211> LENGTH: 1128
<212> TYPE: DNA
<213> ORGANISM: Zea mays

<400> SEQUENCE: 18
```

-continued

```
atggcactca tcatgcagga gagtagtagc catgatttgc tccaagctca cgacgagctc     60

ttgcaccatt ccctgtgctt cgccaaatcg ctcgcgctcg ccgtggcgct ggacctccgc    120

atccccgacg cgatccacca ccacggcgcc ggcggcgcca cccttctcca gatcctcgcc    180

gagactgcgc tccacccaag caagcttcgc gcccttcgcc gcctcatgcg cgtgctcacc    240

gtcacgggca tcttcagcgt cgagcaacca ccagcaggtg gtggtgataa ttcaaccgtc    300

cacacgtcgg acgacgaagc tgtcgtcgtc tacaggttga cggcagcctc ccgcttcctc    360

gtcagcgacg aggtgagcac ggcgaccttg gctccctttg tgagtctggc gctccagcct    420

atcgctgcct gtccgcacgc cctgggtatc tccgcgtggt tccggcagga gcagcacgag    480

ccgtccccgt atggcctggc gttccggcag accccaacga tctgggaaca tgctgacgac    540

gtaaacgcct tgctgaacaa aggcatggcc gcggacagcc gcttcctcat gccaattgtg    600

ctgagggagt gcggcgagac gtttcgtggg atcgactcgt tggttgacgt cggtggtggc    660

catggtggcg ccgccgccgc catcgccgcc gccttccccc acctcaagtg cagcgtgctt    720

gacctcccgc acgttgtcgc cggtgctccg tctgatggca acgtgcactt cgtcgcaggc    780

aatatgtttg agagtattcc acctgcaacc gctgttttcc tcaagaaaac tctacatgac    840

tggggtgacg atgagtgtgt caagatattg aagaattgca agcaagccat atctccacgg    900

gatgcaggtg ggaaggtaat aatcttggac gtggtagttg gatataaaca gtcaaacata    960

aagcatcaag agacacaagt tatgtttgat ttgtatatga tggcggttaa cggagttgag   1020

cgtgatgagc aagagtggaa gaagatcttc attgaagctg gattcaaaga ctacaaaatt   1080

ctacccgtca tcggtgacgt atcagtcatc atcgaggtct atccttga                1128
```

<210> SEQ ID NO 19
<211> LENGTH: 375
<212> TYPE: PRT
<213> ORGANISM: Zea mays

<400> SEQUENCE: 19

```
Met Ala Leu Ile Met Gln Glu Ser Ser Ser His Asp Leu Leu Gln Ala
1               5                   10                  15

His Asp Glu Leu Leu His His Ser Leu Cys Phe Ala Lys Ser Leu Ala
            20                  25                  30

Leu Ala Val Ala Leu Asp Leu Arg Ile Pro Asp Ala Ile His His His
        35                  40                  45

Gly Ala Gly Gly Ala Thr Leu Leu Gln Ile Leu Ala Glu Thr Ala Leu
    50                  55                  60

His Pro Ser Lys Leu Arg Ala Leu Arg Arg Leu Met Arg Val Leu Thr
65                  70                  75                  80

Val Thr Gly Ile Phe Ser Val Glu Gln Pro Pro Ala Gly Gly Gly Asp
                85                  90                  95

Asn Ser Thr Val His Thr Ser Asp Asp Glu Ala Val Val Val Tyr Arg
            100                 105                 110

Leu Thr Ala Ala Ser Arg Phe Leu Val Ser Asp Glu Val Ser Thr Ala
        115                 120                 125

Thr Leu Ala Pro Phe Val Ser Leu Ala Leu Gln Pro Ile Ala Ala Cys
    130                 135                 140

Pro His Ala Leu Gly Ile Ser Ala Trp Phe Arg Gln Glu Gln His Glu
145                 150                 155                 160
```

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```
Pro Ser Pro Tyr Gly Leu Ala Phe Arg Gln Thr Pro Thr Ile Trp Glu
                165                 170                 175

His Ala Asp Asp Val Asn Ala Leu Leu Asn Lys Gly Met Ala Ala Asp
            180                 185                 190

Ser Arg Phe Leu Met Pro Ile Val Leu Arg Glu Cys Gly Glu Thr Phe
        195                 200                 205

Arg Gly Ile Asp Ser Leu Val Asp Val Gly Gly Gly His Gly Gly Ala
    210                 215                 220

Ala Ala Ala Ile Ala Ala Ala Phe Pro His Leu Lys Cys Ser Val Leu
225                 230                 235                 240

Asp Leu Pro His Val Val Ala Gly Ala Pro Ser Asp Gly Asn Val His
                245                 250                 255

Phe Val Ala Gly Asn Met Phe Glu Ser Ile Pro Pro Ala Thr Ala Val
            260                 265                 270

Phe Leu Lys Lys Thr Leu His Asp Trp Gly Asp Asp Glu Cys Val Lys
        275                 280                 285

Ile Leu Lys Asn Cys Lys Gln Ala Ile Ser Pro Arg Asp Ala Gly Gly
    290                 295                 300

Lys Val Ile Ile Leu Asp Val Val Val Gly Tyr Lys Gln Ser Asn Ile
305                 310                 315                 320

Lys His Gln Glu Thr Gln Val Met Phe Asp Leu Tyr Met Met Ala Val
                325                 330                 335

Asn Gly Val Glu Arg Asp Glu Gln Glu Trp Lys Lys Ile Phe Ile Glu
            340                 345                 350

Ala Gly Phe Lys Asp Tyr Lys Ile Leu Pro Val Ile Gly Asp Val Ser
        355                 360                 365

Val Ile Ile Glu Val Tyr Pro
    370                 375


<210> SEQ ID NO 20
<211> LENGTH: 1101
<212> TYPE: DNA
<213> ORGANISM: Zea mays

<400> SEQUENCE: 20

atggcactca tgcaggagag cagccaggac ttgctcgaag cgcacgacga gctcttccac     60

cactgcctgt gcttcgccaa atcgctcgcg ctcgccgtgg cgcaggacct ccgcatcccc    120

gacgcgatcc accaccacgg aggcggcgcc accctccacc agatcctcgc cgaggccgcg    180

ctccacccaa gcaagcttcg cgccctacgc cgcctgatgc gcgtgctcac cgtctcgggc    240

gtcttcaccg tccagtattc ttcaaccgtc gacgcgtcgg acggagctga tgtcgtctac    300

aggctgacgg cagcctcccg cttcctcgtc agcgatagcg acgaggcggg cacggcgtcc    360

ttggctccct ttgcgaacct ggcgctccac cctatcgcca tctccccgca cgccgtgggc    420

atctgcgcgt ggttccggca ggagcagcac gacccgtccc cgtacggcct ggcgttccgc    480

cagatcccga ccatctggga gcatgctgac aacgtaaacg ccctactgaa caaaggcttg    540

ctcgcggaaa gccgcttctt gatgccaatc gtactcaggg agtgcggaga cgaggtgttc    600

cgtgggatcg actcgttggt cgacgtcggc ggtgggcacg gtggcgccgc cgccaccatc    660

gccgccgcat tcccgcacgt caagtgcagc gtgcttgacc tcccgcacgt tgtcgccggt    720

gctccatccg atgcctgcgt gcagttcgtt gcgggcaata tgttccacag tattccacct    780
```

