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EVALUATION OF D-GENOME SYNTHETIC HEXAPLOID WHEATS AND ADVANCED DERIVATIVES FOR POWDERY MILDEW RESISTANCE

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Abstract

The present study was undertaken to characterize 32 D-genome synthetic hexaploid wheats (SHWs) of an Elite-II sub-set and their 60 advanced derivatives. At seedling stage under controlled glasshouse conditions in Murree, the SHWs showed complete resistance/ immune to susceptible disease reactions viz., two (6.25%) were immune, eighteen (56.25%) were resistant, nine (28.12%) were intermediate, whereas three (9.37%) were susceptible. The BW/SH derivatives also demonstrated complete resistance/ immune to intermediate reactions viz., 51 (85%) were immune, 5 (8.33%) were resistant while 4 (6.66%) were intermediate. Adult plant resistance (APR) evaluated at the hill off-station summer site in Kaghan was 100% in SHWs (32/32) and 80% (48/60) in BW/SH derivatives. Several genotypes provided resistance at both plant stages viz., 20 (62.5%) SHWs and 45 (75%) BW/SH derivatives. All the entries were checked for the presence of *Pm4b*, *Pm9*, *Pm16* and *Pm30* resistance genes using linked SSR markers. The marker *Xgwm382.2A* flanked the gene *Pm4b* in one SH and 5 BW/SH derivatives. *Pm9* gene was identified using two markers viz., *Xgwm4.4A* detected *Pm9* in one SH and 27 BW/SH while *Xgwm332.7A* detected same gene in one SH and 13 BW/SH derivatives. *Pm16* and *Pm30* genes were present in one SH entry and in 2 BW/SH derivatives, detected by *Xgwm159.5B*. The findings revealed that 37.5% (12 entries) of the SHs and 5% (3) of the 60 BW/SH derivatives possessed APR. These observations add strength to exploit both intraspecific and interspecific strategies for allelic enrichment within wheat pre-breeding / breeding programs.

Key words: Synthetic hexaploid wheat and derivatives, *Erysiphe graminis* f. sp. *tritici*, Powdery mildew resistance, *Pm* resistance genes, SSR markers.

Introduction

Wheat powdery mildew caused by the biotrophic foliar pathogen *Erysiphe graminis* DC. f. sp. *tritici* Marchal (syn. *Blumeria graminis* f. sp. *tritici*) is an economically important disease of wheat that occurs globally. It has been reported to be widespread across Africa, Asia, Australia, Europe and the Americas. This biotic stress greatly affects grain yield, volume, weight and grain quality characteristics (Everts *et al.*, 2001). Presently, it is becoming an emerging threat to wheat production in Pakistan and is globally causing substantial yield losses that necessitate the development of control strategies. Chemical control methods for powdery mildew aided by cultural practices can be erratic in effectiveness, costly and hazardous to environment (Hardwick *et al.*, 1994). Thus, host genetic resistance is the most convenient control method for powdery mildew and hence attempts are being made to evolve new resistant varieties to eliminate fungicide usage for reducing yield losses and provide environmental safety. There are more than 78 genes/ alleles at 50 genetic loci (*Pm1-Pm53, Pm18=Pm1c*, *Pm22=Pm1e*, *Pm23=Pm4c*, *Pm31=Pm21*) reported for powdery mildew resistance in bread wheat and its relatives (Hao *et al*., 2015; Petersen *et al*., 2015) and out of 50 genetic loci, 11 have been mapped on the A-genome, 26 on the B-genome and 13 on the D-genome (Elkot *et al*., 2015). Synthetic wheat hexaploids (2n=6x=42; AABBDD) have been developed and reportedly (Mujeeb-Kazi, 2003) possess multiple resistances against various biotic and abiotic stresses (Mujeeb-Kazi *et al.*, 2008, Bux *et al.*, 2012, Ogbonnaya *et al*., 2013). These genetic stocks are the product of interspecific hybrids between *Triticum turgidum* and *Aegilops tauschii* accessions that have resulted in over 1000 unique combinations from which various sub-sets have been structured. The SH wheats in general form a rich reservoir for evaluation of various stresses that limit wheat productivity (Mujeeb-Kazi *et al*., 2004). They are also considered as new allelic sources for powdery mildew resistance diversity. This study specifically focused on the evaluation and characterization of the Elite II sub-set of 32 SH entries and 60 bread wheat/synthetic (BW/SH) advanced derivatives involving commercial bread wheat cultivars.

