Supplementary Table 1 | List of *Bgt* isolates used to characterize the resistance spectra of *Pm4a* and *Pm4b* . The first column corresponds to the name of the *Bgt* isolate, followed by the geographic origin and collection site (if available) and the source. The last two columns show the disease reactions of Fed-*Pm4a* and Fed-*Pm4b* NILs distinguishing five classes of host reactions R = resistance (0-10% of leaf area covered), IR (10-25% of leaf area covered), I (25-50% of leaf area covered), IS (50-75 % of leaf area covered) and S (>75% of leaf area covered. Infection test is based on four biological replicates. CHN: China, ISR: Israel; CHE; Switzerland; FRA: France; USA: United States; GRB: Great Britain; JPN; Japan.

Bgt	Origin	Collection site	Source[1-4]	Fed-*Pm4a*	Fed-*Pm4b*
CHE_94202	CHE		Wicker et al. 2013	R	R
CHE_96224	CHE		Wicker et al. 2013	R	R
CHE_96249	CHE		in-house collection	R	R
CHE_97223	CHE		in-house collection	R	R
CHN_10_8	CHN	Yunnan province	Zeng et al. 2014	R	R
CHN_12_50	CHN	Guizhou province	Zeng et al. 2014	R	R
CHN_19_11	CHN	Jiangsu province	Zeng et al. 2014	R	R
CHN_28_9	CHN		Zeng et al. 2014	R	R
CHN_36_70	CHN	Hebei province	Zeng et al. 2014	R	R
CHN_39_1	CHN		Zeng et al. 2014	R	R
CHN_46_31	CHN	Gansu province	Zeng et al. 2014	R	R
CHN_6_69	CHN	Shannxi province	Zeng et al. 2014	R	R
CHN_7_8	CHN	Shannxi province	Zeng et al. 2014	R	R
FRA_B_Stone_95-45	FRA		McNally et al. 2018	R	R
ISR_1	ISR	Hula	McNally et al. 2018	R	R
ISR_103I	ISR	Amiad	Menardo et al. 2016	R	R
ISR_103K	ISR		Menardo et al. 2016	R	R
ISR_113	ISR	Amiad	McNally et al. 2018	R	R
ISR_13	ISR	Hula	McNally et al. 2018	R	R
ISR_16	ISR	Nahal Oz	McNally et al. 2018	R	R
ISR_20	ISR	Ein Hanaziv	McNally et al. 2018	R	R
ISR_204	ISR		Menardo et al. 2016	R	R
ISR_217	ISR		Menardo et al. 2016	R	R
ISR_218	ISR	Tel Far	McNally et al. 2018	R	R
ISR_219	ISR	Bizaron	McNally et al. 2018	R	R
ISR_30P	ISR	Talmei Yafe	McNally et al. 2018	R	R
ISR_30w	ISR	Talmei Yafe	McNally et al. 2018	R	R
ISR_37	ISR	Nahal Oz	McNally et al. 2018	R	R
ISR_43	ISR	Yesodot	McNally et al. 2018	R	R
ISR_44	ISR	Negev	McNally et al. 2018	R	R
ISR_50	ISR	Nahal Oz	McNally et al. 2018	R	R
ISR_52	ISR	DirElBalakh	McNally et al. 2018	R	R
ISR_6	ISR	Hula	McNally et al. 2018	R	R
ISR_67	ISR	Lahav	McNally et al. 2018	R	R
ISR_70	ISR		Menardo et al. 2016	R	R
ISR_94	ISR	Ein Hanaziv	McNally et al. 2018	R	R
ISR_97	ISR		Menardo et al. 2016	R	R

CHN_5_112	CHN		Zeng et al. 2014	R	IR
CHN_5_83	CHN	Shannxi province	Zeng et al. 2014	R	IR
CHN_5_93	CHN		Zeng et al. 2014	R	IR
CHN_51_3	CHN		Zeng et al. 2014	R	IR
CHN_6_21	CHN		Zeng et al. 2014	R	IR
CHN_9_43	CHN		Zeng et al. 2014	R	IR
CHN_HB_22	CHN		Zeng et al. 2014	R	IR
CHN_NJ_16	CHN		Zeng et al. 2014	R	IR
ISR_205	ISR	Kfar-Menahem	McNally et al 2018	R	IR
ISR_209	ISR	K. Revhaya	Menardo et al. 2016	R	IR
ISR_214	ISR	Akko	McNally et al 2018	R	IR
ISR_7	ISR	Hula	Menardo et al. 2016	R	IR
ISR_9	ISR	Hula	McNally et al 2018	R	IR
CHE_97266	CHE		in-house collection	IR	R
ISR_106	ISR	Nahal Oz	McNally et al 2018	IR	R
ISR_107	ISR	Nahal Oz	McNally et al 2018	IR	R
ISR_217	ISR	Kfa Hasidim	Menardo et al. 2016	IR	R
ISR_96	ISR	Negba	McNally et al 2018	IR	R
CHN_13_51	CHN	Guizhou province	Zeng et al. 2014	R	I
CHN_14_32	CHN		Zeng et al. 2014	R	I
CHN_15_9	CHN		Zeng et al. 2014	R	I
CHN_21_1	CHN		Zeng et al. 2014	R	I
CHN_37_38	CHN		Zeng et al. 2014	R	I
CHN_40_2	CHN		Zeng et al. 2014	R	I
CHN_41_5	CHN		Zeng et al. 2014	R	I
ISR_210	ISR	Givat HaMoreh	McNally et al 2018	I	IR
ISR_216	ISR	Ein Shemer	McNally et al 2018	IS	IR
CHN_2_39	CHN		Zeng et al. 2014	R	S
CHE_7004	CHE		Menardo et al. 2016	S	R
CHE_7230	CHE		McNally et al 2018	S	R
CHE_7234	CHE		in-house collection	S	R
CHE_10001	CHE		in-house collection	S	R
CHE_98013	CHE		in-house collection	S	R
CHN_52-27	CHN	Xinjiang	Zeng et al. 2014	S	R
FRA_Syros2000_15	FRA		McNally et al 2018	S	R
ISR_208	ISR	Gilboa	Menardo et al. 2016	S	R
ISR_8	ISR	Hula	Menardo et al. 2016	S	R
JPN_CHIKARA	JPN		McNally et al 2018	S	R
USA_C3-1	USA		McNally et al 2018	S	R
CHN_HB_21	CHN	Hubei province	Zeng et al. 2014	S	IR
CHN_1_47	CHN		Zeng et al. 2014	I	S
CHN_2_5	CHN		Zeng et al. 2014	I	S
CHN_1_19	CHN	Sichuan province	Zeng et al. 2014	S	S
CHN_1_62	CHN		Zeng et al. 2014	S	S
CHN_10_40	CHN		Zeng et al. 2014	S	S
CHN_11_61	CHN		Zeng et al. 2014	S	S
CHN_12_24	CHN		Zeng et al. 2014	S	S