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gcaaccgccg ttttcttcaa gacaactcta tgtgactggg gtgacgacga gtgcatcaag 840

atattgaaga attgcaagca agccatatct ccacgggatg agggtgggaa ggtgataatc 900

atggacgtgg tagtcgggta tgggcagtca aacatgaagc gcctagagac acaagttatg 960

tttgatttgg ttatgatggc ggtcaatgga gtcgagcgcg acgagcaaga gtggaaggag 1020

atgttcattg aagctggatt caaagactac aaaatccgac cagtagctgg cctcatgtcg 1080

gtcatcgagg tctatccatg a 1101

<210> SEQ ID NO 21
<211> LENGTH: 366
<212> TYPE: PRT
<213> ORGANISM: Zea mays

<400> SEQUENCE: 21

Met Ala Leu Met Gln Glu Ser Ser Gln Asp Leu Leu Glu Ala His Asp
1 5 10 15

Glu Leu Phe His His Cys Leu Cys Phe Ala Lys Ser Leu Ala Leu Ala
20 25 30

Val Ala Gln Asp Leu Arg Ile Pro Asp Ala Ile His His His Gly Gly
35 40 45

Gly Ala Thr Leu His Gln Ile Leu Ala Glu Ala Ala Leu His Pro Ser
50 55 60

Lys Leu Arg Ala Leu Arg Arg Leu Met Arg Val Leu Thr Val Ser Gly
65 70 75 80

Val Phe Thr Val Gln Tyr Ser Ser Thr Val Asp Ala Ser Asp Gly Ala
85 90 95

Asp Val Val Tyr Arg Leu Thr Ala Ala Ser Arg Phe Leu Val Ser Asp
100 105 110

Ser Asp Glu Ala Gly Thr Ala Ser Leu Ala Pro Phe Ala Asn Leu Ala
115 120 125

Leu His Pro Ile Ala Ile Ser Pro His Ala Val Gly Ile Cys Ala Trp
130 135 140

Phe Arg Gln Glu Gln His Asp Pro Ser Pro Tyr Gly Leu Ala Phe Arg
145 150 155 160

Gln Ile Pro Thr Ile Trp Glu His Ala Asp Asn Val Asn Ala Leu Leu
165 170 175

Asn Lys Gly Leu Leu Ala Glu Ser Arg Phe Leu Met Pro Ile Val Leu
180 185 190

Arg Glu Cys Gly Asp Glu Val Phe Arg Gly Ile Asp Ser Leu Val Asp
195 200 205

Val Gly Gly Gly His Gly Gly Ala Ala Ala Thr Ile Ala Ala Ala Phe
210 215 220

Pro His Val Lys Cys Ser Val Leu Asp Leu Pro His Val Val Ala Gly
225 230 235 240

Ala Pro Ser Asp Ala Cys Val Gln Phe Val Ala Gly Asn Met Phe His
245 250 255

Ser Ile Pro Pro Ala Thr Ala Val Phe Phe Lys Thr Thr Leu Cys Asp
260 265 270

Trp Gly Asp Asp Glu Cys Ile Lys Ile Leu Lys Asn Cys Lys Gln Ala
275 280 285

Ile Ser Pro Arg Asp Glu Gly Gly Lys Val Ile Ile Met Asp Val Val

-continued

```
    290                 295                 300

Val Gly Tyr Gly Gln Ser Asn Met Lys Arg Leu Glu Thr Gln Val Met
305                 310                 315                 320

Phe Asp Leu Val Met Met Ala Val Asn Gly Val Glu Arg Asp Glu Gln
                325                 330                 335

Glu Trp Lys Glu Met Phe Ile Glu Ala Gly Phe Lys Asp Tyr Lys Ile
            340                 345                 350

Arg Pro Val Ala Gly Leu Met Ser Val Ile Glu Val Tyr Pro
        355                 360                 365


<210> SEQ ID NO 22
<211> LENGTH: 1122
<212> TYPE: DNA
<213> ORGANISM: Zea mays

<400> SEQUENCE: 22

atggtgcagc aagccgcgaa ggaggtgatc ccgctgctga cgccttacaa gatggggcag     60

ttcgagctct ctcaccgggt ggtgctggcg ccgctgacga ggtgccgctc ctacggcaac    120

gtgccgcagc cacacgccgc cgtgtactac tcgcaacgcg ccaccagggg tggcctgctc    180

atcgcggagg ccacgggggt gtcggccacc gcgcaggggt tcccggagtc tcctggcatc    240

tggacgcagg agcaggtcga ggcgtggaag cccatcgtcg acgccgtcca ccgcaagggc    300

gccatcttcg tctgccagat ctggcatgtg ggcagggtct ccaccaacga gctccagccc    360

aacggcgacg cgcctatctc gagcacggac aagcagatct cccccgacgc caagtccgga    420

acggcgtact cgaagccgcg ccggctgcgg accgacgagg tccccgggat cgtcgacgac    480

ttcaggcggg ccgcacggaa cgcgatccag gctggtttcg acgccgtcga gatccacggc    540

gcgcacgggt acctcctgga gcagttcatg aaggacagct gcaacgaccg cacggaccag    600

tacggcggca gcctggagaa ccggtgccgt ctcgccgtgg aggtcgtgga cgccgtcgtc    660

cgcgaggtgg gcgcgcgccg cgtcggcatc aggctgtcgc ccttcgtcga cttcgtggac    720