The use of molecular markers linked to the traits of interest in breeding for disease and pest resistance genes can enhance selection accuracy during breeding. Simple sequence repeat (SSR) markers are highly polymorphic, chromosome arm specific and are being widely used for genetic diversity studies. The two-fold objectives of the present work were to evaluate powdery mildew via seedling and adult plant screening of Elite-II SH wheats and BW/SH advanced derivatives and detecting powdery mildew resistance genes using linked SSR markers.

Materials and Methods

Germplasm: 32 SHWs and 60 bread wheat/ synthetic (BW/SH) advance derivatives were used in this study.

Inoculum: Bulk inoculum from the initial mildew infections that occurred naturally in the Kaghan valley was collected. Inoculum was collected and increased as described by Duggal *et al*. (2000). The same bulk inoculum was used for screening at seedling and adult plant stages.

Evaluation of seedling resistance: The germplasm was evaluated for reaction to powdery mildew and planted in pots under controlled glass house conditions in Murree, Pakistan. A completely randomized design (CRD) with three replications was used. The experiment was inoculated 18 days after planting. After inoculation, seedlings were maintained under regimens of 16-19^{o}C and 12 hr/ day lighting. Disease severity was recorded using 0-9 scale as described by McNeal *et al*. (1971), whereas infection types were characterized as 0 = completely resistant (immune), 1-3 = resistant, 4-6 = intermediate and 7-9 = highly susceptible.

Evaluation of adult plant resistance (APR): For the evaluation of APR, the test material was field planted in Kaghan, Pakistan during the summer crop cycle (June to October) in 2 meter rows with row to row distance of 1 ft and exposed to inoculum present naturally in abundance. The whole experiment was grown in a separate plot bordered by a susceptible wheat and analyzed for powdery mildew diversity. The test material was scored for resistance/ susceptibility and the scoring followed the protocol of Duggal *et al.* (2000) according to a 0 to 9 scale as mentioned under *in vitro* screening.

Molecular diagnostics: Molecular diagnostics of *Pm* resistant genes involved in providing resistance either at adult, seedling or at both stages was applied on all the SH entries and the BW/SH advanced derivatives. SSR markers (Table 1) were employed for detecting the presence of four genes (*Pm4b*, *Pm9*, *Pm16* and *Pm30)* that contribute to powdery mildew resistance in wheat germplasm following standard procedures reported for each marker. DNA extraction was done using the protocol of Weining and Langridge (1991) with minor modifications.

Results

Evaluation of seedling resistance: Different infection types (ITs) were recorded in the SH entries and the BW/SH advanced derivatives at the seedling stage (Table 2).

a. Elite-II SH: The results showed significant variation in disease response of the SH wheats against the inoculated pathogen that ranged from complete resistant/ immune to highly susceptible (Tables 2 and 5). Among 32 SH wheats, 20 (62.5%) were resistant at seedling stage, of which, 2 SH (6.25%) provided complete resistance/ immune response while 18 BW/SH (56.25%) showed resistance. Nine (28.12%) showed intermediate reaction, and three (9.37%) were susceptible.

b. BW/SH advanced derivatives: Similar variable disease response was observed among BW/SH advanced derivatives that ranged from complete resistance/ immune to intermediate (Tables 2 and 6). Out of 60 BW/SH, 56 (93.3%) entries were resistant, 85% (51 entries) showed immune reaction and 8.33% (5) showed resistance. The remaining 4 entries of the 60 (6.66%) were found intermediate in their reaction.

Table 1. Microsatellite (SSR) markers employed for the detection of *Pm* genes in resistant synthetic based germplasm.

No.	Locus	Primer	Sequence	*Pm* gene	References
1.	2A	Xgwm-382-$_{125}$	F: GTCAGATAACGCCGTCCAAT R: CTACGTGCACCACCATTTTG	*Pm4b*	Yi *et al*. (2008)
2.	4A	Xgwm-4-$_{253}$	F: GCTGATGCATATAATGCTGT R:CACTGTCTGTATCACTCTGCT	*Pm9*	Srnic *et al*. (2005)
3.	7A	Xgwm-332-$_{212}$	F: AGCCAGCAAGTCACCAAAAC R:AGTGCTGGAAAGAGTAGTTTTG	*Pm9*	Srnic *et al*. (2005)
4.	5B	Xgwm-159-$_{201}$	F: GGGCCAACACTGGAACAC R: GCAGAAGCTTGTTGGTAGGC	*Pm16*, *Pm30*	Chen *et al*. (2005)

Table 2. Powdery mildew evaluation for seedling and adult plant resistance.

