



Expression of resistance against powdery mildew (*Blumeria graminis* f.sp. *tritici*) in bread wheat (*Triticum aestivum*)

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ABSTRACT

Blumeria graminis f.sp. *tritici* (*Bgt*) causes an important disease of wheat, the powdery mildew to which accessions TA1055 and TA1150 of *Triticum turgidum* L. ssp *dicoccoides* (*T. dicoccoides*) were resistant. These carry *MITd1055* gene for resistance to *Bgt*. The F₁ hybrid plants obtained from crosses between 'Chinese Spring (CS)' and *T. dicoccoides* were sterile or partially sterile. Overall, the mean number of BC₁ seed set was 1.43 seed/spike. Selfed seeds were harvested from 78 BC₁ plants grown to maturity. The levels of fertility were, 33.6% with TA1055 and 26.4% with TA1150. The chromosome numbers of the selfed seed from BC₁ plants ranged from 27 - 44. Fifty eight per cent of their BC₁ progeny had chromosome numbers between 40 and 42. The segregating of BC₁ plants fit a 1:1 ratio and BC₁F₂ fit a 3:1 ratio for resistance to susceptibility supporting further the hypothesis of one dominant gene for resistance. Based on screening of two BC₁F₂ lines carrying powdery mildew resistance derived from TA1055 and TA1150, it appeared that a new gene (*MITd1055*) for powdery mildew resistance has been transferred to bread wheat *Triticum aestivum*. Bread wheat carrying this gene gave intermediate reaction types when inoculated with the tested mixed population of *Bgt*, while the *T. dicoccoides* parents were highly resistant to the mixed population of isolates.

Key words: *Blumeria graminis*, Expression, Powdery mildew, *Triticum dicoccoides*

The powdery mildew is caused by a biotrophic fungus *Blumeria graminis* f. sp. *tritici* (DC) E.O. Speer Em. Marchal, here abbreviated as *Bgt*, is one of the most severe foliar diseases attacking of wheat (*Triticum aestivum* L. emend Fiori & Paol.). In susceptible wheat cultivars and under favourable weather conditions the disease appears on the upper leaf surface and wither and weakens the plants. The losses in yield ranges 5–62% (Parry 1990, Costamilan 2005, Szunics *et al.* 2001). The use of resistant cultivars is most effective and economically feasible means of management of powdery mildew. The pathogen, *Bgt* has the ability to overcome single gene resistance rapidly through evolution of new virulence. Therefore there is a need for different forms of genetic resistance with greater durability (Szunics and Szunics 1999, Peusha *et al.* 2000). There is a considerable variation for resistance available in closely related species of *Triticum*, *Aegilops*, *Agropyron*, *Secale*, *Haynaldia* and *Eremopyron* and also in more distantly related genera including *Hordeum* (Rubiales *et al.* 1993). Therefore, the broadening of the genetic base of cultivated wheat by the introgression of resistance genes from related species or

genera may provide new sources of useful and durable resistance against this disease.

Wild emmer is the only progenitor that is fully interfertile with cultivated *T. turgidum* L. ssp *durum*. *Triticum turgidum* L. ssp. *dicoccoides* (*T. dicoccoides*) also can be hybridized with hexaploid wheat. Thus *T. dicoccoides* can be a value genetic resource to broaden the genetic base of both cultivated species. Genes from *T. dicoccoides* have been successfully transferred into hexaploid wheat (Reader and Miller 1991). The purpose of the present study was to study the expression of the *T. dicoccoides* resistance to wheat powdery mildew and to assess the feasibility of transferring the resistance to wheat.

MATERIALS AND METHODS

Two resistant accessions of *T. turgidum* ssp *dicoccoides* TA1055 and TA1150 (Ahmadifirouzabad 2001) were obtained from Kansas State University. These accessions carried *MITd1055* gene for resistance to *Bgt* (Ahmadi and Moore 2007), Chinese Spring (CS) is known as a susceptible variety of common wheat (*T. aestivum*) to *Bgt*. The *Bgt* isolate Nor2 used for the inheritance and location of gene for resistance to powdery mildew was provided as described by Ahmadi-Firouzabad (2001). The varieties of *T. aestivum* with known genes for resistance were used to identify of genes

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Table 1 Differential reactions of varieties with known genes for resistance against isolate Nor2 of *Bgt*

