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**Origin of host specificity resistance genes of common wheat against non-adapted
pathotypes of *Pyricularia oryzae* inferred from a D genome diversity in synthetic
hexaploid wheat lines**

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Abstract

Wheat resistance genes, *Rwt3* and *Rwt4*, constitute host specificity barrier against non-wheat pathotypes of the blast fungus, *Pyricularia oryzae*. To understand the origin of these host specificity resistance genes, we examined their distribution in *Aegilops tauschii*, a wild wheat progenitor species with the D genome, using synthetic hexaploid lines derived from crosses between *Triticum turgidum* cv. Langdon and 54 *Ae. tauschii* accessions which cover natural habitat regions and lineages of the species. Infection assays with transformants carrying their corresponding avirulence genes (*PWT3* and *PWT4*) revealed different distribution patterns of the two resistance genes. *Rwt3* was present in the TauL1 and TauL2 lineages with wider geographic distribution, while *Rwt4* was mainly present in the TauL2 and TauL3 lineages with narrow geographic distribution. Co-occurrence of *Rwt3* and *Rwt4* was observed exclusively in a TauL2 sublineage which has been suggested to be a probable donor of the D genome to common wheat. This result suggests that *Rwt3* and *Rwt4* in common wheat is likely to have been derived from *Ae. tauschii* individual(s) carrying both genes, and that the common ancestor of common wheat had both genes when it was established through amphidiploidization.

Key words *Pyricularia oryzae*, *Magnaporthe oryzae*, *Aegilops tauschii*, resistance gene, avirulence gene

Introduction

To cope with biotic stresses imposed by pathogenic microorganisms, plants have developed immunity system that prevents infection by most of the potential pathogenic organisms (Cook et al. 2015). Only limited number of successful pathogens, which can suppress or circumvent host immunity by delivering ‘effector’ molecules, cause diseases on a plant species (Ayliffe and Sorensen 2019). As a counter defense against such successful pathogens, plants have resistance (*R*) genes which specifically recognize the presence of pathogen-derived effector molecules encoded by avirulence (*AVR*) genes and induce strong resistance to halt pathogen proliferation (Dodds and Rathjen 2010). *R* genes typically encode NBS-LRR receptors (NLRs) (Jones et al. 2016). In plant genomes, hundreds of NLRs with different signatures of selection are encoded, implying the arms race coevolution with rapidly evolving pathogens (de Vries et al. 2020; Frantzeskakis et al. 2020; Van de Weyer et al. 2019). Although genome sequence-based understanding of plant *R* gene repertoires and evolution are developing, knowledge on how *R* genes have evolved during the evolution of host species from its ancestral species is still limited.

Wheat blast, caused by fungal pathogen *Pyricularia oryzae* (syn. *Magnaporthe oryzae*) *Triticum* pathotype, is one of the most devastating diseases of wheat, which, under favorable climate conditions, leads to up to 100% yield losses (Cruz and Valent 2017). Although now becoming a threat to wheat cultivation in South America and South Asia, wheat blast is a relatively new disease that appeared ~35 years ago in Brazil. Emergence of the wheat blast fungus was attributed to the circumvention of recognition by wheat *R* genes involved in host specificity through loss or mutation of their corresponding *AVR* genes in its ancestral lineages of *P. oryzae* (Inoue et al. 2017). As wheat *R* genes against *Avena* and *Lolium* isolates (pathotypes) of *P. oryzae*, we have

identified *Rwt3* and *Rwt4*, which recognize corresponding AVR genes, *PWT3* and *PWT4*, respectively (Takabayashi et al. 2002; Vy et al. 2014). *Rwt3* was effective against both pathotypes because *PWT3* was present in both, while *Rwt4* was effective against the *Avena* pathotype alone because *PWT4* was present in the *Avena* pathotype but absent in the *Lolium* pathotype. *Rwt3* and *Rwt4* were widely distributed (> 75%) in common wheat landraces from worldwide and thus considered to have served as host specificity barrier against non-wheat pathotypes of *P. oryzae* (Inoue et al. 2017). Our previous researches revealed that both *Rwt3* and *Rwt4* resided on the D genome (chromosome 1D) of common wheat (Hirata et al. 2005; Vy et al. 2014).