IT* range	Reaction	Number of lines tested at seedling stage		Number of lines tested at adult plant stage	
		Elite-II	Advanced derivatives	Elite-II	Advanced derivatives
0	Immune	2	51	-	6
1-3	Resistant	18	5	32	42
4-6	Intermediate	9	4	-	12
7-9	Susceptible	3	-	-	-

* IT = Infection types

Table 3. Lines of Elite-II synthetic hexaploids (SH) and bread wheat/SH advanced derivatives resistant to powdery mildew at both seedling and adult plant stages.

Immune/ Resistant reaction	Accession numbers	
	Elite-II	Advanced derivatives
0-3	2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 16, 17, 18, 19, 22, 26, 29, 30, 31, 32	1, 3, 4, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 52, 54

Table 4. Sources of adult plant resistance (IT <3 on a 0-9 scale) to powdery mildew identified in the synthetic wheat (SH) Elite-II set and bread wheat/SH advanced derivatives tested under field conditions.

Resistant reaction	Accession numbers	
	Elite-II	Advanced derivatives
0	-	-
1	1, 4, 13, 14, 15, 20, 21, 23, 24, 25, 27, 28	5, 28
2	-	6
3	-	-

Evaluation of adult plant resistance: The screening results showed different response of the entries in the field against the pathogen inoculum.

a. Elite-II SH: A significant difference was observed in disease response among the Elite-II SH when tested in the field compared to the seedling stage. All the 32 entries (100%) of Elite-II SH tested at adult stage were similar in their reaction to pathogen inoculum in the field and showed resistance categorized according to 1-3 scale range (Tables 2 and 5).

b. BW/SH Advanced derivatives: BW/SH entries showed similar range of disease response at adult stage in the field as noted at seedling stage i.e. complete resistance/ immune to intermediate (Tables 2 and 6). Based on screening results, the tested entries were categorized as immune, resistant and intermediate. 80% of the BW/SH were resistant, of which, 10% (6 entries) showed complete resistance/ immune response and 70% (42) showed resistance. The entries that showed intermediate response included 12 entries (20%).

Screening at both stages of the crop showed that most of the genotypes resistant at seedling stage were also resistant at the adult plant stage. Such as, 20 (62.5%) SH wheats and 45 (75%) of the BW/SH advanced derivatives were found to be significantly resistant with resistance expressed both at the seedling and adult plant stages (Table 3). The significant findings of the study revealed that out of the tested germplasm, 37.5% (12) of the SH entries and 5% (3) of 60 BW/SH advanced derivatives possessed only APR against powdery mildew (Table 4). These identified SH entries with APR were scored 1-3 (resistant) following disease rating scale at adult stage in the field while categorized intermediate (4-6) and susceptible (7-9) at seedling stage. Similarly, the BW/SH entries with APR were grouped under resistant category (1-3) at adult stage while intermediate (4-6) at seedling stage. These results suggested that these entries with APR are likely an important source for durable resistance in the field.

Molecular diagnostics of *Pm* resistant genes using linked SSR markers: In this study, SSR markers were used for the amplification of *Pm* resistance genes. Marker-assisted selection revealed the presence of four *Pm* genes i.e. *Pm4b, Pm9* (Fig. 1), *Pm16* and *Pm30* responsible for powdery mildew resistance in the SH entries and the BW/SH advanced derivatives. The resistant genotypes that showed the presence of *Pm* genes were scored as (+) and those with the absence of *Pm* genes were indicated as (-) (Tables 5 and 6).