Variety	Powdery mildew resistant genes	Reaction ¹	Infection ² type
Asosan	<i>Pm3a</i>	S	4
Khapli	<i>Pm3c</i>	S	4
Sonora	<i>Pm4a</i>	S	4
Mercia	<i>Pm5</i>	S	4
Disponent	<i>Pm8</i>	R	1
Wembley	<i>Pm12</i>	R	0
Wembley	<i>Pm16</i>	S	3
XX 186	<i>Pm19</i>	R	1
Ovest	<i>Pm22</i>	R	2
Maris Dove	<i>Pm2+Mld</i>	S	4
Chinese Spring	<i>Pm11+15</i>	S	4
Timmo	<i>Pm2+4b+6</i>	S	4
Rendezvous	<i>Pm2+4b+6</i>	S	4
Normandie	<i>Pm1+2+9</i>	S	4

¹S, Susceptible; R, resistant ²0, immune; 1, highly resistant; 2, moderately resistant; 3, moderately susceptible; 4, very susceptible

for virulence in isolate Nor2 (Table 1). Twenty seeds from each variety were separately planted in 12.7 cm diameter pots. After two weeks, the seedlings were inoculated with Nor2 isolate of *Bgt*. Differential reactions of wheat varieties tested are given in Table 1 showing presence of resistant genes *Pm1*, *Pm2*, *Pm3a*, *Pm3c*, *Pm4a*, *Pm4b*, *Pm5*, *Pm9*, *Pm11*, *Pm15*, *Mld* and *Mlk* of *Bgt*.

The accessions of *T. dicoccoides* were crossed as male parents with the susceptible variety Chinese Spring (CS) at Department of Agricultural and Environment Science, The University, Newcastle Upon Tyne, UK. Cytological analysis was carried out at Agricultural Faculty of Lorestan University, Khorramabad, Iran. The F₁ plants of 'CS' × TA1055 and 'CS' × TA1150 were backcrossed as females to 'CS'. The data were recorded on the numbers of spikes crossed and BC₁ seed set for each cross. The BC₁ seeds were germinated on moist filter paper and chromosome numbers were determined. The germinated seeds were then planted in pots of 7.5 cm diameter. The 14-day-old BC₁ seedlings were inoculated with the isolate Nor2 by dusting conidia of the pathogen from previously infected potted plants and

reactions of the plants were recorded after 10 days of inoculation. Classification of reaction on the leaf blade was as follows: 0, immune, no visible signs of infection; 1, highly resistant, necrotic flecks present with no visible development of mycelium; 2, moderately resistant, moderate development of mycelium with slight production of conidia; 3, moderately susceptible, moderate to abundant development of mycelium accompanied by moderate sporulation; 4, very susceptible, abundant mycelium with large pustules profusely sporulating. The BC₁ plants were vernalized and then transplanted in the greenhouse. The BC₁ plants were grown to maturity in the greenhouse and allowed to produce BC₁ self seed. The data were recorded on numbers of selfed seed set/spike. The selfed seeds of each BC₁ plant were harvested and chromosome numbers were determined. The segregating BC₁ selfed progeny from crosses between 'CS' and *T. dicoccoides* accessions (TA1055, TA1150) resistant to powdery mildew were screened for reaction to Nor2 isolate of *Bgt*. The BC₁ progeny and BC₁F₂ from the crosses of 'CS'/TA1055 and 'CS'/TA1150 were inoculated with isolate Nor2 at the seedling stage and heading stage, and the numbers of resistant and susceptible plants for each BC₁ progeny and BC₁F₂ family were recorded.

RESULTS AND DISCUSSION

Crossability of T. dicoccoides with hexaploid wheat

Hybrids (F₁) plants from crosses between 'CS' and *T. dicoccoides* were sterile or partially sterile. Overall, the mean number of BC₁ seed set was 1.43/spike (Table 2). The amount of seed set/spike varied among cross combinations. For example, 55 BC₁ seeds were obtained by backcrossing 34 spikes of the F₁ hybrid between 'CS' × TA1055 to 'CS', whereas only 19 seeds were obtained when 28 spikes of the hybrid between 'CS' × TA1150 were backcrossed to 'CS'. The germination of these seeds was 92%. Seeds obtained from selfing were harvested from 78 BC₁ plants grown to maturity. The majority of these spikes set more than 10 selfed seeds each, representing a fertility of 33.6% with TA1055 and 26.4% with TA1150.