Aegilops tauschii Coss. ($2n = 2x = 14$, genome DD) is a diploid self-pollinating species and the D genome donor of common wheat (Kihara 1944; McFadden and Sears 1944). *Ae. tauschii* has a wide geographic range of distribution from northern Syria and Turkey to western China in Eurasia with its center of distribution in the southern coastal region of the Caspian Sea and Azerbaijan. Previous studies suggested that the population of *Ae. tauschii* was composed of three intraspecific lineages, named TauL1, TauL2, and TauL3 (Matsuoka et al. 2013). TauL1 and TauL2 lineages were further divided into sublineage groups, which markedly differed in their patterns of geographic distributions (Matsuoka et al. 2015). The D genome of *Ae. tauschii* is considered to have been introduced into common wheat (*Triticum aestivum* (L.) Thell., $2n = 6x = 42$, genome AABBDD) through natural hybridization with tetraploid wheat (*Triticum turgidum* L., $2n = 4x = 28$, genome AABB) about 8,000 years ago (Nesbitt and Samuel 1996). Allohexaploid wheat plants can be artificially produced through hybridization of tetraploid wheat and *Ae. tauschii*, which are called synthetic hexaploid wheat (Kihara 1944; Kihara and Lilienfeld 1949; McFadden and Sears 1944). Recently, Matsuoka, Takumi, and their colleagues developed synthetic hexaploid lines by crossing *Ae. tauschii*

core collections with *T. turgidum* cv. Langdon and used them to analyze a phenotypic diversity derived from *Ae. tauschii* D genomes in the hexaploid background (Kajimura et al. 2011; Matsuoka et al. 2007; Nishijima et al. 2014; Takumi et al. 2009).

In the present study, we surveyed the distribution of the two host specificity resistance genes, *Rwt3* and *Rwt4*, in *Ae. tauschii* accessions using those synthetic hexaploid lines. Based on responses of the synthetic hexaploid lines against fungal strains either with or without their corresponding *AVR* gene, we determined the presence/absence of *Rwt3* and *Rwt4* in 54 *Ae. tauschii* accessions which covered natural habitat regions and lineages of this species. We found accessions carrying both genes exclusively in a TauL2 sublineage at a high frequency. This result provided a clue to infer evolutionary trajectories of the host specificity *R* genes widely distributed in common wheat landraces.

Materials and Methods

Plant materials

Wheat cultivars used were *T. aestivum* cv. Norin 4 (N4), Chinese Spring (CS), Transfed (Tfed), Hope, and *T. turgidum* ssp. *durum* cv. Langdon (Ldn). Synthetic hexaploid wheat lines derived from crosses between Ldn and 54 *Ae. tauschii* accessions (Kajimura et al. 2011; Matsuoka et al. 2007; Takumi et al. 2009) were provided by Dr. S. Takumi, Kobe University.

Fungal materials

Fungal materials used were *Pyricularia oryzae* *Triticum* isolate Br48 [*pwt3;pwt4*], *Avena* isolate Br58 [*PWT3;PWT4*], and their derivatives, Br48+3 (a transformant of Br48 carrying transgene *PWT3* derived from Br58, strain M-16), Br48+4 (a transformant of Br48 carrying transgene *PWT4* derived from Br58, strain XB-6) (Inoue et al. 2017), and 61M2 [*pwt3;PWT4*] (an F₁ culture derived from a cross, Br58 x Br48) (Takabayashi et al. 2002). 61M2 had been used for the identification of *Rwt4* (Takabayashi et al. 2002). They were transferred from PDA slants to oatmeal agar media just before use and incubated at 25°C.

Infection assay

Wheat seeds were sown in vermiculite supplied with liquid fertilizer in a seedling case (5.5 × 15 × 10 cm) and grown at 22°C in a controlled-environment room or growth chambers with a 12-h photoperiod of fluorescent lighting (50-100 μmol m⁻² s⁻¹) at 50-70% relative humidity for 7-8 days. Primary leaves of the seedlings were fixed onto a hard plastic board with rubber bands just before inoculation.