CHN_12_3	CHN		Zeng et al. 2014	S	S
CHN_12_82	CHN		Zeng et al. 2014	S	S
CHN_13_76	CHN		Zeng et al. 2014	S	S
CHN_15_11	CHN		Zeng et al. 2014	S	S
CHN_17_40	CHN	Anhui province	Zeng et al. 2014	S	S
CHN_18_1	CHN		Zeng et al. 2014	S	S
CHN_18_11	CHN		Zeng et al. 2014	S	S
CHN_18_38	CHN		Zeng et al. 2014	S	S
CHN_18_45	CHN		Zeng et al. 2014	S	S
CHN_2_25	CHN	Sichuan province	Zeng et al. 2014	S	S
CHN_2_65	CHN		Zeng et al. 2014	S	S
CHN_24_4	CHN	Jiangsu province	Zeng et al. 2014	S	S
CHN_30_1	CHN	Anhui province	Zeng et al. 2014	S	S
CHN_35_1	CHN		Zeng et al. 2014	S	S
CHN_35_18	CHN		Zeng et al. 2014	S	S
CHN_36_3	CHN		Zeng et al. 2014	S	S
CHN_39_19	CHN		Zeng et al. 2014	S	S
CHN_39_5	CHN		Zeng et al. 2014	S	S
CHN_44_3	CHN	Shandong province	Zeng et al. 2014	S	S
CHN_45_10	CHN	Gansu province	Zeng et al. 2014	S	S
CHN_45_6	CHN	Gansu province	Zeng et al. 2014	S	S
CHN_46_25	CHN	Gansu province	Zeng et al. 2014	S	S
GRB_JIW2	GRB		Wicker et al. 2013	S	S

Supplementary Table 2 | List of EMS-induced Pm4a and Pm4b mutants used in this study. The given name of each mutant (first column) is followed by the donor line, Fed-*Pm4a* or Fed-*Pm4b*, where the EMS treatment was performed. In the column Mutation, the first letter indicates the amino acid in the wild-type followed by the position and the amino acid change in the corresponding mutant. Last column denotes the predicted domain based delimited based on Conserved Domain Database (CDD) from NCBI, where S_TKc (cl21453) corresponds to the serine/threonine kinase domain, C2C and C2D (cl14603) to C2 domain third and fourth repeat found in Multiple C2 domain and Transmembrane regions Proteins (MCTP). Finally, PRT_C (pfam08372) denotes the plant phosphoribosyltransferase C-terminal domain. The last three columns display the reactions of the EMS-derived mutants after inoculation with *Bgt94202*, *Bgt96224* and *BgtJIW2*. Values refer to percentage of the surface are of tested leaf segments infected (means of four biological replicates ± SE).

