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Powdery mildew resistance in some new wheat amphiploids ($2n = 6x = 42$) derived from A- and S-genome diploid progenitors

Khola Rafique[1], Awais Rasheed[2]*, Alvina Gul Kazi[3], Hadi Bux[4], Farah Naz[1], Tariq Mahmood[2] and Abdul Mujeeb-Kazi[5]

[1]*Department of Plant Pathology, PMAS Arid Agriculture University, Rawalpindi, Pakistan,* [2]*Department of Plant Sciences, Quaid-i-Azam University, Islamabad, Pakistan,* [3]*Atta-ur-Rehman School of Applied Biosciences (ASAB), National University of Science and Technology (NUST), Islamabad, Pakistan,* [4]*Institute of Plant Sciences, Sindh University, Jamshoro, Pakistan and* [5]*National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad, Pakistan*

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Abstract

Triticum urartu possesses the A^u genome common to bread wheat. Similarly, *Triticum monococcum* contains the A^m genome, which is closely related to the A-genome donor of bread wheat. *Aegilops speltoides* of the Sitopsis section has the S genome, which is most similar to the B genome of bread and durum wheat when compared with all other wild grasses. Amphiploids developed through bridge crossing between A^m/A^u and S-genome diploid resources and elite durum cultivars demonstrate enormous diversity to improve both bread and durum wheat cultivars. We evaluated such A-genome amphiploids (*Triticum turgidum* × *T. urartu* and *T. turgidum* × *T. monococcum*, $2n = 6x = 42$; $BBAAA^mA^m/A^uA^u$) and S-genome amphiploids (*T. turgidum* × *Ae. speltoides*, $2n = 6x = 42$; AABBSS) along with their durum parents (AABB) for their resistance to powdery mildew (PM) at the seedling stage. The results indicated that 104 accessions (53.6%) of A-genome amphiploids ($AABBA^mA^m/A^uA^u$) were resistant to PM at the seedling stage. Of their 24 durum parents, five (20.83%) were resistant to PM and 16 (66.6%) were moderately tolerant. Similarly, ten (50%) accessions of S-genome amphiploids (BBAASS) possessed seedling PM resistance, suggesting a valuable source of major resistance genes. PM screening of the amphiploids and parental durum lines showed that resistance was contributed either by the diploid progenitors or durum parents, or both. We also observed the suppression of resistance in several cases; for example, resistance in durum wheat was suppressed in respective amphiploids. The results from this germplasm screening will facilitate their utilization to genetically control PM and widen the genetic base of wheat.

Keywords: *Aegilops speltoides*; amphiploids; *Erysiphe graminis* f. sp. tritici; powdery mildew resistance; *Triticum turgidum*; *Triticum urartu*

Introduction

Powdery mildew (PM) of wheat caused by an obligate biotrophic fungus, *Erysiphe graminis* DC. f. sp. tritici Marchal, is an important and devastating disease problem worldwide, resulting in both yield losses and quality deterioration (Griffey *et al*., 1993). Resistance breeding requires constant efforts to enrich the reservoir of resistance genes in wheat. Wild species have been widely used as genetic resources for introgression of useful genes into cultivated species by wide hybridization (Mujeeb-Kazi, 2003; Yao *et al*., 2007). So far,

* Corresponding author. E-mail: awaispbg@gmail.com

58 genes/alleles for resistance to PM in wheat at 39 loci have been identified and located on 16 different chromosomes, of which 21 resistance genes/alleles have been tagged with molecular markers (Huang and Roder, 2004; McIntosh *et al.*, 2010).