Conidial suspensions (1 × 10⁵ spores/ml) with 0.01% (v/v) Tween 20 prepared as described previously (Vy et al. 2014) were sprayed on the adaxial surface of primary leaves (5-10 ml suspension / 20 leaves) with an air compressor. The inoculated plants were put in trays, covered by cling wrap to maintain high humidity, and incubated in darkness for 24 h at 22-25°C. They were then transferred to the dry condition with the fluorescent lighting and incubated further at 22-25°C. Symptoms were evaluated at five days after inoculation based on the size and the color of lesions. The size was rated using six progressive grades from 0 to 5: 0, no visible evidence of infection; 1, pinhead spots; 2, small lesions (<1.5 mm); 3, lesions with an intermediate size (<3 mm); 4, large typical

lesions; and 5, complete shriveling of leaf blades. These lesions were classified into two categories based on their color: B, brown; and G, green. Infection types were represented by the combination of the size score and the color code. For example, infection type 2B indicated small lesions accompanied by brown tissues. Infection types 0 and those with “B” were considered resistant (avirulent), while infection types with “G” were considered susceptible (virulent). Five seedlings were employed for each fungal strain/wheat line combination in one replication. All infection assays were repeated twice.

Results

Establishment of a system for wheat genotyping based on specific recognition of corresponding *AVR* genes

To survey the distribution of *Rwt3* and *Rwt4*, we first established a system for rapid genotyping based on responses to transformants carrying avirulence genes. Inoue et al. (2017) already applied this system to the survey of these resistance genes in common wheat accessions, but did not mention the process of its establishment. Here, we describe the process in detail. *Rwt3* and *Rwt4* correspond to *PWT3* and *PWT4*, respectively, in a one-to-one manner. Therefore, if an accession responds to *PWT3*, we can infer that it carries *Rwt3*. If another accession responds to *PWT4*, we can infer that it carries *Rwt4*. The presence/absence of these specific responses can be detected by comparing a response of an accession against Br48 with its responses against a Br48 transformant carrying *PWT3* (strain M-16, hereafter called Br48+3) and a Br48 transformant carrying *PWT4* (strain XB-6, hereafter called Br48+4). Actually, N4, which is known to carry both resistance genes (Takabayashi et al. 2002; Vy et al.

2014), is susceptible to Br48 but resistant to Br48+3 and Br48+4 (Table 1). CS, which is known to carry *Rwt3* but lack *Rwt4* (Takabayashi et al. 2002; Vy et al. 2014), is susceptible to Br48 and Br48+4 but resistant to Br48+3 (Table 1).

To complete this system, we looked for a cultivar that responded to Br48+4 but not to Br48+3. Consequently, we found Tfed, a cultivar that showed this type of response (Table 1). Tfed should obviously lack *Rwt3* because it is susceptible to Br48+3, but is expected to carry *Rwt4* because it is resistant to Br48+4. To check if this inference is correct, a segregation analysis was performed using 61M2 which had been used for the identification of *Rwt4* in N4 (Takabayashi et al. 2002). 61M2 is an F₁ culture derived from Br58 x Br48 that carries *PWT4* alone. Against this culture, Tfed was resistant while Hope was susceptible (Table 1). When F₂ seedlings derived from Tfed x Hope were inoculated with 61M2, resistant and susceptible seedlings segregated in a 3:1 ratio (Table 2), suggesting that the resistance of Tfed to 61M2 is controlled by a single gene. However, a cross between Tfed and N4 produced no susceptible seedlings even when the population size was expanded to more than 400 (Table 2). These results suggest that Tfed carries *Rwt4* as expected from its contrastive reactions to Br48 and Br48+4. Taken together, we concluded that the presence/absence of *Rwt3* and *Rwt4* can be determined based on the infection assay with the set of tester strains (Br48, Br48+3, and Br48+4).

Genotyping of *Ae. tauschii* accessions through infection assays with synthetic wheat lines

Rwt3 and *Rwt4* are both located on the 1D chromosome of common wheat (Hirata et al. 2005; Vy et al. 2014), and therefore assumed to be derived from the D genome donor, *Ae.*

tauschii. *Ae. tauschii* is a wild wheat progenitor, and due to its wild nature, difficult to handle compared with cultivated wheat. In addition, its first leaf is too thin and small to determine infection types. To overcome these shortcomings of *Ae. tauschii*, we employed synthetic hexaploid wheat lines derived from crosses between a tetraploid wheat cultivar Ldn and *Ae. tauschii* accessions (Kajimura et al. 2011; Matsuoka et al. 2007; Takumi et al. 2009) for infection assays with the tester strains. Because Ldn is susceptible to the tester strains (Table 1), resistance responses in synthetic hexaploid lines, if they occur, should be attributable to *Ae. tauschii* accessions used for their production. Actually, we detected differential responses against the tester strains in the synthetic hexaploid lines, which enabled us to determine the presence/absence of *Rwt3* and *Rwt4* in *Ae. tauschii* accessions used for the crosses. Reactions of four representative lines are shown in Fig. 1 as examples.