tgcgtggact cggacccggt ggcgctcggc cactacatgg tgcagcagct caacaggcat    780

agcggcttgc tctactgcca catggtggag ccccgcatta ccgcccacgg gcgcaggcag    840

atccctcacg ggctcttgcc gttccgaaag gcgtttcatg gaacgttcat cgccgccggt    900

gggtacgatc gggaggaagg caacaaggtg gtggcagaag ggtatgctga cctcgtcgcg    960

tatgggaagc tgttcttggc taacccggac ctgcctagga ggttcgagct ggatgtggca   1020

ctcaacaaat acgaccgctc caccttctac acgcaagatc ctattgttgg ctacacagat   1080

taccctttct ttgaagaaga agatggcaag aatgagaggt ga                       1122


<210> SEQ ID NO 23
<211> LENGTH: 373
<212> TYPE: PRT
<213> ORGANISM: Zea mays

<400> SEQUENCE: 23

Met Val Gln Gln Ala Ala Lys Glu Val Ile Pro Leu Leu Thr Pro Tyr
1               5                   10                  15

Lys Met Gly Gln Phe Glu Leu Ser His Arg Val Val Leu Ala Pro Leu
            20                  25                  30

Thr Arg Cys Arg Ser Tyr Gly Asn Val Pro Gln Pro His Ala Ala Val
        35                  40                  45
```

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```
Tyr Tyr Ser Gln Arg Ala Thr Arg Gly Gly Leu Leu Ile Ala Glu Ala
    50                  55                  60

Thr Gly Val Ser Ala Thr Ala Gln Gly Phe Pro Glu Ser Pro Gly Ile
65                  70                  75                  80

Trp Thr Gln Glu Gln Val Glu Ala Trp Lys Pro Ile Val Asp Ala Val
                85                  90                  95

His Arg Lys Gly Ala Ile Phe Val Cys Gln Ile Trp His Val Gly Arg
            100                 105                 110

Val Ser Thr Asn Glu Leu Gln Pro Asn Gly Asp Ala Pro Ile Ser Ser
        115                 120                 125

Thr Asp Lys Gln Ile Ser Pro Asp Ala Lys Ser Gly Thr Ala Tyr Ser
    130                 135                 140

Lys Pro Arg Arg Leu Arg Thr Asp Glu Val Pro Gly Ile Val Asp Asp
145                 150                 155                 160

Phe Arg Arg Ala Ala Arg Asn Ala Ile Gln Ala Gly Phe Asp Ala Val
                165                 170                 175

Glu Ile His Gly Ala His Gly Tyr Leu Leu Glu Gln Phe Met Lys Asp
            180                 185                 190

Ser Cys Asn Asp Arg Thr Asp Gln Tyr Gly Gly Ser Leu Glu Asn Arg
        195                 200                 205

Cys Arg Leu Ala Val Glu Val Val Asp Ala Val Val Arg Glu Val Gly
    210                 215                 220

Ala Arg Arg Val Gly Ile Arg Leu Ser Pro Phe Val Asp Phe Val Asp
225                 230                 235                 240

Cys Val Asp Ser Asp Pro Val Ala Leu Gly His Tyr Met Val Gln Gln
                245                 250                 255

Leu Asn Arg His Ser Gly Leu Leu Tyr Cys His Met Val Glu Pro Arg
            260                 265                 270

Ile Thr Ala His Gly Arg Arg Gln Ile Pro His Gly Leu Leu Pro Phe
        275                 280                 285

Arg Lys Ala Phe His Gly Thr Phe Ile Ala Ala Gly Gly Tyr Asp Arg
    290                 295                 300

Glu Glu Gly Asn Lys Val Val Ala Glu Gly Tyr Ala Asp Leu Val Ala
305                 310                 315                 320

Tyr Gly Lys Leu Phe Leu Ala Asn Pro Asp Leu Pro Arg Arg Phe Glu
                325                 330                 335

Leu Asp Val Ala Leu Asn Lys Tyr Asp Arg Ser Thr Phe Tyr Thr Gln
            340                 345                 350

Asp Pro Ile Val Gly Tyr Thr Asp Tyr Pro Phe Phe Glu Glu Glu Asp
        355                 360                 365