There are numerous examples of successful transfer of genes carrying resistance to various pathogens, environmental stresses and nutritionally useful characteristics from wild diploid relatives into the genome of polyploid wheats (Jiang *et al.*, 1994). At the diploid level, there are two species of einkorn wheat: *Triticum monococcum* L. and *Triticum urartu*. Biosystematic treatments and sterility of their hybrids (Johnson and Dhaliwal, 1976) indicated that they are valid biological species. *T. monococcum* comprises both wild and cultivated forms and has important wild subspecies. *T. urartu* was identified in 1937 and, later, Johnson (1975) investigated this species as a possible donor of the B genome to polyploid wheats. However, the results revealed that *T. urartu* chromosomes pair with A-genome chromosomes of the hexaploid wheat. Strong evidence has been reported in favour of *Aegilops speltoides* (SS) as the female parent of all wild tetraploid wheats (Kilian *et al.*, 2011); however, the B-genome origin of wheat remains to be confirmed. Of all the genomes analysed, the *Ae. speltoides* genome is most closely related to the B genome of wheat (Eilam *et al.*, 2007).

The maintenance of resistance to obligate pathogens of common wheat over many years has been dependent on the ongoing availability, identification and utilization of resistance genes. As resistance genes became ineffective due to increased virulence in pathogen populations, breeders introduced new genes. These initially came from common wheat, but, subsequently, there was increasing use of resistance genes introduced from wheat with lower ploidy or from related species. Common wheat is an allohexaploid species and genes introgressed into it from species of lower ploidy often confer lower levels of resistance than in the original source genotypes (McIntosh *et al.*, 2011). In some cases, such introgressions do not have adequate resistance to protect hexaploid cultivar derivatives from significant losses. In other instances, the original hybrids with wheat are fully susceptible, indicating evidence of the genetic suppression of resistance. The possibility of the genetic suppression of phenotypic effects in wide crosses of wheat has been a recurrent topic for many years; however, there is no plausible explanation of how suppression actually occurs, assuming that the presumed genes are present.

The present paper reports seedling screening of amphiploids derived from A^u/A^m genome and S-genome diploid ($2n = 2x = 14$) progenitor species against PM to identify resistant germplasm.

Materials and methods

Germplasm

The germplasms for this study encompass amphiploid wheats that are genomically $AABBA^mA^m$, $AABBA^uA^u$ and AABBSS. The production protocol of these amphiploids has been reported earlier (Mujeeb-Kazi, 2006). Amphiploids derived from the crosses of *T. durum* with either A^m or A^u genome progenitor species (194 accessions) along with their 24 durum parents (*Triticum turgidum*, AABB) and 20 accessions of amphiploids derived from the crosses of *T. durum* with *Ae. speltoides* (AABBSS) were screened against PM. The pedigree, accessions numbers of *T. urartu*, *T. monococcum* and *Ae. speltoides* along with disease scores are given in Supplementary Table S1 (available online only at http://journals.cambridge.org).

Seedling screening for PM resistance

For the evaluation of seedling resistance, *in vitro* screening was performed on all genotypes at the seedling stage under glass house conditions at the Crop Diseases Research Station, Murree, Pakistan. The planting had three replicates to form a completely randomized design and replication means were taken. Artificial inoculation was done using bulk inoculum collected during the 2007–8 cropping season. From the initial infections, the inoculum collected was applied on the test materials, and this served as the source of all further testing. Test procedures relative to inoculum collection and increase were essentially similar to those reported by Duggal *et al.* (2000). After inoculations, seedlings were maintained at 16–19°C with light for 21 h/d. Infection types were recorded after the appearance of mildew symptoms on a 0–9 scale (McNeal *et al.*, 1971). Plants having infection type 0 were considered as completely resistant (immune), those having infection types 1–3 were considered resistant, those with a 4–6 score as intermediate and 7–9 as highly susceptible.

Results

Seedling resistance evaluation

PM development was found to be satisfactory in greenhouse evaluations and readily identifiable variations in disease reactions between resistant and susceptible seedlings were observed. The frequency of genotypes among the A-genome amphiploids, S-genome amphiploids and durum parents is depicted in Table 1. The PM data for

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Table 1. Seedling screening for powdery mildew resistance in amphiploids and their durum parents

		Number of lines tested		
Seedling IT range	Reaction	A-genome amphiploids	Durums	S-genome amphiploids
0	Immune	15	0	5
1–3	Resistant	93	5	5
4–6	Intermediate	56	16	2
7–9	Susceptible	30	3	8

IT, infection types.

individual amphiploids are given in Supplementary Table S1 (available online only at http://journals.cambridge.org).