Distinct patterns of the distribution of *Rwt3* and *Rwt4* in *Ae. tauschii* lineages

To reveal the distribution of *Rwt3* and *Rwt4* in *Ae. tauschii* population, the tester strains were sprayed onto synthetic hexaploid lines with the D genomes derived from 54 *Ae. tauschii* accessions that covered the diversity of natural habitat ranges and intraspecific lineages/sublineages of the species (Table 3). *Rwt3* and *Rwt4* were detected in 17 (31.5%) and 26 (48.1%) lines, respectively (Table 4). *Rwt3* was detected in two major lineages, TauL1 and TauL2, and distributed across the species habitat range, while *Rwt4* was mainly detected in TauL2 and TauL3, and restricted to the Transcaucasus and Middle East region (Fig. 2).

Detection of accessions carrying both genes at a high frequency and exclusively in the TauL2b sublineage

Our previous research with ~500 common wheat landraces suggested that both *Rwt3* and *Rwt4* were widely distributed in more than 75% of the landraces (Inoue et al. 2017). The rate of co-occurrence of the two genes (percentage of accessions carrying both genes) was ~70%, which led us to a hypothesis that both genes were harbored by a common ancestor of common wheat when it was established by amphidiploidization of the AB and D genomes, but have been degraded differentially in ~30% of landraces. If this is the case, the actual D genome donor should carry both genes. To verify this hypothesis, we checked if any accession/lineage/sublineage of *Ae. tauschii* carried both *Rwt3* and *Rwt4*. The co-occurrence of *Rwt3* and *Rwt4* was observed at high frequency (58.8%) and exclusively in the TauL2b sublineage (Table 4). The accessions carrying both genes were mainly distributed in the southern coast of the Caspian Sea (Fig. 2b).

Discussion

In the present study, the distribution of *Rwt3* and *Rwt4* in *Ae. tauschii*, a progenitor species of common wheat, was verified by using synthetic hexaploid wheat lines derived from crosses between a tetraploid wheat cultivar (Ldn) and a panel of *Ae. tauschii* accessions covering the natural habitat and lineages. We found accessions carrying both *R* genes among those collected in the southern coast of the Caspian Sea (Fig. 2), which had been suggested to be the birthplace of common wheat based on comparative gene analysis (Tsunewaki 1966). In addition, all these two gene-carriers belonged to the TauL2b sublineage which has been suggested to be involved in the establishment of common

wheat. Nishijima et al. (2014) reported, through the genetic analysis of glaucous phenotype and cuticular wax loci in *Ae. tauschii*, that glaucous *Ae. tauschii* accessions in the TauL2 lineage (including the TauL2b sublineage in the present study) were likely involved in the origin of hexaploid common wheat. Recently, Matsuoka and Takumi (2017) showed that TauL2 accessions had actually higher potential for natural hybridization with *T. turgidum*. Considering their geographical distribution together, they concluded that the southern coastal region of the Caspian Sea is a good candidate for the place of origin of common wheat. Taken together, we suggest that the D genome donor to common wheat was *Ae. tauschii* individual(s) carrying both *R* genes. This implies that the common ancestor of common wheat gained the two *R* genes simultaneously from such *Ae. tauschii* individual(s). This idea may explain why *Rwt3* and *Rwt4* are widely distributed in common wheat landraces with a high rate of co-occurrence (Inoue et al. 2017). An alternative scenario is that a common ancestor of common wheat gained *Rwt3* from an accession of *Ae. tauschii* carrying *Rwt3* alone while another common ancestor gained *Rwt4* from an accession of *Ae. tauschii* carrying *Rwt4* alone, and that following hybridization between these two common ancestors resulted in the establishment of common wheat carrying both genes. However, this seems unlikely because *Ae. tauschii* accessions carrying *Rwt3* alone were not detected in the TauL2 lineage (Table 3, Fig. 2).