Gly Lys Asn Glu Arg
    370


<210> SEQ ID NO 24
<211> LENGTH: 2595
<212> TYPE: DNA
<213> ORGANISM: Zea mays

<400> SEQUENCE: 24

atgctgagcg ggatcatcga cgggctgacg ggggcgaaca agcatgcgcg gctcaagggc     60

acggtggtgc tcatgcgcaa gaacgtgctg gacctcaacg acttcggcgc caccgtcgtt    120
```

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gacagcatca gcgagttcct cggcaagggg gtcacctgcc agctcatcag ctccaccctc 180

gtcgacgcca acaacggcaa ccgcgggcgg gtcggggcgg aggcgaacct ggagcagtgg 240

ctgacgagcc tgccgtcgct gacgaccggc gagtccaagt tcggcgtcac gttcgactgg 300

gaggtggaga agctgggagt gccgggggcc gtcgtcgtca agaacaacca cgccgccgag 360

ttcttcctca agacaatcac cctcgacgac gtgcccggcc gcggcgccgt caccttcgtc 420

gccaactcct gggtctaccc cgcgggcaag taccgctaca accgcgtctt cttctccaac 480

gatacgtacc tgccaagcca gatgccggcg gcgctgaagc cgtaccgcga cgacgagctc 540

cgcaacctcc gcggcgacga ccagcagggc ccctaccagg agcacgaccg cgtgtaccgc 600

tacgacgtct acaacgacct cggcgagccc gacggcggca acccgcgccc catcctcggc 660

ggctccgccg accacccgta cccgcgccgc tgccgcacgg gccgcaagcc caccaaaacc 720

gaccccaact cggagagccg actgtcgctg gtggagcaga tctacgtgcc gcgggacgag 780

cgcttcggcc acctcaagat gtccgacttc ctgggctact ccatcaaggc catcacgcag 840

ggcatcatcc cggcggtgcg cacgtacgtg gacaccaccc cgggcgagtt cgactccttc 900

caggacatca tcaacctgta cgagggcggg atcaagctgc ccaagatcca ggcgctcgag 960

gacatgcgca agctcttccc gctccagctc gtcaaggacc tcctccccgc cggcggggac 1020

tacctgctca agctccccat cccacagatc atccaagagg acaagaacgc gtggaggacc 1080

gacgaggagt tcgcgcggga ggtgctcgcc ggcgtcaacc cgatggtgat cacgcgcctc 1140

acggagttcc cgcccaagag cacgctggac cccagcaagt acggcgacca caccagcacg 1200

atcacggcgg agcacatcga gaagaacctc gagggcctca cggtgcagca ggcgctggac 1260

ggcaacaggc tctacatcct ggaccaccac gaccgcttca tgccgttcct catcgacgtc 1320

aacaacctgg agggcaactt catctacgcc accaggacgc tcttcttcct gcgcggcgac 1380

ggcaggctcg cgcccctcgc catcgagctc agcgagccgt acatcgacgg ggacctcacc 1440

gtggccaaga gcaaggtcta cacgccggcg tccagcggcg tcgaggcctg ggtgtggcag 1500

ctcgccaagg cctatgtcgc cgtcaacgac tctggctggc accaactcgt cagccactgg 1560

ctgaacaccc acgcggtgat ggagccgttc gtgatcgcga cgaaccggca gctgagcgtg 1620

acgcacccgg tgcacaagct cctgagctcg cacttccgcg acaccatgac catcaacgcg 1680

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aagtacgcgc tgggcatgtc ctccgtggtg tacaagagct ggaacttcac cgagcagggc 1800

ctccccgccg acctcgtcaa gaggggcgtg gcggtggcgg acccgtccag cccgtacaag 1860

gtgcggctgc tgatcgagga ctacccgtac gcgagcgacg ggctggccat ctggcacgcc 1920

atcgagcagt gggtgggcga gtacctggcc atctactacc ccgacgacgg cgcgctgcgg 1980

ggcgacgagg agctgcaggc gtggtggaag gaggtgcgcg aggtcgggca cggcgaccac 2040

aaggacgcgc cctggtggcc caagatgcag gccgtgtcgg agctcgccag cgcctgcacc 2100

accatcatct ggatcgcgtc ggcgctccac gccgccgtca acttcggcca gtacccgtac 2160

gcggggtacc tcccgaacag gcccacggtg agccggcgcc ggatgccgga gcccggcagc 2220

aaggagtacg aggagctgga gcgcgacccg gagcgcggct tcatccacac catcacgagc 2280

cagatccaga ccatcatcgg catctcgctc atcgagatcc tctccaagca ctcctccgac 2340

gaggtgtacc tcggccagcg cgacaccccc gagtggacct ccgacgcccg ggcgctggcg 2400

-continued

gcgttcaaga ggttcagcga cgcgctggtc aagatcgagg gcaaggtggt gggcgagaac 2460

cgcgacccgc agctgaggaa caggaacggc cccgccgagt tcccctacat gctgctctat 2520

cccaacacct ctgaccacag tggcgccgcc gcagggctca ctgccaaggg catccccaac 2580

agcatctcca tctga 2595

<210> SEQ ID NO 25
<211> LENGTH: 864
<212> TYPE: PRT
<213> ORGANISM: Zea mays

<400> SEQUENCE: 25

Met Leu Ser Gly Ile Ile Asp Gly Leu Thr Gly Ala Asn Lys His Ala
1 5 10 15

Arg Leu Lys Gly Thr Val Val Leu Met Arg Lys Asn Val Leu Asp Leu
20 25 30

Asn Asp Phe Gly Ala Thr Val Val Asp Ser Ile Ser Glu Phe Leu Gly
35 40 45

Lys Gly Val Thr Cys Gln Leu Ile Ser Ser Thr Leu Val Asp Ala Asn
50 55 60

Asn Gly Asn Arg Gly Arg Val Gly Ala Glu Ala Asn Leu Glu Gln Trp
65 70 75 80

Leu Thr Ser Leu Pro Ser Leu Thr Thr Gly Glu Ser Lys Phe Gly Val
85 90 95

Thr Phe Asp Trp Glu Val Glu Lys Leu Gly Val Pro Gly Ala Val Val
100 105 110

Val Lys Asn Asn His Ala Ala Glu Phe Phe Leu Lys Thr Ile Thr Leu
115 120 125

Asp Asp Val Pro Gly Arg Gly Ala Val Thr Phe Val Ala Asn Ser Trp
130 135 140

Val Tyr Pro Ala Gly Lys Tyr Arg Tyr Asn Arg Val Phe Phe Ser Asn
145 150 155 160

Asp Thr Tyr Leu Pro Ser Gln Met Pro Ala Ala Leu Lys Pro Tyr Arg
165 170 175

Asp Asp Glu Leu Arg Asn Leu Arg Gly Asp Asp Gln Gln Gly Pro Tyr
180 185 190

Gln Glu His Asp Arg Val Tyr Arg Tyr Asp Val Tyr Asn Asp Leu Gly
195 200 205

Glu Pro Asp Gly Gly Asn Pro Arg Pro Ile Leu Gly Gly Ser Ala Asp
210 215 220

His Pro Tyr Pro Arg Arg Cys Arg Thr Gly Arg Lys Pro Thr Lys Thr
225 230 235 240

Asp Pro Asn Ser Glu Ser Arg Leu Ser Leu Val Glu Gln Ile Tyr Val
245 250 255

Pro Arg Asp Glu Arg Phe Gly His Leu Lys Met Ser Asp Phe Leu Gly
260 265 270

Tyr Ser Ile Lys Ala Ile Thr Gln Gly Ile Ile Pro Ala Val Arg Thr
275 280 285

Tyr Val Asp Thr Thr Pro Gly Glu Phe Asp Ser Phe Gln Asp Ile Ile
290 295 300

Asn Leu Tyr Glu Gly Gly Ile Lys Leu Pro Lys Ile Gln Ala Leu Glu
305 310 315 320

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Asp Met Arg Lys Leu Phe Pro Leu Gln Leu Val Lys Asp Leu Leu Pro
                325                 330                 335

Ala Gly Gly Asp Tyr Leu Leu Lys Leu Pro Ile Pro Gln Ile Ile Gln
            340                 345                 350

Glu Asp Lys Asn Ala Trp Arg Thr Asp Glu Glu Phe Ala Arg Glu Val
        355                 360                 365