Chromosome number

The BC₁ plants had chromosome numbers ranging from

Table 2 Numbers of BC₁ seed/spike of hybrids between *T. dicoccoides* and common wheat and numbers of BC₁ segregating for resistance to powdery mildew

<i>T. dicoccoides</i> parent	Mean no. of BC ₁ seed/F ₁ spike	No. of BC ₁ plants	BC ₁ plants selfed seed set	Reaction to powdery mildew			<i>P</i> value
				Resistant	Susceptible	X ² value 1:1	
TA1055	2.33	51	33.6	22	29	0.96	> 0.01
TA1150	0.52	27	26.4	15	12	0.33	> 0.01
Total	1.43 ¹	78	30.0 ²	37	41		

¹Mean no. of BC₁ seed/spike, ²Mean number of seed set of BC₁ plants which set seed

Table 3 Segregation for seedling reaction to mildew isolate Nor2 in BC₁F₂ families from crosses of 'CS' with *T. dicoccoides* accessions

<i>T. dicoccoides</i> parent	Reaction to powdery mildew no. of BC ₁ F ₂ families			
	Resistant	susceptible	X ² value 3:1	P value
TA1055	15	5	0	> 0.01
TA1150	5	5		
Total	20	10		

36 to 47 with a mean chromosome number 41.7. The chromosome numbers of BC₁F₂ plants ranged from 27 to 44. The BC₁ plants with chromosome numbers ranging from 40 to 43 on an average, in selfed seeds and 58% of their progeny had chromosome numbers ranging from 40 to 42. There were the most viable combination in BC₁ plants, the majority of BC₁ plants produced tetraploid (2n=28), and hexaploid (2n=42) progeny thus indicated that the BC₁ plants tend to possess the chromosome number of their parents.

Transfer of powdery mildew resistance to hexaploid wheat

The BC₁ progeny and BC₁F₂ from the crosses of CS/TA1055 and CS/TA1150 were screened for reaction to powdery mildew. When BC₁ plants were inoculated with isolate Nor2, only 22 of the 51 BC₁ plants from the cross of CS/TA1055 were scored as resistance, and 29 BC₁ plants were susceptible, and segregated in a 1:1 ratio (X² = 0.96, P. 0.50 - 0.25). From the cross of CS/TA1150, 15 out of 27 BC₁ plants were resistance and 12 of them were susceptible and the pooled data fit a 1:1 ratio (X² = 0.33, P. 0.75 - 0.50) (Table 2).

The susceptible and resistant BC₁F₂ plants from each cross were inoculated with isolate Nor2. The progeny of all of the

susceptible BC₁F₂ plants were susceptible. Progeny of the resistant BC₁F₂ plants derived from CS/TA1055 segregated in a 3 resistant: 1 susceptible for powdery mildew resistance and the heterogeneity X² value for families from this cross was not significant (Table 3).

Small quantities of BC₁F₂ plants derived from 'CS' × TA1150 were available for powdery mildew screening and segregation ratios within BC₁F₂ families were not determined. The infection type of the resistant plants was different; some showed infection type 0-1 and others showed infection type 2. The infection types of the homozygous resistant plants were lower than the heterozygous ones. Thus, resistance which is conditioned by an incompletely dominant gene was transmitted from *T. dicoccoides* into bread wheat.

When Cox *et al.* (1991) estimated the probability of gene transfer to hexaploid wheat from diploid species after one backcross, it was slightly greater than the 0.5 probability expected in a hexaploid × hexaploid cross. This was a result of production of unreduced gametes by the interspecific hybrid. Although unreduced gametes were not produced by *T. dicoccoides* × *T. aestivum* hybrids at a high frequency, there was a large variation in chromosome numbers of BC₁ plants. The formula for computing the probability of gene transfer to wheat from a diploid species after one backcross can be modified to calculate the probability of gene transfer from a tetraploid species.

Cox *et al.* (1991) noted that because the frequency at which each chromosome is incorporated into functioning gametes is not known, the estimate of the probability of gene transfer is not precise. However, we can calculate the probability P_{BC₁} that a single gene from the tetraploid parent is transferred to a single backcross plant using the following formula, given certain assumptions: (i) gametes with 21 or more chromosomes have at least one copy of each A, B and D genome chromosome, (ii) gametes with 14 to 21

Table 4 Distribution of somatic chromosome number among BC₁ plants and BC₁F₂ families from crosses between common wheat and *T. dicoccoides*

BC ₁ chromosome no.	No. of BC ₁ plants examined	Chromosome no of BC ₁ F ₂																	Total	
		27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43		44
36	1	0	3	0	0	0	0	0	3	1	0	0	0	0	0	0	0	0	0	7
37	1	1	2	1	0	0	1	0	2	1	0	0	1	0	0	1	0	0	0	10
38	2	0	1	0	0	0	0	1	1	0	1	1	2	0	0	4	1	1	1	14
39	3	0	2	0	1	0	0	0	2	3	0	0	0	1	3	11	4	0	0	27
40	2	0	0	0	0	1	0	1	3	1	0	0	1	0	6	3	6	2	0	24
41	5	0	0	0	0	1	0	0	1	1	0	1	0	0	7	8	11	1	0	31
42	12	0	5	2	0	0	0	0	2	0	0	0	0	1	4	1	19	2	1	37
43	2	0	2	0	1	0	1	0	1	3	0	0	1	1	0	5	8	0	0	23
44	1	0	1	1	0	1	0	0	1	2	0	0	1	0	1	0	13	1	0	22
45	1	0	0	0	0	0	2	1	0	1	0	0	0	0	0	2	0	0	0	6
46	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	1	3
47	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1
Total	32	1	16	4	2	3	4	3	17	13	1	2	6	3	21	35	63	8	3	205

chromosomes are deficient for each of the 21 chromosomes with equal probability, and (iii) during non-disjunction, all disomic chromosomes are equally likely to be lost.