Mutant name	Source	Mutation	Affected domain	*Bgt94202*	*Bgt96224*	*BgtJIW2*
pm4b_m7	Fed-*Pm4b*	S390F	spacer	82.5 ± 4.3	85.0 ± 5.0	85.0 ± 5.0
pm4b_m89	Fed-*Pm4b*	P497L	C2C	80.0 ± 0.0	82.5 ± 4.3	85.0 ± 5.0
pm4b_m123[€]	Fed-*Pm4b*	G132D	S_TKc	80.0 ± 0.0	77.5 ± 8.3	85.0 ± 5.0
pm4b_m125	Fed-*Pm4b*	G234D	S_TKc	80.0 ± 0.0	80.0 ± 10.0	87.5 ± 4.3
pm4b_m151[€]	Fed-*Pm4b*	P184L	S_TKc	80.0 ± 0.0	80.0 ± 7.1	90.0 ± 0.0
pm4b_m180	Fed-*Pm4b*	G665S	PRT_C	70.0 ± 7.1	80.0 ± 7.1	85.0 ± 5.0
pm4b_m207[€]	Fed-*Pm4b*	D170N	S_TKc	72.5 ± 8.3	80.0 ± 7.1	85.0 ± 5.0
pm4b_m244	Fed-*Pm4b*	Q588*; R737W	PRT_C	72.5 ± 8.3	80.0 ± 7.1	87.5 ± 4.3
pm4b_m256[€]	Fed-*Pm4b*	G659D	PRT_C	75.0 ± 11.2	85.0 ± 5.0	85.0 ± 8.7
pm4b_m324	Fed-*Pm4b*	T622M	PRT_C	82.5 ± 8.3	85.0 ± 5.0	90.0 ± 0.0
pm4b_m360	Fed-*Pm4b*	G659D	PRT_C	80.0 ± 7.1	85.0 ± 5.0	90.00 ± 0.0
pm4b_m445	Fed-*Pm4b*	Q14*	spacer	85.0 ± 5.0	85.0 ± 5.0	90.0 ± 0.0
pm4b_m467	Fed-*Pm4b*	Y626N	PRT_C	85.0 ± 5.0	85.0 ± 5.0	80.0 ± 0.0
pm4b_m495[€]	Fed-*Pm4b*	Q274*	S_TKc	82.5 ± 4.3	82.5 ± 4.3	80.0 ± 0.0
pm4b_m510	Fed-*Pm4b*	V477M	C2C	85.0 ± 5.0	80.0 ± 7.1	87.5 ± 4.3
pm4b_m526[€]	Fed-*Pm4b*	R291K	S_TKc	80.0 ± 7.1	85.0 ± 5.0	90.0 ± 0.0
pm4b_m532[€]	Fed-*Pm4b*	G104E	S_TKc	82.5 ± 8.3	80.0 ± 7.1	77.5 ± 4.3
pm4b_m641[€]	Fed-*Pm4b*	G45E	S_TKc	80.0 ± 7.1	82.5 ± 8.3	85.0 ± 5.0
pm4a_m077	Fed-*Pm4a*	D188N	S_TKc	85.0 ± 5.0	80.0 ± 7.1	85.0 ± 5.0
pm4a_m102	Fed-*Pm4a*	Q719R	PRT_C	87.5 ± 4.3	75.0 ± 5.0	85.0 ± 5.0
pm4a_m113	Fed-*Pm4a*	E183K	S_TKc	85.0 ± 5.0	80.0 ± 7.1	87.5 ± 4.3
pm4a_m177	Fed-*Pm4a*	T204I;P688L	S_TKc	70.0 ± 0.0	80.0 ± 7.1	85.0 ± 5.0
pm4a_m188	Fed-*Pm4a*	G562D	spacer	70.0 ± 0.0	82.5 ± 4.3	85.0 ± 5.0
pm4a_m226	Fed-*Pm4a*	W681*	PRT_C	75.0 ± 5.0	80.0 ± 7.1	85.0 ± 5.0
pm4a_m247	Fed-*Pm4a*	L261F	S_TKc	80.0 ± 7.1	77.5 ± 8.3	85.0 ± 5.0
pm4a_m280	Fed-*Pm4a*	P617S	PRT_C	72.5 ± 8.3	77.5 ± 4.3	85.0 ± 5.0
pm4a_m293	Fed-*Pm4a*	G190D	S_TKc	75.0 ± 5.0	80.0 ± 7.1	85.0 ± 5.0
pm4a_m366	Fed-*Pm4a*	G317S	C2D	80.0 ± 7.1	80.0 ± 7.1	85.0 ± 5.0
pm4a_m398	Fed-*Pm4a*	E217K	S_TKc	80.0 ± 7.1	82.5 ± 4.3	85.0 ± 5.0
pm4a_m425	Fed-*Pm4a*	V118I	S_TKc	85.0 ± 5.0	77.5 ± 4.3	85.0 ± 5.0
pm4a_m448	Fed-*Pm4a*	A100T	S_TKc	80.0 ± 7.1	77.5 ± 4.3	85.0 ± 5.0
pm4a_m507	Fed-*Pm4a*	P617L	PRT_C	80.0 ± 7.1	80.0 ± 7.1	82.5 ± 4.3

[€]Mutants subjected to chromosome flow sorting and MutChromSeq, and then confirmed by Sanger sequencing

Supplementary Table 3 | Disease reactions of selected T2 families challenged with selected *Bgt* isolates. The first column displays the name of each progeny. Second and third column indicates the presence (+) or absence (-) of the transgenes *Pm4b_V1CDS*- and *Pm4b_V2CDS* (See Methods). The remaining columns show the disease reaction of each T2 line challenged with two *Pm4a/b*-avirulent (*Bgt96224* and *Bgt94202*) and two *Pm4a/b*-virulent (*BgtJIW2* and *Bgt97251*) isolates. Top four rows show the disease reactions of the Fed-*Pm4a* and the Fed-*Pm4b* NILs genotypes, Bobwhite S26, the susceptible background where transgenic complementation assays were performed, and Kanzler, a highly susceptible cultivar to *Bgt*. Five classes of host reactions were considered. R = resistance (0-10% of leaf area covered), IR (10-25% of leaf area covered), I (25-50% of leaf area covered), IS (50-75 % of leaf area covered) and S (>75% of leaf area covered. Evaluation was done 7-9 dpi.

T2_line	Pm4b_V1CDS	Pm4b_V2CDS	*Bgt96224*	*Bgt94202*	*BgtJIW2*	*Bgt97251*
Fed-*Pm4a*	-	-	R	R	S	S
Fed-*Pm4b*	-	-	R	R	S	S
Bobwhite S26	-	-	S	S	S	S
Kanzler	-	-	S	S	S	S
T2#3-2.12_1.1	+	+	R	R	S	IS
T2#3-2.12_1.2	+	+	R	R	S	S
T2#3-2.12_1.4	+	+	R	R	IS	IS
T2#3-2.12_1.5	+	+	IR	R	S	S
T2#3-2.12_1.6	+	+	R	R	IS	IS
T2#3-2.12_1.7	+	+	R	R	IS	IS
T2#3-2.12_1.8	+	+	R	R	IS	S
T2#3-2.12_1.9	+	+	R	R	S	S
T2#3-2.12_1.10	+	+	R	R	S	S
T2#3-2.12_1.11	+	+	R	R	S	S
T2#3-2.12_1.12	+	+	R	R	S	IS
T2#3-2.12_1.13	+	+	R	R	S	S
T2#3-2.12_1.14	+	+	R	R	S	S
T2#3-2.12_1.15	+	+	R	R	S	S
T2#3-2.12_1.16	+	+	R	R	S	S
T2#3-2.13_1.1	-	-	S	S	S	IS
T2#3-2.13_1.2	+	+	IR	IS	S	S
T2#3-2.13_1.3	-	-	S	S	S	S
T2#3-2.13_1.4	+	+	IS	IR	S	S
T2#3-2.13_1.5	-	-	S	S	S	IS
T2#3-2.13_1.6	+	+	R	R	S	S
T2#3-2.13_1.7	+	+	IR	IR	IS	IS
T2#3-2.13_1.8	+	+	R	R	S	S
T2#3-2.13_1.9	+	+	R	R	S	S
T2#3-2.13_1.10	+	+	IR	R	S	S
T2#3-2.13_1.11	+	+	R	R	S	S
T2#3-2.13_1.12	+	+	R	IR	S	S
T2#3-2.13_1.13	+	+	R	IR	S	S
T2#3-2.13_1.14	+	+	R	R	S	S
T2#3-2.13_1.16	+	+	IS	S	S	S
T2#25-1.8_1.1	+	+	IS	S	S	S