Leu Ala Gly Val Asn Pro Met Val Ile Thr Arg Leu Thr Glu Phe Pro
    370                 375                 380

Pro Lys Ser Thr Leu Asp Pro Ser Lys Tyr Gly Asp His Thr Ser Thr
385                 390                 395                 400

Ile Thr Ala Glu His Ile Glu Lys Asn Leu Glu Gly Leu Thr Val Gln
                405                 410                 415

Gln Ala Leu Asp Gly Asn Arg Leu Tyr Ile Leu Asp His His Asp Arg
            420                 425                 430

Phe Met Pro Phe Leu Ile Asp Val Asn Asn Leu Glu Gly Asn Phe Ile
        435                 440                 445

Tyr Ala Thr Arg Thr Leu Phe Phe Leu Arg Gly Asp Gly Arg Leu Ala
    450                 455                 460

Pro Leu Ala Ile Glu Leu Ser Glu Pro Tyr Ile Asp Gly Asp Leu Thr
465                 470                 475                 480

Val Ala Lys Ser Lys Val Tyr Thr Pro Ala Ser Ser Gly Val Glu Ala
                485                 490                 495

Trp Val Trp Gln Leu Ala Lys Ala Tyr Val Ala Val Asn Asp Ser Gly
            500                 505                 510

Trp His Gln Leu Val Ser His Trp Leu Asn Thr His Ala Val Met Glu
        515                 520                 525

Pro Phe Val Ile Ala Thr Asn Arg Gln Leu Ser Val Thr His Pro Val
    530                 535                 540

His Lys Leu Leu Ser Ser His Phe Arg Asp Thr Met Thr Ile Asn Ala
545                 550                 555                 560

Leu Ala Arg Gln Thr Leu Ile Asn Gly Gly Gly Ile Phe Glu Met Thr
                565                 570                 575

Val Phe Pro Gly Lys Tyr Ala Leu Gly Met Ser Ser Val Val Tyr Lys
            580                 585                 590

Ser Trp Asn Phe Thr Glu Gln Gly Leu Pro Ala Asp Leu Val Lys Arg
        595                 600                 605

Gly Val Ala Val Ala Asp Pro Ser Ser Pro Tyr Lys Val Arg Leu Leu
    610                 615                 620

Ile Glu Asp Tyr Pro Tyr Ala Ser Asp Gly Leu Ala Ile Trp His Ala
625                 630                 635                 640

Ile Glu Gln Trp Val Gly Glu Tyr Leu Ala Ile Tyr Tyr Pro Asp Asp
                645                 650                 655

Gly Ala Leu Arg Gly Asp Glu Glu Leu Gln Ala Trp Trp Lys Glu Val
            660                 665                 670

Arg Glu Val Gly His Gly Asp His Lys Asp Ala Pro Trp Trp Pro Lys
        675                 680                 685

Met Gln Ala Val Ser Glu Leu Ala Ser Ala Cys Thr Thr Ile Ile Trp
    690                 695                 700

Ile Ala Ser Ala Leu His Ala Ala Val Asn Phe Gly Gln Tyr Pro Tyr
705                 710                 715                 720

Ala Gly Tyr Leu Pro Asn Arg Pro Thr Val Ser Arg Arg Arg Met Pro
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               725                 730                 735

Glu Pro Gly Ser Lys Glu Tyr Glu Glu Leu Glu Arg Asp Pro Glu Arg
            740                 745                 750

Gly Phe Ile His Thr Ile Thr Ser Gln Ile Gln Thr Ile Ile Gly Ile
        755                 760                 765

Ser Leu Ile Glu Ile Leu Ser Lys His Ser Ser Asp Glu Val Tyr Leu
    770                 775                 780

Gly Gln Arg Asp Thr Pro Glu Trp Thr Ser Asp Ala Arg Ala Leu Ala
785                 790                 795                 800

Ala Phe Lys Arg Phe Ser Asp Ala Leu Val Lys Ile Glu Gly Lys Val
                805                 810                 815

Val Gly Glu Asn Arg Asp Pro Gln Leu Arg Asn Arg Asn Gly Pro Ala
            820                 825                 830

Glu Phe Pro Tyr Met Leu Leu Tyr Pro Asn Thr Ser Asp His Ser Gly
        835                 840                 845

Ala Ala Ala Gly Leu Thr Ala Lys Gly Ile Pro Asn Ser Ile Ser Ile
    850                 855                 860


<210> SEQ ID NO 26
<211> LENGTH: 717
<212> TYPE: DNA
<213> ORGANISM: Zea mays

<400> SEQUENCE: 26

atggccgccg cgctcaggtg cccggcctcc gtcagggtct cgggccccgc cgccgcgggg     60

ctcgccaagg tgcgccaggc gtccagagtc gtcgcggtga gcggggcccg gcagagccgc    120

ggcggcggcg tcgccgtgcg ggcgtcgctg ttctcgccca agcccgcggc ggccaaggac    180

gcgcggccga ccaaggtgca ggagctgtac gtgtacgaga tcaacgagcg ggaccgcgag    240

agccccgcgt acctgcgcct cagcgccaag cagacggaga acgcgctggg cgacctcgtc    300

cccttcacca acaaggtgta caacggcagc ctggacaagc ggctgggcgt caccgcgggc    360

atctgcgtgc tcatccagca cgtccccgac cgcaacggcg accgctacga ggccatctac    420

agcttctact tcggagacta cggccacatc tcggtgcagg gcccgtacct gacctacgag    480

gagtcgtacc tggcggtcac cggcggctcc ggcgtcttcg agggcgtcta cggccaggtc    540

aagctcaacc agatcgtctt ccccttcaag atcttctaca ccttctacct ccggggcatc    600

ccggacctgc ccagggacct gctctgcacg cccgtcccgc cgtcgcccac cgtcgagccc    660

acccccgccg ccagggccgc cgagccgcac gcatccctcg acaactacac caactga       717


<210> SEQ ID NO 27
<211> LENGTH: 238
<212> TYPE: PRT
<213> ORGANISM: Zea mays

<400> SEQUENCE: 27

Met Ala Ala Ala Leu Arg Cys Pro Ala Ser Val Arg Val Ser Gly Pro
1               5                   10                  15

Ala Ala Ala Gly Leu Ala Lys Val Arg Gln Ala Ser Arg Val Val Ala
            20                  25                  30

Val Ser Gly Ala Arg Gln Ser Arg Gly Gly Gly Val Ala Val Arg Ala
        35                  40                  45

Ser Leu Phe Ser Pro Lys Pro Ala Ala Ala Lys Asp Ala Arg Pro Thr
```