In contrast to *Rwt4*, *Rwt3* was found not only in the TauL2 lineage but also in the TauL1 lineage, including the TauL1b sublineage which has spread eastwards (Fig. 2). This may suggest that *Rwt3* may have provided some adaptive advantages to *Ae. tauschii* in eastern habitats. Since *PWT3* corresponding to *Rwt3* is widely conserved in the population of *P. oryzae*, *Rwt3* may have prevented various strains of the blast fungus from infecting *Ae. tauschii*. Alternatively, *Rwt3* may have defended this species against other pathogens than *P. oryzae*. This may be one factor which enabled the TauL1 lineage to

expand its distribution from the Transcaucasus-Middle East region to Central Asia (Matsuoka et al. 2015; Mizuno et al. 2010). Further analyses are needed to understand the evolutionary trajectories or the origin of the two *R* genes in wheat and its wild relatives. Cloning of these *R* genes would facilitate the evolutionary analysis based on nucleotide sequences.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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Figure legends

Fig. 1. Reactions of wheat primary leaves to *Pyricularia oryzae* Br48 (*Triticum* isolate), a Br48 transformant carrying *PWT3* (Br48+3, strain M-16), and a Br48 transformant carrying *PWT4* (Br48+4, strain XB-6). Ldn, *T. turgidum* ssp. *durum* cv. Langdon. The other four wheat lines are synthetic hexaploids derived from crosses between Ldn and different *Aegilops tauschii* accessions. Wheat (on the left) and fungal (on the top) genotypes are indicated in the parentheses. The minus (-) symbols represent recessive/nonfunctional alleles. Inoculated leaves were incubated at 25°C for five days.

Fig. 2. Geographic distribution of *Aegilops tauschii* accessions with different *Rwt3/Rwt4* genotypes. (a) The TauL1 sublineages. TauL1a, TauL1b, and TauL1x are represented by square, circle, and triangle symbols, respectively. (b) The TauL2 and TauL3 sublineages. TauL2a, TauL2b, TauL2x, and TauL3 are represented by square, circle, triangle, and diamond symbols, respectively. Each accession is color-coded according to its *Rwt3* and *Rwt4* genotypes. Numbers on x and y axes indicate Longitude and Latitude, respectively.

Table 1. Reaction of representative hexaploid and tetraploid wheat cultivars to *Triticum* isolate Br48 of *Pyricularia oryzae* and its transformants carrying *PWT3* or *PWT4*.

Species	Cultivar	Genomes	Genotype	Infection type ^a with			
				Br48	Br48+3 ^b	Br48+4 ^b	61M2
<i>T. aestivum</i>	Norin 4 (N4)	ABD	<i>Rwt3/Rwt4</i>	4-5G	1B	0	1B
<i>T. aestivum</i>	Chinease Spring (CS)	ABD	<i>Rwt3/rwt4</i>	4G	1-2B	4G	4G
<i>T. aestivum</i>	Transfed (Tfed)	ABD	<i>rwt3/Rwt4</i>	5G	4-5G	1B	0
<i>T. aestivum</i>	Hope	ABD	<i>rwt3/rwt4</i>	5G	3G	5G	4G
<i>T. turgidum</i> ssp. <i>durum</i>	Langdon (Ldn)	AB	<i>rwt3/rwt4</i>	5G	4G	4-5G	ND

^a After 5-day incubation at 22°C. 0, no visible infection; 1, pinhead spots; 2, small lesions (<1.5 mm); 3, scattered lesions of intermediate size (<3 mm); 4, large typical lesions; and 5, complete shriveling of leaf blades. B, brown lesion; G, green lesion. Resistant reactions are shown in bold. Representative infection type scores from two independent experiments (total n = 10) are shown. ND, not determined.

^b Br48+3, a Br48 transformant (M-16) carrying *PWT3*; Br48+4, a Br48 transformant (XB-6) carrying *PWT4*; 61M2, an F₁ culture derived from a cross, Br58 (*Avena* isolate) x Br48.

Table 2. Segregation of reactions to 61M2, an F_1 culture derived from Br58 x Br48, in F_2 populations derived from crosses between wheat cultivars

Cross	No. of F_2 seedlings			χ^2 (3:1)	P
	Resistant ^a	Susceptible ^b	Total		
Tfed x Hope (<i>rwt3/rwt4</i>)	148	38	186	2.1	0.15
Tfed x N4 (<i>Rwt3/Rwt4</i>)	419	0	419	-	-

^a Infection type 0-5B

^b Infection type 3G-5G

Table 3. Reaction of synthetic hexaploid wheat lines derived from crosses between *Triticum turgidum* cv. Langdon and *Aegilops tauschii* accessions against *Triticum* isolate Br48 of *Pyricularia oryzae* and its transformants carrying *PWT3* or *PWT4*.