T2#25-1.8_1.2	+	+	I	S	S	S
T2#25-1.8_1.3	+	+	R	R	S	S
T2#25-1.8_1.4	+	+	I	IS	S	S
T2#25-1.8_1.5	+	+	R	IR	S	S
T2#25-1.8_1.6	+	+	R	I	S	S
T2#25-1.8_1.8	+	+	R	R	S	IS
T2#25-1.8_1.10	+	+	S	S	S	S
T2#25-1.8_1.11	+	+	R	IR	S	S
T2#25-1.8_1.12	+	+	R	R	S	IS
T2#25-1.8_1.13	+	+	R	R	S	S
T2#25-1.8_1.14	+	+	R	IS	S	S
T2#25-1.8_1.16	-	-	IS	S	S	S
T2#25-1.11_1.1	+	+	I	R	S	S
T2#25-1.11_1.2	+	+	R	R	S	S
T2#25-1.11_1.3	+	+	IS	R	S	S
T2#25-1.11_1.4	+	+	IR	R	S	IS
T2#25-1.11_1.6	+	+	R	IR	IS	S
T2#25-1.11_1.7	+	+	R	R	S	S
T2#25-1.11_1.8_	-	-	S	S	S	S
T2#25-1.11_1.9	+	+	R	R	S	S
T2#25-1.11_1.11	+	+	IR	IR	S	S
T2#25-1.11_1.12	+	+	R	R	S	S
T2#25-1.11_1.13	-	-	IR	IS	S	IS
T2#25-1.11_1.14	-	-	S	S	S	S
T2#25-1.11_1.15	+	+	R	R	IS	IS
T2#25-1.11_1.16	+	+	R	R	IS	IS
T2#52-1.4_1.1	-	-	S	S	S	S
T2#52-1.4_1.2	-	-	S	S	S	S
T2#52-1.4_1.3	-	-	S	S	S	S
T2#52-1.4_1.4	-	-	S	S	S	S
T2#52-1.4_1.5	-	-	I	S	S	S
T2#52-1.4_1.6	-	-	I	S	S	IS
T2#52-1.4_1.7	-	-	IS	S	S	IS
T2#52-1.4_1.8	-	-	IS	S	S	IS
T2#52-1.4_1.9	+	+	R	R	S	S
T2#52-1.4_1.10	+	+	R	R	S	IS
T2#52-1.4_1.11	-	-	I	S	S	S
T2#52-1.4_1.12	+	+	R	R	S	IS
T2#52-1.4_1.13	+	+	R	R	S	S
T2#52-1.4_1.14	+	+	R	R	S	IS
T2#52-1.4_1.15	+	+	R	R	S	S
T2#52-1.4_1.16	+	+	R	R	S	IS
T2#52-3.11_1.2	+	+	R	R	S	IS
T2#52-3.11_1.3	+	+	R	R	S	IS
T2#52-3.11_1.5	+	+	R	R	S	S
T2#52-3.11_1.7	+	+	R	R	S	S
T2#52-3.11_1.8	+	+	R	R	S	S

T2#52-3.11_1.11	+	+	R	R	S	IS
T2#52-3.11_1.12	+	+	R	R	S	S
T2#52-3.11_1.13	+	+	R	R	S	S
T2#52-3.14_1.2	+	+	R	R	S	IS
T2#52-3.14_1.3	+	+	R	R	IS	IS
T2#52-3.14_1.4	+	+	R	R	S	S
T2#52-3.14_1.7	+	+	R	R	IS	S
T2#52-3.14_1.9	+	+	R	R	IS	S
T2#52-3.14_1.12	+	+	R	R	S	S
T2#52-3.14_1.14	+	+	R	R	I	IS
T2#52-3.14_1.16	+	+	R	R	S	IS

Supplementary Table 4 | Disease reactions of selected T1 transgenic lines overexpressing *Pm4b_V1* or *Pm4b_V2* challenged with selected *Bgt* isolates. The first column displays the name of each progeny. Second column displays the *Pm4b* splicing variant transformed: either *Pm4b_V1CDS* or *Pm4b_V2CDS*. The third column, named detection, indicates the presence (+) or absence (-) of the corresponding transgenes: *Pm4b_V1CDS* or *Pm4b_V2CDS*. The remaining columns show the disease reaction of each T1 transgenic line challenged with two *Pm4a/b*-avirulent (*Bgt96224* and *Bgt94202*) and one *Pm4a/b*-virulent (*BgtJIW2*). Top four rows show the disease reactions of the Fed-*Pm4a*, Fed-*Pm4b*, Bobwhite S26, the susceptible background where transgenic complementation assays were performed, and Kanzler, a highly susceptible cultivar to Bgt. Five classes of host reactions were considered. R = resistance (0-10% of leaf area covered), IR (10-25% of leaf area covered), I (25-50% of leaf area covered), IS (50-75 % of leaf area covered) and S (>75% of leaf area covered)