-continued

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    50                  55                  60

Lys Val Gln Glu Leu Tyr Val Tyr Glu Ile Asn Glu Arg Asp Arg Glu
65                  70                  75                  80

Ser Pro Ala Tyr Leu Arg Leu Ser Ala Lys Gln Thr Glu Asn Ala Leu
                85                  90                  95

Gly Asp Leu Val Pro Phe Thr Asn Lys Val Tyr Asn Gly Ser Leu Asp
            100                 105                 110

Lys Arg Leu Gly Val Thr Ala Gly Ile Cys Val Leu Ile Gln His Val
        115                 120                 125

Pro Asp Arg Asn Gly Asp Arg Tyr Glu Ala Ile Tyr Ser Phe Tyr Phe
    130                 135                 140

Gly Asp Tyr Gly His Ile Ser Val Gln Gly Pro Tyr Leu Thr Tyr Glu
145                 150                 155                 160

Glu Ser Tyr Leu Ala Val Thr Gly Gly Ser Gly Val Phe Glu Gly Val
                165                 170                 175

Tyr Gly Gln Val Lys Leu Asn Gln Ile Val Phe Pro Phe Lys Ile Phe
            180                 185                 190

Tyr Thr Phe Tyr Leu Arg Gly Ile Pro Asp Leu Pro Arg Asp Leu Leu
        195                 200                 205

Cys Thr Pro Val Pro Pro Ser Pro Thr Val Glu Pro Thr Pro Ala Ala
    210                 215                 220

Arg Ala Ala Glu Pro His Ala Ser Leu Asp Asn Tyr Thr Asn
225                 230                 235


<210> SEQ ID NO 28
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 28

taccatgttc cctagggatt g                                              21


<210> SEQ ID NO 29
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 29

gtggcgcaat cactttaacc                                                20


<210> SEQ ID NO 30
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 30

atctcgttgg ggatgtcttg                                                20


<210> SEQ ID NO 31
<211> LENGTH: 20
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 31

agcaccgttc aaatgtctcc 20

<210> SEQ ID NO 32
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 32

tattgttggt gctgttgccg 20

<210> SEQ ID NO 33
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 33

ggactcaatc cttgtccctg 20

<210> SEQ ID NO 34
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 34

cccgagcacg taaagcagat 20

<210> SEQ ID NO 35
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 35

cttcatgccc ctggcatact 20

<210> SEQ ID NO 36
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 36

gacgaggacg acgataagga ctt 23

<210> SEQ ID NO 37
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 37

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ggccatactc cttctgaaga gacag 25

<210> SEQ ID NO 38
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 38

atggccgagc tcatcaacaa 20

<210> SEQ ID NO 39
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 39

tcgtcctcac ctccgtctgt 20

<210> SEQ ID NO 40
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 40

tgttcctccg gatcatctgc 20

<210> SEQ ID NO 41
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 41

aagaggctgt cccaccgct 19

<210> SEQ ID NO 42
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 42

ccatttcgac tgggaggtcc 20

<210> SEQ ID NO 43
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 43

gtccatgctc accttccagc 20

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<210> SEQ ID NO 44
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 44

aagttcaaca ccataggact cgatg 25

<210> SEQ ID NO 45
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 45

caggtagcta gagcctgaag tggtc 25

<210> SEQ ID NO 46
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 46

ggctgggttc cgtgactaca 20

<210> SEQ ID NO 47
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 47

gacctcgatg atggacggg 19

<210> SEQ ID NO 48
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 48

ggaagaggat gaacgagctc aa 22

<210> SEQ ID NO 49
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 49

gacccagcag attcatcgat g 21

<210> SEQ ID NO 50
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 50

gggaccagtt cggcaacat 19

<210> SEQ ID NO 51
<211> LENGTH: 18
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 51

tgcccacctt ccacacgt 18

<210> SEQ ID NO 52
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 52

cagcaggtgg tggtgataat 20

<210> SEQ ID NO 53
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 53

agcgccagac tcacaaagg 19

<210> SEQ ID NO 54
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 54

gcccacccaa gtaagcttcg 20

<210> SEQ ID NO 55
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 55

agagacagcg ataggatgga 20

<210> SEQ ID NO 56
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 56

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catcgtgtgc cagtactacc 20

<210> SEQ ID NO 57
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 57

accggtcatt cgtcacaagc 20

<210> SEQ ID NO 58
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 58

gcctcatagt tcccgacctc 20

<210> SEQ ID NO 59
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 59

gaatcctcgt gaagctcgtg 20

<210> SEQ ID NO 60
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 60

cctcatgatg tgggtgcag 19

<210> SEQ ID NO 61
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 61

atgcatgaca aggccagact 20

<210> SEQ ID NO 62
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 62

aaaaacatgg gacctcgtga 20

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<210> SEQ ID NO 63
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 63

attgcatgac gacaatgcta ga 22

<210> SEQ ID NO 64
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 64

ggggacaagt ttgtacaaaa aagcaggctt catggctgct caccagcctc acct 54

<210> SEQ ID NO 65
<211> LENGTH: 54
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Primer