Code of synthetic hexaploid	Ae. tauschii accession				Infection type ^c with			Genotype ^e	
	Code	Locality	Source ^a	Lineage ^b	Br48	Br48+3 ^d	Br48+4 ^d	Rwt3	Rwt4
6026	KU-2816	Armenia	KYOTO	TauL1a	3-5G	2B	3-4G	+	–
6067	KU-2810	Armenia	KYOTO	TauL1a	5G	3B	5G	+	–
6068	KU-2814	Armenia	KYOTO	TauL1a	5G	3B	5G	+	–
6069	KU-2824	Armenia	KYOTO	TauL1a	5G	2B	5G	+	–
6057	KU-2144	Iran	KYOTO	TauL1a	5G	4-5G	0-1B	–	+
6086	KU-2152	Iran	KYOTO	TauL1a	5G	4G	5G	–	–
6027	PI 476874	Afghanistan	USDA	TauL1b	5G	3-4G	5G	–	–
6031	KU-2059	Afghanistan	KYOTO	TauL1b	5G	2B	4G	+	–
6064	KU-2022	Afghanistan	KYOTO	TauL1b	5G	2B	4G	+	–
6029	PI 499262	China	USDA	TauL1b	5G	3G	5G	–	–
6030	PI 508262	China	USDA	TauL1b	5G	4G	5G	–	–
6001	AE 1090	Kazakhstan	IPK	TauL1b	5G	3-4G	5G	–	–
6045	IG 131606	Kyrgyzstan	ICARDA	TauL1b	4-5G	2B	4-5G	+	–
6088	IG 46663	Pakistan	ICARDA	TauL1b	5G	3-4G	5G	–	–
6093	CGN 10768	Pakistan	CGN	TauL1b	5G	3G	5G	–	–
6094	CGN 10770	Pakistan	CGN	TauL1b	5G	4G	5G	–	–
6091	IG 48554	Tajikistan	ICARDA	TauL1b	5G	4G	5G	–	–
6044	IG 126387	Turkmenistan	ICARDA	TauL1b	5G	3-4G	5G	–	–
6047	IG 48042	India	ICARDA	TauL1x	5G	5G	5G	–	–
6087	KU-2157	Iran	KYOTO	TauL1x	5G	4G	5G	–	–
6002	IG 47259	Syria	ICARDA	TauL1x	5G	4-5G	5G	–	–
6032	KU-2811	Armenia	KYOTO	TauL2a	5G	4G	0	–	+
6023	KU-2155	Iran	KYOTO	TauL2a	5G	3-4G	0	–	+
6024	KU-2156	Iran	KYOTO	TauL2a	5G	4-5G	0	–	+
6051	KU-20-8	Iran	KYOTO	TauL2a	5G	4G	5G	–	–
6075	KU-2083	Iran	KYOTO	TauL2a	5G	3-5G	5G	–	–
6083	KU-2118	Iran	KYOTO	TauL2a	5G	4G	5G	–	–
6021	KU-2132	Turkey	KYOTO	TauL2a	5G	4-5G	5G	–	–
6022	KU-2136	Turkey	KYOTO	TauL2a	5G	4-5G	5G	–	–
6004	KU-20-10	Iran	KYOTO	TauL2b	4-5G	2B	0	+	+
6010	KU-2088	Iran	KYOTO	TauL2b	5G	2B	0	+	+
6012	KU-2090	Iran	KYOTO	TauL2b	5G	2-3B	0	+	+
6013	KU-2091	Iran	KYOTO	TauL2b	5G	2B	0	+	+
6014	KU-2093	Iran	KYOTO	TauL2b	5G	4G	0	–	+
6016	KU-2098	Iran	KYOTO	TauL2b	5G	3G	0	–	+
6017	KU-2103	Iran	KYOTO	TauL2b	3-4G	2B	0	+	+
6019	KU-2109	Iran	KYOTO	TauL2b	5G	4G	5G	–	–
6025	KU-2158	Iran	KYOTO	TauL2b	4G	2B	0	+	+
6035	KU-2106	Iran	KYOTO	TauL2b	5G	2B	0	+	+
6052	KU-2092	Iran	KYOTO	TauL2b	5G	4G	0	–	+
6053	KU-2105	Iran	KYOTO	TauL2b	3-4G	2B	0	+	+
6059	KU-2159	Iran	KYOTO	TauL2b	5G	3G	0	–	+
6060	KU-2160	Iran	KYOTO	TauL2b	5G	3-4G	0	–	+
6080	KU-2102	Iran	KYOTO	TauL2b	5G	4-5G	0	–	+
6082	KU-2108	Iran	KYOTO	TauL2b	5G	2B	0	+	+
6089	IG 46623	Syria	ICARDA	TauL2b	3G	2B	0	+	+
6006	KU-2076	Iran	KYOTO	TauL2x	5G	3-4G	5G	–	–
6007	KU-2078	Iran	KYOTO	TauL2x	5G	3-5G	5G	–	–
6008	KU-2079	Iran	KYOTO	TauL2x	5G	4G	0	–	+
6011	KU-20-9	Iran	KYOTO	TauL2x	5G	3-4G	0	–	+
6050	KU-2075	Iran	KYOTO	TauL2x	5G	4G	0	–	+
6039	AE 454	Georgia	IPK	TauL3	5G	4G	0	–	+
6040	AE 929	Georgia	IPK	TauL3	5G	4G	0	–	+
6063	KU-2829A	Georgia	KYOTO	TauL3	5G	4-5G	0	–	+