Line	Transgene	Detection	*Bgt96224*	*Bgt94202*	*BgtJIW2*
Fed-*Pm4a*	-		R	R	R
Fed-*Pm4b*	-		R	R	R
Bobwhite S26	-		S	S	S
Kanzler	-		S	S	S
T1#9_2.1	*Pm4b_V1CDS*	+	S	S	S
T1#9_2.2	*Pm4b_V1CDS*	+	S	S	S
T1#9_2.3	*Pm4b_V1CDS*	+	S	S	S
T1#9_2.4	*Pm4b_V1CDS*	+	S	S	S
T1#9_2.5	*Pm4b_V1CDS*	+	S	S	S
T1#9_2.8	*Pm4b_V1CDS*	+	S	S	S
T1#9_2.9	*Pm4b_V1CDS*	+	S	S	S
T1#9_2.10	*Pm4b_V1CDS*	+	S	S	S
T1#9_2.11	*Pm4b_V1CDS*	+	S	S	S
T1#9_2.12	*Pm4b_V1CDS*	+	S	S	S
T1#9_2.13	*Pm4b_V1CDS*	+	S	S	S
T1#9_2.14	*Pm4b_V1CDS*	-	S	S	S
T1#9_2.15	*Pm4b_V1CDS*	+	S	S	S
T1#9_2.16	*Pm4b_V1CDS*	+	S	S	S
T1#12_2.1	*Pm4b_V1CDS*	+	S	S	S
T1#12_2.2	*Pm4b_V1CDS*	+	S	S	S
T1#12_2.3	*Pm4b_V1CDS*	+	S	S	S
T1#12_2.4	*Pm4b_V1CDS*	+	S	S	S
T1#12_2.5	*Pm4b_V1CDS*	-	S	S	S
T1#12_2.6	*Pm4b_V1CDS*	-	S	S	S
T1#12_2.7	*Pm4b_V1CDS*	+	S	S	S
T1#12_2.8	*Pm4b_V1CDS*	+	S	S	S
T1#12_2.9	*Pm4b_V1CDS*	-	S	S	S
T1#12_2.10	*Pm4b_V1CDS*	+	S	S	S
T1#12_2.11	*Pm4b_V1CDS*	+	S	S	S
T1#12_2.12	*Pm4b_V1CDS*	+	S	S	S
T1#12_2.13	*Pm4b_V1CDS*	+	S	S	S

T1#12_2.14	Pm4b_V1CDS	+	S	S	S
T1#12_2.15	Pm4b_V1CDS	+	S	S	S
T1#12_2.16	Pm4b_V1CDS	-	S	S	S
T1#19_1.1	Pm4b_V1CDS	+	S	S	S
T1#19_1.2	Pm4b_V1CDS	+	S	S	S
T1#19_1.4	Pm4b_V1CDS	+	S	S	S
T1#19_1.5	Pm4b_V1CDS	+	S	S	S
T1#19_1.6.1	Pm4b_V1CDS	-	S	S	S
T1#19_1.6.2	Pm4b_V1CDS	-	S	S	S
T1#19_1.7	Pm4b_V1CDS	-	S	S	S
T1#19_1.9	Pm4b_V1CDS	+	S	S	S
T1#19_1.10	Pm4b_V1CDS	+	S	S	S
T1#19_1.11	Pm4b_V1CDS	+	S	S	S
T1#19_1.12	Pm4b_V1CDS	-	S	S	S
T1#19_1.13	Pm4b_V1CDS	-	S	S	S
T1#19_1.14	Pm4b_V1CDS	-	S	S	S
T1#19_1.15	Pm4b_V1CDS	+	S	S	S
T1#19_1.16	Pm4b_V1CDS	+	S	S	S
T1#6_3.2	Pm4b_V2CDS	-	S	S	S
T1#6_3.3	Pm4b_V2CDS	+	S	S	S
T1#6_3.4	Pm4b_V2CDS	-	S	S	S
T1#6_3.5	Pm4b_V2CDS	+	S	S	S
T1#6_3.6	Pm4b_V2CDS	+	S	S	S
T1#6_3.7	Pm4b_V2CDS	+	S	S	S
T1#6_3.8	Pm4b_V2CDS	-	S	S	S
T1#6_3.11	Pm4b_V2CDS	+	S	S	S
T1#6_3.12	Pm4b_V2CDS	+	S	S	S
T1#6_3.13	Pm4b_V2CDS	+	S	S	S
T1#6_3.14	Pm4b_V2CDS	-	S	S	S
T1#6_3.16	Pm4b_V2CDS	-	S	S	S
T1#24_1.1	Pm4b_V2CDS	+	S	S	S
T1#24_1.2	Pm4b_V2CDS	+	S	S	S
T1#24_1.3	Pm4b_V2CDS	-	S	S	S
T1#24_1.4	Pm4b_V2CDS	+	S	S	S
T1#24_1.5	Pm4b_V2CDS	+	S	S	S
T1#24_1.6	Pm4b_V2CDS	+	S	S	S
T1#24_1.7	Pm4b_V2CDS	+	S	S	S
T1#24_1.8	Pm4b_V2CDS	+	S	S	S
T1#24_1.10	Pm4b_V2CDS	+	S	S	S
T1#24_1.11	Pm4b_V2CDS	+	S	S	S
T1#24_1.12	Pm4b_V2CDS	+	S	S	S
T1#24_1.13	Pm4b_V2CDS	+	S	S	S
T1#24_1.14	Pm4b_V2CDS	+	S	S	S
T1#24_1.15	Pm4b_V2CDS	+	S	S	S

T1#24_1.16	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.1	*Pm4b_V2CDS*	-	S	S	S
T1#29_2.2	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.3	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.4	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.5	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.6	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.7	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.8	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.9	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.10	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.12.1	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.12.2	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.13	*Pm4b_V2CDS*	-	S	S	S
T1#29_2.14.1	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.14.2	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.15	*Pm4b_V2CDS*	+	S	S	S
T1#29_2.16	*Pm4b_V2CDS*	+	S	S	S

Supplementary Table 5 | Disease reactions of wheat cultivars carrying the *Pm4* locus challenged with selected *Bgt* isolates. In the first column, WW refers to Whealbi Wheat lines from Pont et al6. Detailed passport information is available at https://urgi.versailles.inra.fr/download/iwgsc/IWGSC_RefSeq_Annotations/v1.0/iwgsc_refseqv1.0_Whealbi_GWAS.zip. Second column specifies the *Pm4* allele. From third column on, disease reaction of each wheat line to selected *Bgt* isolates, where values refer to percentage of the surface area of tested leaf segments (means of four biological replicates). Note that disease reactions of the Fed-*Pm4a* and the Fed-*Pm4b* NILs genotypes are included in the top to facilitate the comparison of resistance spectra among *Pm4* alleles. In general, *Pm4b*-, *Pm4d*- and *Pm4h*-containing lines exhibit a very similar pattern that Fed-*Pm4a* and the Fed-*Pm4b* NILs, for example susceptible to *BgtJIW2* and *Bgt97251* but resistant to *Bgt96224*, *Bgt94202*, *Bgt97223* *Bgt97266*. Five classes of host reactions R = resistance (0-10% of leaf area covered), IR (10-25% of leaf area covered), I (25-50% of leaf area covered), IS (50-75 % of leaf area covered) and S (>75% of leaf area covered. Infection test is based on four biological replicates.