$$P_{BC_1} = 1/2 \left[1 + \frac{\sum_{n=43}^{n=56} f(n)(n-42)/14 - \sum_{n=34}^{n=42} f(n)(42-n)/21}{\sum_{n=43}^{n=56} f(n)(n-42)/14 + \sum_{n=34}^{n=42} f(n)(42-n)/21} \right]$$

Where f (n) is the sample frequency of BC₁ plants with 'n' chromosomes.

In the current study, the probability of capturing a gene in one BC₁ plant from a *T. aestivum* × *T. dicoccoides* cross is 0.495, virtually equal to the expected probability of 0.5 from a cross between plants of the same ploidy level. This occurs because the number of BC₁ plants with chromosome numbers less than 42 is greater than that of plants with chromosome numbers greater than 42. Given this probability, producing five BC₁ plants from a wheat and *T. dicoccoides* cross gives a greater than 95% chance of transferring a single gene. Taking into consideration the average number of BC₁ hybrid seed set per spike (1.19) and the viability of BC₁ seed (92%) and from *T. aestivum* and *T. dicoccoides* hybrids, one BC₁ seed can be obtained for every one spike. Therefore, producing an adequate number of BC₁ seed would require pollinating approximately ten F₁ spikes.

Based on screening of two BC₁F₂ lines carrying powdery mildew resistance derived from TA1055 and TA1150, it appears that a new gene (*MITd1055*) for powdery mildew resistance has been transferred to bread wheat *T. aestivum*. Bread wheat carrying this gene gave intermediate reaction types when inoculated with the tested mixed population of *Bgt*, while the *T. dicoccoides* parents were highly resistant to the mixed population of isolates.

It has been demonstrated that introgression through direct crossing of *T. dicoccoides* and hexaploid wheat is a valuable technique for germplasm enhancement (Reader and Miller 1991). In this study, the production of the initial interspecific hybrid between hexaploid wheat and *T. dicoccoides* was not

problematic, but the major problem was the production of the backcross seed (BC₁), because the number of backcrossed seed was inadequate.

REFERENCES

- Ahmadi-Firouzabad A. 2001. 'Introgression of resistance to powdery mildew (*Erysiphe graminis* f.sp. *tritici*) from alien species into bread wheat (*Triticum aestivum*)'. PhD thesis, University of Newcastle upon Tyne, Newcastle.
- Ahmadi H and Moore K. 2007. Inheritance and chromosomal location of powdery mildew resistance gene in wild wheat *Triticum turgidum* var. *dicoccoides*. *Plant Pathology Journal* **6**: 164–8.
- Costamilan L M. 2005: Variability of the wheat powdery mildew pathogen *Blumeria graminis* f. sp. *tritici*. *Fitopatologia Brasileira* **30**: 420–2
- Cox T S, Harrell L G, chen P and Gill B S. 1991. Reproductive behaviour of hexaploid/diploid wheat hybrids. *Plant Breeding* **107**: 105–18.
- Parry D. 1990. *Plant Pathology in Agriculture*, pp 47. Cambridge University Press, Cambridge.
- Peusha H, Enno T and Priilinn O. 2000. Chromosomal location of powdery mildew resistance genes and cytogenetic analysis of meiosis in common wheat cultivar Meri. *Hereditas* **132**: 29–34.
- Reader S M and Miller T E. 1991. The introduction into bread wheat of a major gene for resistance to powdery mildew from wild emmer wheat. *Euphytica* **53**: 57–60.
- Rubiales D, Brown J K M and Martin A. 1993. *Hordeum chilense* resistance to powdery mildew and its potential use in cereal breeding *Euphytica* **67**: 215–20.
- Szunics L and Szunics L. 1999. Wheat powdery mildew resistance genes and their application in practise. *Acta Agronomica Hungarica* **47**: 69–89.
- Szunics L, Szunics L U, Vida G, Bedö Z and Svec M. 2001. Dynamics of changes in the races and virulence of wheat powdery mildew in Hungary between 1971 and 1999. *Sixth International Wheat Conference*, pp 373–9, held during 5–9 June 2000 at Budapest, Hungary.