^a CGN, Centre for Genetic Resources, The Netherlands; ICARDA, International Center for Agricultural Research in the Dry Areas; IPK, Institut für Pflanzengenetik und Kulturpflanzenforschung; KYOTO, Plant Germ-plasm Institute of Kyoto University; USDA, US Department of Agriculture.

^b Refer to Matsuoka et al. (2015).

^c After 5-day incubation at 22°C. 0, no visible infection; 1, pinhead spots; 2, small lesions (<1.5 mm); 3, scattered lesions of intermediate size (<3 mm); 4, large typical lesions; and 5, complete shriveling of leaf blades. B, brown lesion; G, green lesion. Resistant reactions are shown in bold. Representative infection type scores from two independent experiments (total n = 9-10) are shown.

^d Br48+3, a Br48 transformant (M-16) carrying *PWT3*; Br48+4, a Br48 transformant (XB-6) carrying *PWT4*.

^e +, present; –, absent. Genotypes were determined based on the reactions to Br48+3 and Br48+4.

Table 4. Distribution of *Rwt3* and *Rwt4* in *Aegilops tauschii* accessions inferred from genotyping of synthetic hexaploid lines

Lineage ^a	Number (percentage) of accessions			Total
	<i>Rwt3</i>	<i>Rwt4</i>	<i>Rwt3 and Rwt4</i>	
TauL1a	4 (66.7 %)	1 (16.7 %)	0 (0.0 %)	6
TauL1b	3 (25.0 %)	0 (0.0 %)	0 (0.0 %)	12
TauL1x	0 (0.0 %)	0 (0.0 %)	0 (0.0 %)	3
TauL2a	0 (0.0 %)	3 (37.5 %)	0 (0.0 %)	8
TauL2b	10 (58.8 %)	16 (94.1 %)	10 (58.8 %)	17
TauL2x	0 (0.0 %)	3 (60.0 %)	0 (0.0 %)	5
TauL3	0 (0.0 %)	3 (100.0 %)	0 (0.0 %)	3
Total	17 (31.5 %)	26 (48.1 %)	10 (18.5 %)	54

^a Refer to Matsuoka et al. (2015).