Line	Pm4 allele	BgtJIW2	Bgt94202	Bgt96224	Bgt96229	Bgt97028	Bgt97223	Bgt97251	Bgt97266	Bgt98013	Bgt98230	Bgt98250
Fed-*Pm4a*	*Pm4a*	S	R	R	S	S	R	S	R	R	S	S
Fed-*Pm4b*	*Pm4b*	S	R	R	IS	S	R	S	R	R	IS	IS
WW-001		S	R	R	R	I	R	I	R	S	I	I
WW-009		S	R	R	S	IS	R	R	R	IR	IS	IS
WW-012		S	R	R	IS	I	R	R	R	IR	IS	IS
WW-017		R	R	R	S	R	R	R	R	R	R	R
WW-018		R	R	R	R	R	R	R	R	R	R	R
WW-019		S	R	R	S	IS	R	IS	R	IS	I	IS
WW-021		S	R	R	S	IS	R	IS	R	IS	IS	IS
WW-024		R	R	R	S	R	R	R	R	S	R	R
WW-048		S	R	R	R	S	R	I	R	S	S	IS
WW-049	*Pm4b*	IS	S	IS	S	S	IR	I	R	IS	S	I
WW-156		R	R	R	IR	IR	R	S	R	S	IS	IR
WW-161		S	R	R	S	I	R	S	R	S	IS	R
WW-282		S	R	R	S	IR	R	I	R	S	IS	IS
WW-286		S	R	R	IS	I	R	IS	R	IS	I	I
WW-291		S	R	R	S	I	R	IS	R	IS	R	I
WW-356		S	R	S	S	I	R	S	R	IS	I	S
WW-399		S	IS	S	S	IS	IS	IS	IS	S	S	S
WW-451		R	R	R	IR	IR	R	IS	R	I	I	I
WW-508		IS	R	R	R	I	R	I	R	R	S	I
WW-003		S	R	R	R	I	R	I	R	I	IS	I
WW-007		IS	R	R	S	R	R	I	R	IS	R	IS
WW-014		S	R	R	S	I	R	IS	R	IS	IS	I
WW-037		S	R	R	S	S	R	R	R	I	S	S
WW-042	*Pm4d*	S	R	R	R	S	R	R	R	I	S	IS
WW-157		S	R	R	S	IS	R	IS	R	S	IS	I
WW-162		S	R	R	R	I	R	I	R	IS	IS	IR
WW-164		S	R	R	I	IS	R	IS	R	IS	IS	I
WW-166		S	R	R	R	I	R	S	R	S	IS	I
WW-085		S	S	S	S	S	S	S	I	S	S	IS
WW-110		S	S	S	S	S	S	IS	S	S	S	S
WW-143		S	S	IS	IS	IS	S	S	S	S	S	IR
WW-149		S	S	IS	IS	S	S	S	S	IS	S	R
WW-243		R	R	R	R	R	IR	R	R	R	R	R
WW-262	*Pm4f*	IS	I	I	IS	R	IR	IS	R	I	I	I
WW-265		I	IR	IR	IR	IR	IR	I	I	IR	IR	IR
WW-335		S	IS	I	S	S	S	IS	I	I	S	IS
WW-336		R	IS	S	IS	IS	I	S	R	I	S	I
WW-341		S	S	IS	IS	S	S	S	S	S	IS	IS
WW-445		S	S	IS	IR	R	S	S	S	IS	IS	I
WW-093		S	IS	IS	S	S	S	S	S	S	S	S
WW-213	*Pm4g*	IS	S	R	I	IR	R	R	R	R	R	R
WW-470		S	S	S	S	I	IS	S	I	IS	S	I
WW-474	*Pm4h*	S	R	R	I	R	IR	S	R	I	R	R

Supplementary Table 6 | List of *Pm4* homologues found in different species within the Triticeae tribe The first column displays the given name used in Supplementary Fig. 4. If annotated in the corresponding reference assembly (last column), the real name of each *Pm4* homologue is given in the second column. Third column specifies the species where is found the *Pm4* homologue, followed by the chromosome and its length and the hit positions corresponding to the beginning and end of the gene. chr: chromosome. Note that if a homologue does not have assigned a chromosome is due to the fact that that homologue was located in the "unknown" (Un) chromosome. If this was the case, the given name includes "Un".