Discussion

Genomic diversity is paramount for wheat yield sustainability and for this, stress durability is vital. Various biotic and abiotic stresses are production constraints and often genetic diversity limitations emerge that necessitate new alleles to be identified and exploited. Synthetic wheats are such novel resources that have emerged about two decades ago (Mujeeb-Kazi & Hettel, 1995) and have been in pre-breeding usage globally across multiple facets that embrace basic, strategic and applied research scenarios. They have multiple disease resistances (MDR) and are of prime value in wheat breeding (Ogbonnaya *et al*., 2008). Synthetic hexaploid wheats resulting from *Ae. tauschii* crosses with durum wheat cultivars, exhibit the genetic diversity for various biotic stresses (Mujeeb-Kazi *et al*., 2008). It is further attributed that *Ae. tauschii*, the diploid D genome progenitor of hexaploid bread wheat is an arsenal for providing diversity for numerous major/ minor biotic and abiotic stresses that limit wheat productivity globally and therefore currently occupies a very high priority in wheat breeding (Coghlan, 2006; Simonite, 2006). This view had earlier also been advocated by Mujeeb-Kazi (2003, 2005 and 2006), Mujeeb-Kazi *et al.* (1996) that unequivocally recognized *Ae. tauschii* as being a rich source of valuable genes for resistance to various wheat diseases including powdery mildew and pests. In this study, the high frequency of mildew resistance was observed in D-genome synthetic hexaploid wheats (SH's) and in D genome derived advanced derivatives (BW/SH) likely the presence of diverse resistance sources against powdery mildew in the D genome donor accessions coupled with complimentation of resistance sources that might be within the A and B genomes of the durum parents. Several mildew resistance genes from *Aegilops species* have been introduced into common wheat (Heun & Friebe, 1990).

The results of this study demonstrate that novel sources of powdery mildew resistance are available in D-genome synthetic sets and the BW/SH advanced derivatives. It has been recognized that lines resistant at seedling stage also provide a good level of field or APR (Ma *et al*., 1995). A similar result was found in the SH wheats (62.5%) and the BW/SH advanced derivatives (75%). All these SH and BW/SH lines have shown excellent resistance against powdery mildew at both stages. It is well recognized that most genes, which confer mildew resistance at the seedling stage, also confer good level of APR. This supports our observations for these new genetic stocks under study.

Table 5. *Pm* genes detection and screening disease response in resistant 32 Elite-II synthetic hexaploid wheats (SH).

No.	Pedigree	DR		*Pm* Gene				No.	Pedigree	DR		*Pm* Gene			
		S	A	*Pm 4b*	*Pm9*	*Pm 16*	*Pm 30*			S	A	*Pm 4b*	*Pm9*	*Pm 16*	*Pm 30*
1.	SORA/AE.SQUARROSA (192) *	I	R	-	-	-	-	17	CETA/AE.SQUARROSA (1031)	Im	R	-	-	-	-
2.	CROC_1/AE.SQUARROSA (210)	R	R	-	-	-	-	18	CETA/AE.SQUARROSA (1038)	R	R	-	-	-	-
3.	DVERD_2/AE.SQUARROSA (214)	R	R	-	-	-	-	19	CETA/AE.SQUARROSA (1046)	R	R	-	-	-	-
4.	ARLIN_1/AE.SQUARROSA (218)	I	R	-	-	-	-	20	CETA/AE.SQUARROSA (1053)	S	R	-	-	-	-
5.	GAN/AE.SQUARROSA (236)	R	R	-	-	-	-	21	CROC_1/AE.SQUARROSA (212)	S	R	-	-	-	-
6.	SORA/AE.SQUARROSA (323)	R	R	-	-	-	-	22	CETA/AE.SQUARROSA (368)	R	R	-	-	-	-
7.	D67.2/P66.270//AE.SQUARROSA (308)	R	R	-	-	-	-	23	ARLIN_1/AE.SQUARROSA (430)	I	R	-	-	-	-
8.	STY-US/CELTA//PALS/3/ SRN_5/4/AE.SQUARROSA (431)	R	R	-	-	-	-	24	D67.2/P66.270// AE.SQUARROSA (497)	I	R	-	-	-	-
9.	LCK59.61/AE.SQUARROSA (693)	R	R	-	-	-	-	25	D67.2/P66.270// AE. SQUARROSA (1015)	I	R	-	-	-	-
10.	SKARV_2/AE.SQUARROSA (304)	R	R	-	-	-	-	26	GAN/AE.SQUARROSA (206)	R	R	-	-	-	-
11.	CETA/AE.SQUARROSA (1025)	R	R	-	-	-	-	27	ARLIN_1/AE.SQUARROSA (335)	I	R	-	-	-	-
12.	DOY1/AE.SQUARROSA (1027)	R	R	-	-	-	-	28	GAN/AE.SQUARROSA (335)	I	R	-	-	-	-
13.	CETA/AE.SQUARROSA (386)	I	R	-	-	-	-	29	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (385)	R	R	-	-	-	-
14.	CETA/AE.SQUARROSA (392)	S	R	+	+	+	+	30	CETA/AE.SQUARROSA (417)	Im	R	-	-	-	-
15.	CETA/AE.SQUARROSA (533)	I	R	-	-	-	-	31	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (431)	R	R	-	-	-	-
16.	CPI/GEDIZ/3/GOO//JO/CRA/4/ AE.SQUARROSA (1018)	R	R	-	-	-	-	32	DOY1/ AE.SQUARROSA (534)	R	R	-	-	-	-