<400> SEQUENCE: 65

ggggaccact ttgtacaaga aagctgggtg gctcaagaga actctgggtg gcaa 54

1.-**15**. (canceled)

16. A method for producing a plant having increased fungal resistance, wherein the fungal resistance is regulated by at least one wall-associated kinase, the method comprising:

(i) (a) providing at least one plant cell, tissue, organ, or whole plant having a genotype with respect to the presence of at least one gene encoding a wall-associated kinase in the genome of said plant cell, tissue, organ, or whole plant; or

(i) (b) introducing at least one gene encoding at least one wall-associated kinase into the genome of at least one cell of at least one of a plant cell, tissue, organ, or whole plant; and

(ii) (a) modifying at least one gene encoding at least one wall-associated kinase in the at least one plant cell, tissue, organ, or whole plant; and/or

(ii) (b) modulating the expression level of at least one wall-associated kinase and/or the transcription level, the expression level, or a function of at least one molecule within a signalling pathway from the at least one wall-associated kinase to the synthesis of at least one benzoxazinoid or within a synthesis pathway of at least one benzoxazinoid in the at least one plant cell, tissue, organ, or whole plant;

(iii) producing a population of plants from the at least one plant cell, tissue, organ, or whole plant; and

(iv) selecting a plant having increased fungal resistance from the population of plants, optionally based on a determination of a reduced synthesis of at least one benzoxazinoid in response to a fungal pathogen infection, wherein the synthesis of the at least one benzoxazinoid is regulated by the at least one wall-associated kinase.

17. The method according to claim **16**, wherein the at least one wall-associated kinase is a WAK-RLK1 gene.

18. The method of claim **17**, wherein the at least one wall-associated kinase

a) is encoded by a nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO: 1 or 7, or a functional fragment thereof,

b) is encoded by a nucleic acid molecule comprising the nucleotide sequence having at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to nucleotide sequence of SEQ ID NO: 1 or 7,

c) is encoded a nucleic acid molecule hybridizing with a complementary sequence to a) or b) under stringent conditions,

d) is encoded by a nucleic acid molecule comprising the nucleotide sequence coding for an amino acid sequence of SEQ ID NO: 2 or 8, or a functional fragment thereof,

e) is encoded by a nucleic acid molecule comprising the nucleotide sequence coding for an amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to amino acid sequence of SEQ ID NO: 2 or 8,

f) comprising the amino acid sequence of SEQ ID NO: 2 or 8, or

g) comprising an amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%,

85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to amino acid sequence of SEQ ID NO: 2 or 8,

provided that any sequence of a) to g), optionally after expression, still encodes at least one functional Htn1, Ht2, Ht3, or an allelic variant, a mutant, or a functional fragment thereof.

19. The method according to claim **16**, wherein the benzoxazinoid whose synthesis is regulated by the at least one wall-associated kinase, is selected from at least one of DIMBOA, DIMBOA, HMBOA, HM2BOA, HDMBOA, HDM2BOA, HBOA, DHBOA, DIBOA or TRIBOA, the aforementioned benzoxazinoid being in the glucoside or aglucone form, or a benzoxazolinone, or any combination of the aforementioned benzoxazinoids.

20. The method according to claim **16**, wherein the fungal resistance against which resistance to a fungus is increased, or a disease caused by said fungus is selected from a fungus of the order of Pleosporales, comprising *Helminthosporium turcicum* causing northern corn leaf blight (NCLB), particularly affecting maize and wheat plants, or comprising *Bipolaris maydis* causing southern corn leaf blight, the order of Pucciniales causing rust disease, comprising *Puccinia sorghi* causing common rust, or *Diploida macrospora* causing *Diploida* leaf streak/blight, or *Colletotrichum graminicola* causing Anthracnose, or *Fusarium* spp. causing *Fusarium* stalk rot, or *Gibberella* spp., causing *Giberella* stalk rot, or *Sphacelotheca reiliana* causing maize head smut.

21. The method according to claim **16**, wherein the at least one gene encoding at least one wall-associated kinase is stably integrated into the genome of the at least one plant cell, tissue, organ, or whole plant, or wherein the at least one gene encoding at least one wall-associated kinase is transiently introduced into a plant cell, tissue, organ, or whole plant, or wherein the at least one gene encoding at least one wall-associated kinase is stably integrated into the genome of the at least one plant cell, tissue, organ, or whole plant, wherein the introduction of the at least one gene encoding at least one wall-associated kinase comprises the introgression of the at least one gene during plant breeding.

22. The method according to claim **16**, wherein the at least one molecule within the signalling pathway from the at least one wall-associated kinase to the synthesis of at least one benzoxazinoid or within the synthesis pathway of at least one benzoxazinoid is selected from the group consisting of the genes bx1 (SEQ ID NO: 10), bx2 (SEQ ID NO: 12), igl (SEQ ID NO: 14), bx6 (SEQ ID NO: 16), bxl1 (SEQ ID NO: 18), bxl4 (SEQ ID NO: 20), opr2 (SEQ ID NO: 22), lox3 (SEQ ID NO: 24) or aoc1 (SEQ ID NO: 26), or a homologous genes thereof, or the proteins BX1 (SEQ ID NO: 11), BX2 (SEQ ID NO: 13), IGL (SEQ ID NO: 15), BX6 (SEQ ID NO: 17), BX11 (SEQ ID NO: 19), BX14 (SEQ ID NO: 21), OPR2 (SEQ ID NO: 23), LOX3 (SEQ ID NO: 25) or AOC1 gene (SEQ ID NO: 27), or a homolog thereof.

23. The method according to claim **16**, wherein the reduced synthesis of at least one benzoxazinoid is achieved by providing at least one wall-associated kinase, an allelic variant, a mutant or a functional fragment thereof, or a gene encoding the same, wherein the at least one wall-associated kinase comprises a sequence which can directly or indirectly influence the benzoxazinoid pathway and at least one further plant metabolic pathway, wherein the plant metabolic pathway is selected from the group consisting of the jasmonic acid pathway, the ethylene pathway, the lignin synthesis pathway, a defense pathway, a receptor-like kinase pathway, and a cell wall associated pathway.