Given name	Real name	Species	chr	chr length	blast_hit_1	blast_hit_2	Assembly mapping
HORVU2Hr1G126810	HORVU2Hr1G126810	*Hordeum vulgare*	2H	686565487	675091299	675096975	Barley HC Proteins May2016[7]
AET2Gv21296200	AET2Gv21296200	*Aegilops tauschii*	2	658177745	648456981	648448441	ASM34733v1 → Aet_v4.0
AET2Gv21296800	AET2Gv21296800	*Aegilops tauschii*	2	658177745	648669491	648660155	ASM34733v1 → Aet_v4.0
AET2Gv21297100	AET2Gv21297100	*Aegilops tauschii*	2	658177745	649380150	649375185	ASM34733v1 → Aet_v4.0
Pm4_Scer_2R-H1	gene not annotated	*Secale cereale*	2R	946003182	942144497	942135749	Scer_Lo7_v1p1p0
Pm4_Scer_2R-H2	gene not annotated	*Secale cereale*	2R	946003182	942196000	942188331	Scer_Lo7_v1p1p1
Pm4_Scer_2R-H3	SECCE2Rv1G0142720.1	*Secale cereale*	2R	946003182	942510789	942518886	Scer_Lo7_v1p1p1
Pm4_DW_2B-H1	gene not annotated	*Triticum turgidum durum*	2B	803510855	783236667	783242710	Tdur_Svevo_v2
Pm4_DW_Un-H1	gene not annotated	*Triticum turgidum durum*	-	-	-	-	
Pm4_DW_Un-H2	gene not annotated	*Triticum turgidum durum*	-	-	-	-	Tdur_Svevo_v2
Pm4_Tu-H1	gene not annotated	*Triticum urartu*	-	-	-	-	Tura
Pm4_WEW_2A-H1	gene not annotated	*Triticum turgidum dicoccoides*	2A	788103699	772507911	772501710	Ttur_Zavitan_v2
Pm4_WEW_2A-H2	TRITDC2AG081930	*Triticum turgidum dicoccoides*	2A	788103699	772732306	772727283	Ttur_Zavitan_v2
Pm4_WEW_2A-H3	gene not annotated	*Triticum turgidum dicoccoides*	2A	788103699	772765384	772758678	Ttur_Zavitan_v2
Pm4_WEW_2B-H1	TRITDC2BG090970	*Triticum turgidum dicoccoides*	2B	816754914	801015698	801021217	Ttur_Zavitan_v2
Pm4_WEW_2B-H2	TRITD2Bv1G265730	*Triticum turgidum dicoccoides*	2B	816754914	802467722	802462401	Ttur_Zavitan_v2
TraesCS2A01G558500	TraesCS2A01G558500	*Triticum aestivum*	2A	796414552	761903162	761896325	Taes_HC_2017_proteins[8]
TraesCS2B01G621800	TraesCS2B01G621800	*Triticum aestivum*	2A	817281873	795988821	795978311	Taes_HC_2017_proteins