Number in parenthesis is the accession number of the *Ae. squarrosa* syn. *Ae. tauschii*

+, presence of the *Pm* gene; -, absence of the *Pm* gene.

DR, disease reaction; S, seedling stage; A, adult stage; Im, Immune; R, resistant; I, intermediate; S, susceptible.

Table 6. *Pm* genes detection and screening disease response in 60 bread wheat (BW)/ Synthetic wheat (SH) advanced derivatives.

No.	Pedigree	DR		*Pm* gene				No.	Pedigree	DR		*Pm* gene			
		S	A	*Pm 4b*	*Pm 9*	*Pm 16*	*Pm 30*			S	A	*Pm 4b*	*Pm 9*	*Pm 16*	*Pm 30*
1.	BCN//CETA/AE. SQUARROSA (895)*	R	R	-	+	-	-	31.	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE. SQUARROSA (498)/5/OPATA	Im	R	-	-	-	-
2.	ALTAR 84/ AE. SQUARROSA//OPATA (219)	I	I	-	+	-	-	32.	GAN/AE. SQUARROSA (897)//OPATA	Im	R	-	-	-	-
3.	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE. SQUARROSA (409) CIGM93.388	R	R	-	+	-	-	33.	-	Im	R	-	+	-	-
4.	ALTAR 84/ AE. SQUARROSA (224)	Im	R	-	+	-	-	34.	-	Im	R	-	+	-	-
5.	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE. SQUARROSA (809)	I	R	-	+	-	-	35.	-	Im	R	-	-	-	-
6.	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE. SQUARROSA (878)	I	R	-	-	-	-	36.	-	Im	R	-	-	-	-
7.	BOTNO	Im	R	-	-	-	-	37.	-	Im	R	-	-	-	-
8.	AJAIA_9	Im	R	-	+	-	-	38.	-	Im	R	-	-	-	-
9.	-	Im	R	-	+	-	-	39.	-	Im	Im	-	+	-	-
10.	CETA/AE.SQUARROSA (533)	R	R	-	-	-	-	40.	-	Im	Im	-	+	-	-
11.	CETA/AE.SQUARROSA (1038)	R	R	-	-	-	-	41.	-	Im	R	-	+	-	-
12.	YS/PASTOR	Im	R	+	+	-	-	42.	-	Im	R	-	-	-	-
13.	YS/PASTOR	Im	R	-	+	-	-	43.	-	Im	R	-	-	-	-
14.	YS/PASTOR	Im	R	-	-	-	-	44.	-	Im	Im	-	-	-	-
15.	YS/PASTOR	Im	R	-	-	-	-	45.	-	Im	R	-	+	+	+
16.	YS/PASTOR	Im	R	-	-	-	-	46.	-	Im	Im	-	-	-	-
17.	YS/PASTOR	Im	R	-	+	-	-	47.	-	Im	R	-	+	-	-
18.	YS/PASTOR	Im	R	-	-	-	-	48.	-	Im	R	-	+	-	-

Table 6. (Cont'd.).