24. The method according to claim **16**, wherein the modification of the at least one gene encoding at least one wall-associated kinase within step (ii) (a) or (ii) (b) is performed by at least one of a site-specific nuclease (SSN) or a catalytically active fragment thereof, or a nucleic acid sequence encoding the same; oligonucleotide directed mutagenesis; chemical mutagenesis; or TILLING.

25. The method according to claim **16**, wherein the at least one plant cell, tissue, organ, or whole plant provided in step (i) is selected from the group consisting of *Hordeum vulgare, Hordeum bulbusom, Sorghum bicolor, Saccharum officinarium, Zea* spp., including *Zea mays, Setaria italica, Oryza minuta, Oryza saliva, Oryza australiensis, Oryza alta, Triticum aestivum, Triticum durum, Secale cereale*, Triticale, *Malus domestica, Brachypodium distachyon, Hordeum marinum, Aegilops tauschii, Daucus glochidiatus, Beta* spp., including *Beta vulgaris, Daucus pusillus, Daucus muricatus, Daucus carota, Eucalyptus grandis, Nicotiana sylvestris, Nicotiana tomentosiformis, Nicotiana tabacum, Nicotiana benthamiana, Solanum lycopersicum, Solanum tuberosum, Coffea canephora, Vitis vinifera, Erythrante guttata, Genlisea aurea, Cucumis sativus, Marus notabilis, Arabidopsis arenosa, Arabidopsis lyrata, Arabidopsis thaliana, Crucihimalaya himalaica, Crucihimalaya wallichii, Cardamine nexuosa, Lepidium virginicum, Capsella bursa pastoris, Olmarabidopsis pumila, Arabis hirsute, Brassica napus, Brassica oleracea, Brassica rapa, Raphanus sativus, Brassica juncacea, Brassica nigra, Eruca vesicaria* subsp. *sativa, Citrus sinensis, Jatropha curcas, Populus trichocarpa, Medicago truncatula, Cicer yamashitae, Cicer bijugum, Cicer arietinum, Cicer reticulatum, Cicer judaicum, Cajanus cajanifolius, Cajanus scarabaeoides, Phaseolus vulgaris, Glycine max, Gossypium* sp., *Astragalus sinicus, Lotus japonicas, Torenia fournieri, Allium cepa, Allium fistulosum, Allium sativum, Helianthus annuus, Helianthus tuberosus* and *Allium tuberosum*, or any variety or subspecies belonging to one of the aforementioned plants.

26. A plant cell, tissue, organ, whole plant or plant material, or a derivative or a progeny thereof, obtainable by a method according to claim **16**.

27. A method for identifying at least one gene involved in increased pathogen resistance in a plant cell, tissue, organ, whole plant, or plant material the method comprising:

(i) determining the genotype of at least one plant cell, tissue, organ, whole plant, or plant material with respect to the presence of at least one gene encoding a wall-associated kinase in the genome of said plant cell, tissue, organ, whole plant or plant material;

(ii) optionally determining the benzoxazinoid signature of the at least one plant cell, tissue, organ, whole plant, or plant material of step (i);

(iii) exposing the at least one plant cell, tissue, organ, whole plant, or plant material of step (i) or (ii) to a stimulus, optionally wherein the stimulus is correlated with the benzoxazinoid signature in the at least one plant cell, tissue, organ, whole plant, or plant material;

(iv) performing an analysis of at least one analyte obtained from the at least one plant cell, tissue, organ, whole plant, or plant material of step (i) or (ii) after exposition to the stimulus;

(v) determining at least one gene being regulated upon exposition to a stimulus according to step (iii) in at least one cell of the at least one plant cell, tissue, organ, whole plant, or plant material as derivable from the analysis of at least one analyte as defined in step (iv),

(vi) subjecting the at least one gene as determined in step (v) to a functional characterization; and

(vii) providing at least one gene involved in increased pathogen resistance in a plant cell, tissue, organ, whole plant, or plant material.

28. A plant cell, tissue, organ, whole plant or plant material, or a derivative or a progeny thereof, obtainable by introducing at least one gene as provided by the method according to claim **27** into at least one cell of at least one of a plant cell, tissue, organ, or whole plant.

29. A plant cell, tissue, organ, whole plant or plant material, or a derivative or a progeny thereof, wherein the introduction of at least one gene into at least one cell of at least one of a plant cell, tissue, organ, or whole plant according to the method according to claim **27** is a stable introduction.

30. A method of increasing pathogen resistance in a plant cell, tissue, organ, whole plant, or plant material, the method comprising:

(i) providing at least one plant cell, tissue, organ, whole plant or plant material;

(ii) (a) treating the at least one plant cell, tissue, organ, whole plant or plant material according to step (i) with a substance neutralizing an effect of at least one benzoxazinoid, and/or

(ii) (b) treating the at least one plant cell, tissue, organ, whole plant or plant material according to step (i) with a substance activating the signalling pathway downstream of at least one wall-associated kinase; and/or

(ii) (c) treating the at least one plant cell, tissue, organ, whole plant or plant material according to step (i) with a substance modulating or modifying the activity of at least one promoter or at least one regulatory sequence of at least one gene of the at least one plant cell, tissue, organ, whole plant or plant material of step (i), wherein said promoter or said regulatory sequence is involved in the regulation of transcription of at least on gene involved in the signalling pathway of, or downstream of at least one wall-associated kinase or involved in the synthesis pathway of at least one benzoxazinoid;

(ii) (d) treating the at least one plant cell, tissue, organ, whole plant or plant material according to step (i) with a substance inhibiting the synthesis of at least one benzoxazinoid; and

(iii) reducing an amount of at least one benzoxazinoid and thereby increasing pathogen resistance in at least one plant cell, tissue, organ, whole plant, or plant material.

31. The method of claim **30**, wherein the method is effective to increase fungal resistance in at least one plant cell, tissue, organ, whole plant, or plant material.

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