Supplementary Table 7 | Primers used in this study

Primer	Sequence	Description	Function
GH438 (TI GH dT25VN)	CTATCAGCAACCATTGAGTCACGTCCTCAAAGATGCTCAdT25VN		5' RACE
GH439 (U-GH)	CTATCAGCAACCATTGAGTCACG		3' RACE
GH377	AGAGTGCAGAGACTTCAATCCA		3' RACE
GH432	GCACGTTCCCCACTCACGATTTGCATTGCT		5' RACE
GH398	CCTTCACACGGCAAATCTGAA	Fw long-range	Full-length amp. *Pm4b_V1* transcript
GH399	GATGTGCACCCAACACTAACT	Rv long-range	Full-length amp. *Pm4b_V1* transcript
GH400	ATCAGAGTCTCTATCGCCCT	Fw nested	Full-length amp. *Pm4b_V1* transcript
GH401	CACCCAACACTAACTGAAAGGAG	Rv nested	Full-length amp. *Pm4b_V1* transcript
GH382	GTTCCCCACTCACGATTTGC	Sequencing	Seq of full-length *Pm4b_V1/V2* transcript
GH385	TCGACGATAACATGGAACCCAA	Sequencing	Seq of full-length *Pm4b_V1/V2* transcript
GH387	CACCATTGGAAGGATGAGCTG	Sequencing	Seq of full-length *Pm4b_V1/V2* transcript
GH397	TAAAGATACAGATGGGCGGC	Sequencing	Seq of full-length *Pm4b_V1* transcript
JS233	ACTTTGCAATAGGGCGGTTG	Sequencing	Seq of full-length *Pm4b_V1/V2* transcript
JS293	AGTCACCACCAACATGAAGTC	Sequencing	Seq of full-length *Pm4b_V1* transcript
GH398	CCTTCACACGGCAAATCTGAA	Fw long-range	Full-length amp. *Pm4b_V2* transcript
GH407	AGTAATAACTCTACGCAACATGAAG	Rv long-range/semi-nested	Full-length amp. *Pm4b_V2* transcript
GH400	ATCAGAGTCTCTATCGCCCT	Fw semi-nested	Full-length amp. *Pm4b_V2* transcript
JS280	CGCACATAGACATGACGCTG	Sequencing	Seq of full-length *Pm4b_V2* transcript
JS292	TGCATTCTGGACCCTGACTC	Sequencing	Seq of full-length *Pm4b_V2* transcript
JS298	TGGTCTCTAGCGTCATGGTC	Sequencing	Seq of full-length *Pm4b_V2* transcript
JS540	GACCATGACGCTAGAGACCA	Sequencing	Seq of full-length *Pm4b_V2* transcript
JS717	AGGTGGACATCCTAGGCGCT	Forward	Haplotype marker
JS718	GATCTGGGTACCACAGCACCG	Reverse	Haplotype marker
JS256	GCTGAGTGATGTTAATTTGTTCGG	Fw long-range	Amp. Exon1-5 gDNA
JS257	AGAAAAAGGCAACTATAGCCCAT	Rv long-range/nested	Amp. Exon1-5 gDNA
JS251	TCTGACAAGTATATGTAGCAACCC	Fw nested	Amp. Exon1-5 gDNA
GH382	GTTCCCCACTCACGATTTGC	Sequencing	Seq Exon1-5 gDNA
GH384	AAGCAGCTAGTTGGCTCATAC	Sequencing	Seq Exon1-5 gDNA
GH385	TCGACGATAACATGGAACCCAA	Sequencing	Seq Exon1-5 gDNA
JS255	GTAGCAACCCAATTAAAGGAAGAA	Sequencing	Seq Exon1-5 gDNA
JS278	ACTAACGCATGACTCTGCCC	Fw long-range/nested	Amp. Exon6-7 gDNA
JS261	CTTGCGTGGAGAAAGGAACAA	Rv long-range	Amp. Exon6-7 gDNA
GH407	AGTAATAACTCTACGCAACATGAAG	Fw nested	Amp. Exon6-7 gDNA
JS280	CGCACATAGACATGACGCTG	Sequencing	Seq Exon 6-7 gDNA
JS292	TGCATTCTGGACCCTGACTC	Sequencing	Seq Exon 6-7 gDNA
GH387	CACCATTGGAAGGATGAGCTG	Sequencing	Seq Exon 6-7 gDNA
GH397	TAAAGATACAGATGGGCGGC	sequencing	Seq Exon 6-7 gDNA
GH402	ACCACATTTCACAAGAGAGCTA	Sequencing	Seq Exon 6-7 gDNA
GH414	TAGGTTGGAGAGATCACAACGA	Fw; Exon5-6; 179-bp	qRT-PCR Pm4 expression
GH415	CTGAGGTAGAGGAGGCAACTT	Rv; Exon5-6; 179-bp	qRT-PCR Pm4 expression
GH377	AGAGTGCAGAGACTTCAATCCA	Fw; Exon5-7; 159-bp	qRT-PCR Pm4 expression
GH417	TTCTTCGTACCCAGCAGGTC	Rv; Exon5-7; 159-bp	qRT-PCR Pm4 expression
JS483	CACCATGGAACACAAAACTAGTACCACAC	Universal forward	TOPO cloning Pm4b_V1/2
JS486	TCAGGTCAGCAGGTGGTACT	Rv; stop codon	TOPO cloning Pm4b_V1
JS487	GGTCAGCAGGTGGTACTCC	Rv; no stop codon	TOPO cloning Pm4b_V1
JS484	TCACAGGAGCACGTCCC	Rv; stop codon	TOPO cloning Pm4b_V2
JS485	CAGGAGCACGTCCCC	Rv; no stop codon	TOPO cloning Pm4b_V2
JS274	TTAATTGGCGCGCCCCATGGAACACAAAACTAGTACCACA	Universal forward (*Asc* I)	Biolistic bombardment
JS275	CTCTCTTAATTAATTTCACAGGAGCACGTCCC	Rv (*Pac* I)	Biolistic bombardment Pm4b_V2CDS
JS276	TCTCTCTTAATTAATTTCAGGTCAGCAGGTGGTAC	Rv (*Pac* I)	Biolistic bombardment Pm4b_V1CDS
JS295	CATCTGAGCCTTGAGACGGA	Fw sitting on Exon 6	Detection of transgene Pm4b_V1CDS
JS297	GAGGAAATGAAACTGCGCCT	Fw sitting on Exon 7	Detection of transgene Pm4b_V2CDS
HZ010	ATGTATAATTGCGGGACTCT	Universal Rv (nos terminator)	Detection of transgene Pm4b_V1/2CDS
JS189	GCTTCGCAAGAGCGCCAT	Fw Pm4b_V1_target_1 (Exon 6)	VIGS of *Pm4b_V1*
JS190	CCTTGCCCATCTGTTGGTCTC	Rv Pm4b_V1_target_1 (Exon 6)	VIGS of *Pm4b_V1*
JS498	GGCAGAAGTTGCCTCCTCTA	Fw Pm4b_V2_target_1 (Exon 7)	VIGS pf *Pm4b_V2*
JS499	GTTGTAGCGTGTGTCGTTGG	Rv Pm4b_V2_target_1 (Exon 7)	VIGS pf *Pm4b_V2*
JS589	GAACACAAAACTAGTACCACAC	N-terminal Flag tagging Pm4b_V1	Epitope tagging
JS590	CTTGTCGTCATCGTCCTTGTAGTCCATGGTGAAGGG	N-terminal Flag tagging Pm4b_V1	Epitope tagging
JS601	GATTATGCTGAACACAAAACTAGT	N-terminal HA-tagging Pm4b_V1	Epitope tagging
JS602	TGGAACATCGTATGGATACATGGT	N-terminal HA-tagging Pm4b_V1	Epitope tagging
JS593	GATGACGACAAGTGAAAGGGTGGGCGCGCC	C-terminal Flag tagging Pm4b_V2	Epitope tagging
JS594	GTCCTTGTAGTCGGTCAGCAGGTGGTACTCCG	C-terminal Flag tagging Pm4b_V2	Epitope tagging

JS488	TTCCAGATTATGCTTGAAAGGGTGGGCGCGCCG	C-terminal Flag tagging Pm4b_V2	Epitope tagging
JS489	CATCGTATGGATACAGGAGCACGTCCCCC	C-terminal Flag tagging Pm4b_V2	Epitope tagging

Supplementary Table 8 | Target-specific amplification efficiencies of the splicing variants *Pm4b_V1* and *Pm4b_V2* and the reference genes used in this study.

gene / Target	gene ID	position	primer	amplicon length bp	efficiency (E) slope r2 of calibration curve	reference
Pm4_V1		Exon 5-6	F: TAGGTTGGAGAGATCACAACGA (GH414) R: CTGAGGTAGAGGAGGCAACTT (GH415)	179	E: 97.6 % slope: -3.381 r2: 0.999	this work
Pm4_V2		Exon 5-7	F: AGAGTGCAGAGACTTCAATCCA (GH377) R: TTCTTCGTACCCAGCAGGTC (GH417)	159	E: 93.1 % slope: -3.500 r2: 0.991	this work
ADP	TraesCS3B01G368600, TraesCS3D01G330500 (TA.2291)	Exon 2	F: TCTCATGGTTGGTCTCGATG (GH094) R: GGATGGTGGTGACGATCTCT (GH095)	80	E: 98.2 % slope: -3.365 r2: 0.999	Giménez et al[9]
ZFL	TraesCS3D01G432800, TraesCS3A01G440000	Exon 1	F: CAGGCATCTCACTGGAGACT (GH105) R: TGGCATCTCTCTTGCTTCTG (GH106)	79	E: 96.7 % slope: -3.403 r2: 0.989	this work