No.	Pedigree	DR		*Pm* gene				No.	Pedigree	DR		*Pm* gene			
		S	A	*Pm 4b*	*Pm 9*	*Pm 16*	*Pm 30*			S	A	*Pm 4b*	*Pm 9*	*Pm 16*	*Pm 30*
19.	MAYOOR//TK SN1081/AE. SQUARROSA (222)/3/FCT	Im	I	-	-	-	-	49.	-	Im	I	+	+	-	-
20.	MAYOOR//TK SN1081/AE. SQUARROSA (222)/3/FCT	Im	R	-	+	-	-	50.	-	Im	I	-	-	-	-
21.	MAYOOR//TK SN1081/AE. SQUARROSA (222)/3/BCN	Im	R	-	+	+	+	51.	-	Im	I	-	-	-	-
22.	MAYOOR//TK SN1081/AE. SQUARROSA (222)/3/BCN	Im	Im	-	-	-	-	52.		Im	R	+	+	-	-
23.	TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE. SQUARROSA (205) /3/3*BUC/6/OPATA	Im	R	-	-	-	-	53.	-	Im	I	-	+	-	-
24.	TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE. SQUARROSA (205)/3/3*BUC/6/CNO	Im	R	-	-	-	-	54.	-	Im	R	-	+	-	-
25.	TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE. SQUARROSA (205)/3/3*BUC/6/BCN	Im	R	-	-	-	-	55.	-	Im	I	-	+	-	-
26.	TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE. SQUARROSA	Im	R	-	-	-	-	56.	-	Im	I	-	+	-	-
27.	SABUF/3/BCN//CETA/AE. SQUARROSA (895)/4/BCN	R	R	-	+	-	-	57.	-	Im	I	-	-	-	-
28.	SABUF/3/BCN//CETA/AE. SQUARROSA (895)/4/BCN	I	R	-	-	-	-	58.	-	Im	I	-	+	-	-
29.	SABUF/3/BCN//CETA/AE. SQUARROSA (895)/4/FCT	Im	Im	-	-	-	-	59.	-	Im	I	+	+	-	-
30.	SABUF/3/BCN//CETA/AE. SQUARROSA (895)/4/FCT	Im	R	-	-	-	-	60.	-	Im	I	+	+	-	-

Number in parenthesis is the accession number of the *Ae. squarrosa* syn. *Ae. tauschii*

+, presence of the *Pm* gene; -, absence of the *Pm* gene

DR, disease reaction; S, seedling stage; A, adult stage; Im, Immune; R, resistant; I, intermediate; S, susceptible

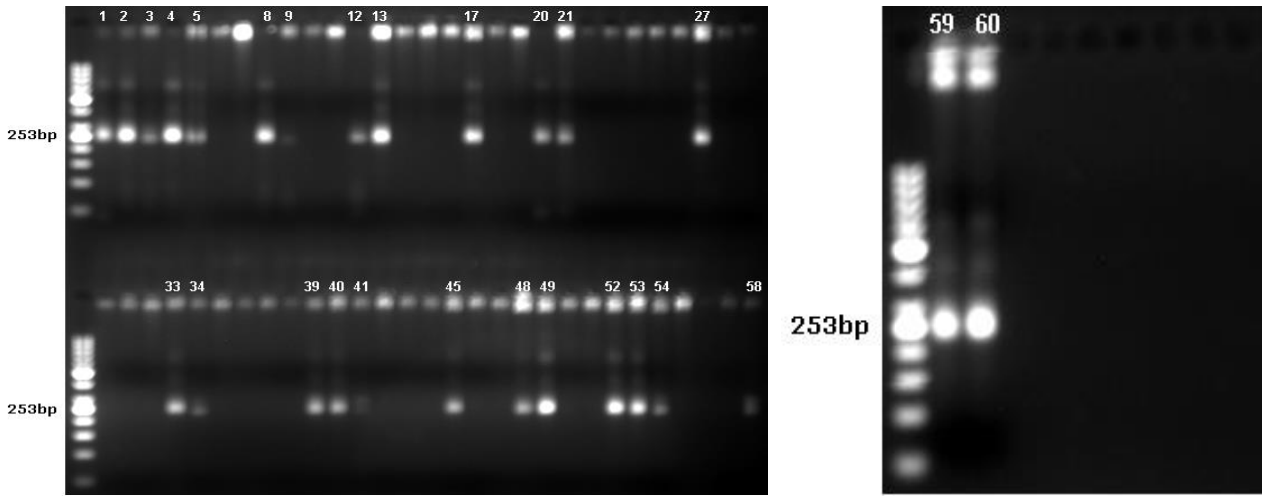

Fig. 1. SSR amplification of *Pm9* (253bp) in BW/SH advanced derivatives using primer Xgwm-4.