A-genome amphiploids

The results revealed that among the 194 A-genome amphiploids, 15 accessions (7.7%) were immune, 93 (47.9%) were resistant, 56 (28.8%) showed an intermediate response and the remaining 30 (15.4%) were found to be completely susceptible to PM. These 194 amphiploids were developed by exploiting 24 durum wheat genotypes and PM evaluation of these durum wheats enabled us to propose putative resistance sources in these synthesized amphiploids. Among the 24 durum wheats, five (20.8%) were completely resistant to PM, 16 (66.6%) showed an intermediate response and the remaining 3 (12.5%) were completely susceptible to PM (Table 2). The comparative analysis of amphiploids and their durum parents identified that in 41 amphiploids, resistance was encoded by an A-genome diploid parent and in 102 cases, both parents were found to be tolerant, and it was difficult to dissect

Table 2. Comparison of powdery mildew (PM) resistance in durum wheats and their derived A-genome amphiploids

Durums	PM status	No. of amphiploids derived	Resistant	Intermediate	Susceptible	Durum resistance suppression (*n*)
CROC_1	S	5	1	–	4	–
ARLIN_1	I	29	14	7	8	8
ALTAR84	S	1	1	–	–	–
DVERD_2	R	6	4	1	1	1
68.111/RGB-U//WARD/3/FGO/4/RABI	R	7	7	–	–	0
CPI/GEDIZ/3/GOO//JO/CRA	R	31	19	7	5	5
D67.2/P66.270	I	9	5	2	2	2
CERCETA	I	10	3	5	2	2
STERNA-DW	I	2	–	2	–	0
SCAUP	I	13	3	8	2	0
YAV_2/TEZ	R	17	12	4	1	1
YARMUK	I	2	2	–	–	0
DECOY 1	S	13	6	2	5	–
GARZA/BOY	R	4	2	2	–	0
ARAOS	I	3	2	1	–	0
GAN	I	2	1	1	–	0
SCOOP_1	I	16	9	7	–	0
STY-US/CELTA//PALS/3/SRN_5	I	2	2	–	–	0
FGO/USA2111	I	2	–	2	–	0
ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE	I	6	4	2	–	0
BOTNO	I	4	4	–	–	0
LCK59.61	I	1	–	1	–	0
AJAIA_9	I	2	2	–	–	0
SHAG_22	I	7	5	1	1	1

S, susceptible; I, intermediate; R, resistant.

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the donor resistance source. The suppression of resistance was identified in 20 amphiploids, in which resistance of the durum parent was not manifested in the amphiploids (Table 2). In 23 amphiploids, both parents (diploid and durum) were susceptible to PM and the amphiploids also did not show any resistance.

Among the 194 A-genome amphiploids, 120 were developed from the hybridization of 93 *T. monococcum* ssp. *boeoticum* accessions and 20 durum cultivars. Similarly, 38 were developed from the hybridization of *T. monococcum* ssp. *monococcum* with 12 durum cultivars and 36 from *T. urartu*. So, the resistance sources from different subspecies of A-genome-related species possess allelic diversity that could enrich the existing *Triticum* gene pool with the potential to improve both durum and bread wheat.

S-genome amphiploids

The results revealed that among the 20 S-genome amphiploids, five were found to be immune, five were resistant, two showed an intermediate response and eight were found to be completely susceptible to PM. The comparative analysis of the durum parents and respective amphiploids indicated that the resistance source in eight amphiploids was from *Ae. speltoides* (Table 3). In seven amphiploids, resistance suppression was observed, i.e. the resistant durum parents failed to express resistance in respective amphiploids. In two amphiploids, an additive response was observed, in which the amphiploids showed a higher level of resistance than the durum parent, suggesting that both parents were resistant. In six susceptible amphiploids, both parents were proposed to have susceptibility because the susceptible durum parents and their respective amphiploids did not show any resistance (Table 3).