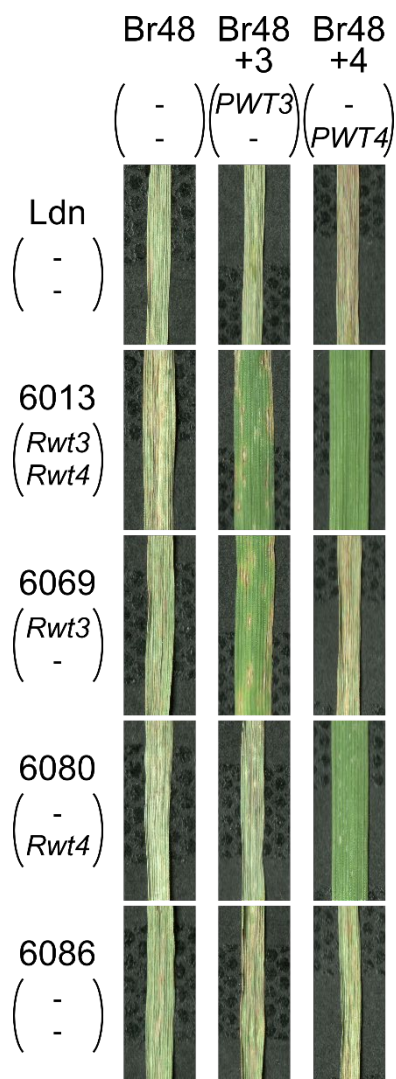


Fig. 1

Fig. 1. Reactions of wheat primary leaves to *P. oryzae* Br48 (*Triticum* isolate), a Br48 transformant carrying *PWT3* (Br48+3, strain M-16), and a Br48 transformant carrying *PWT4* (Br48+4, strain XB-6). Ldn, *T. turgidum* ssp. *durum* cv. Langdon. The other four wheat lines are synthetic hexaploids derived from crosses between Ldn and different *Ae. tauschii* accessions. Wheat (on the left) and fungal (on the top) genotypes are indicated in the parentheses. The minus (-) symbols represent recessive/nonfunctional alleles. Inoculated leaves were incubated at 25°C for five days.

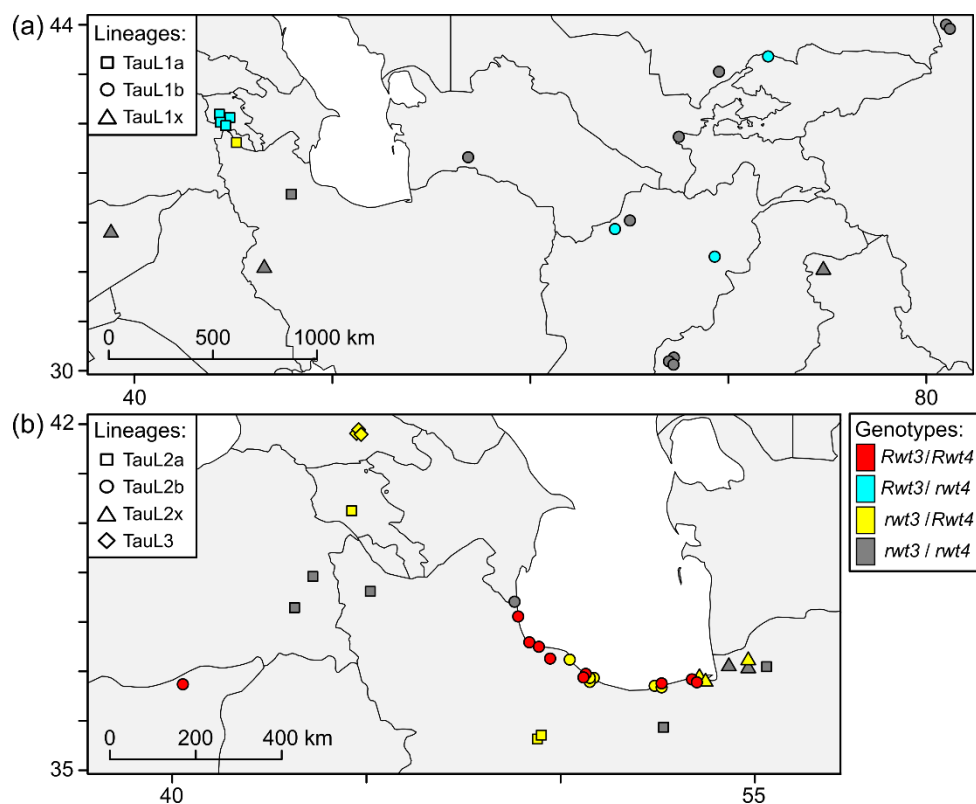


Fig. 2

Fig. 2. Geographic distribution of *Ae. tauschii* accessions with different *Rwt3/Rwt4* genotypes. (a) The TauL1 sublineages. TauL1a, TauL1b, and TauL1x are represented by square, circle, and triangle symbols, respectively. (b) The TauL2 and TauL3 sublineages. TauL2a, TauL2b, TauL2x, and TauL3 are represented by square, circle, triangle, and diamond symbols, respectively. Each accession is color-coded according to its *Rwt3* and *Rwt4* genotypes. Numbers on x and y axes indicate Longitude and Latitude, respectively.