Discussion

Previous studies have identified PM resistance in wild relatives and several genes have been transferred to cultivated wheat such as *Pm12* (6B) and *Pm32* (1B) from *Ae. speltoides* (Jia, 1996; Hsam *et al.*, 2003), *Pm29* (7D) from *Aegilops geniculata* (Zeller *et al.*, 2002), *Pm34* and *Pm35* (5DL) from *Aegilops tauschii* (Miranda *et al.*, 2006, 2007; Qiu *et al.*, 2006), *Pm39* from *Aegilops umbellulata* (Zhu *et al.*, 2006), and some undesignated genes from *Aegilops longissima*, *Aegilops searsii*, *Ae. umbellulata* (Buloichik *et al.*, 2008), *Aegilops comosa* (Bennett, 1984) and *Aegilops sharonensis* (Olivera *et al.*, 2007). From *T. monococcum*, *Pm25* and three temporarily designated genes, *Pm2026*, *Mlm3033* and *Mlm80*, have been introduced in wheat (McIntosh *et al.*, 2010). The present investigation identified some new valuable sources of PM resistance which can be introgressed into cultivated wheats through wide hybridization. This initial screening identified novel genes/alleles which need to be further validated at the molecular level. A competitive advantage of studying amphiploids is the availability and identification of user-friendly, genetically compatible germplasm having the potential to improve both durum and bread wheats. A moderate frequency of seedling resistant accessions in both A-genome amphiploids (56%) and S-genome amphiploids (50%) was observed, which could provide diverse sources of resistance to this disease.

Amphiploids that probably had received resistance genes from either of the parents and expressed successfully are important to further use for protecting wheat from PM. The comparative advantage of using a higher number of diploid progenitors with each durum cultivar was to get the optimum number of amphiploids expressing resistance. This is important due to the phenomenon of resistance suppression that has been widely observed in wheat and wide crosses for various biotic stresses. This phenomenon of the dilution of resistance was earlier reported by Kerber and Dyck (1969) who found a reduced expression of resistance to leaf rusts in amphiploids with *T. durum* compared with the diploid resistant parent *Ae. tauschii*. Similar results were obtained by Trottet *et al.* (1982) with PM, leaf and stripe rust, and glume blotch. Bai and Knott (1992) described the

Table 3. Comparison of powdery mildew (PM) resistance in durum wheats and their derived S-genome amphiploids

Durums	PM status	No. of amphiploids derived	Resistant	Intermediate	Susceptible	Durum resistance suppression (*n*)
CROC_1	S	1	1	–	–	–
ARLIN_1	I	10	4	2	4	4
ALTAR84	S	1	1	–	–	–
CPI/GEDIZ/3/GOO//JO/CRA	R	4	2	0	2	2
D67.2/P66.270	I	1	–	–	1	1
CERCETA	I	3	3	–	–	0

S, susceptible; I, intermediate; R, resistant.

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presence of at least four suppressor genes on chromosomes of the D genome inhibiting leaf and stem rust resistance from tetraploid wheats in hexaploid synthetics, and proposed the search for non-suppressing alleles to facilitate the transfer of potentially good sources of genes from relatives into common wheat. There are also certain evidences where resistance in durum is suppressed by the diploid progenitor in amphiploids, e.g. *Lr23* (Nelson *et al.*, 1997). However, the complete expression of diploid resistance in common wheat has been reported for green bug resistance (Harvey *et al.*, 1980), Hessian fly (Gill *et al.*, 1987), cereal cyst nematode (Eastwood *et al.*, 1991) and resistance to wheat curl mite (Thomas and Connor, 1986). In several cases, PM resistance of the same durum parent was expressed or suppressed when hybridized with different diploid accessions (Supplementary Table S1, available online only at http://journals.cambridge.org). Therefore, it is not clear whether resistance gene suppressors operated in durum or diploid accessions, which is due to the lack of knowledge about the function, structure, variability and operation of suppressor genes in *Triticeae*. Thus, prior knowledge of the dilution effect and the search for non-suppressor alleles would enhance the success of transfer of beneficial genes from wild diploid progenitors to common wheat and their use in practical wheat breeding programmes.

In conclusion, this initial PM screening at the seedling stage identified several amphiploids that carry resistance from A^m, A^u, AB and/or S genomes and justifies the expansion of genetic diversity for PM resistance.

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