Synthetics with both seedling and APR are more attractive and preferred for utilization in wheat breeding programs. However, we must consider race specific and non-specific resistance that impinges upon resistance durability that is crucial for sustainable agriculture. Race-specific resistance against wheat mildew has been widely studied and utilized in breeding programs. It does have less affectivity for disease control as a result of the rapid adaptation of pathogen populations on cultivars possessing such type of disease resistance based upon major gene presence. Race-specific resistant cultivars and varieties offer temporary control of powdery mildew that lasts only two to five years (Brown *et al*., 1997). On the other hand, non-race specific resistance is often durable and remains effective for longer periods under disease conducive environments (Johnson, 1984). Previous studies have identified and demonstrated APR as durable against wheat powdery mildew. Similar observations were also found in this study where twelve entries viz. 1, 4, 13, 14, 15, 20, 21, 23, 24, 25, 27 and 28 of the Elite II SH and three (5, 6 and 28) BW/SH advanced derivatives possessed APR under field conditions. Therefore, the identification of APR to mildew in the SH's and the BW/SH advanced derivatives validates them as being new sources of resistance, which could be durable and highly desirable for agricultural practicality. These could be an essential source for utilization in wheat breeding against mildew.

Molecular markers are powerful tools to identify genes of interest and have been used to genetically and physically locate *Pm* genes in the wheat genome (Huang & Roder, 2004). In the present study, the SSR marker *Xgwm-382* amplified the PCR fragment in one SH wheat and in five BW/SH advanced derivatives with a size of 125bp in *Pm4b*. The results were in accordance with Yi *et al.* (2008) in which it was confirmed that the 125bp allele was the indicator for the presence of *Pm4b* gene located on the chromosome 2AL. The gene was derived from *T. carthlicum* (Alam *et al*., 2011). The entries detected with *Pm4b* gene showed varied reactions viz. complete resistance/ immune (3 BW/SH advanced derivatives) at seedling stage and resistance (one SH wheat and two BW/SH advanced derivative) at later growth stage (APR) in the study (Tables 5 and 6). Similarly, Hysing *et al*. (2007) observed complete resistance against powdery mildew and identified the presence of *Pm4b* alone and in combination with other genes in several land races and cultivars. Recently, according to Emara *et al*. (2016), complete efficacy of *Pm4b* gene in bread wheat cultivars against 42 powdery mildew isolates was observed in 2013 while intermediate efficacy was revealed in 2014. Two SSR markers were employed to detect the presence of *Pm9* resistance gene found in the hexaploid common wheat (Ahmadi & Moore, 2007) and located on the long arm of chromosome 7AL. Polymorphism between SH wheats and the BW/SH advanced derivatives were observed at the *Xgwm-4* and *Xgwm-332* SSR loci. A 253bp fragment was observed at the *Xgwm-4* locus in only one SH entry and 27 BW/SH advanced derivatives. A 212 bp fragment was observed at the *Xgwm-332* locus in only one SH and in 13 genotypes of BW/SH advanced derivative entries. Srnic *et al.* (2005) reported that *Pm9* was linked with the SSR locus *Xgwm-4* at 253bp and *Xgwm-332* at 212bp on chromosome 4AL and 7AL respectively. The SH entry displayed APR while BW/SH advanced derivatives showed varied (complete resistance/ immune to intermediate) responses at both stages. Hysing *et al*. (2007) observed that gene combinations *Pm1a+Pm2+Pm9* conferred resistance in bread wheat landraces and cultivars against 11 mildew isolates. Powdery mildew resistance genes *Pm16* and *Pm30* share common origin and chromosome location i.e. short arm of chromosome 5B linked to SSR locus Xgwm-159 at 201bp (Chen *et al.*, 2005). Both genes have been derived from *T. turgidum* var. *dicoccoides* (Alam *et al*., 2013). The results indicated that both genes (201bp) were present in one SH wheat with APR and two BW/SH advanced derivative genotypes possessing complete resistance/ immune response at seedling and resistance at adult stage. Likewise, Emara *et al*. (2016) reported complete (100%) resistance of *Pm16* gene against 42 mildew isolates at both seedling and adult plant stages of bread wheats. Earlier, *Pm30* gene has also been identified as a single dominant gene associated with mildew resistance at seedling stage of hexaploid wheat (Liu *et al*., 2002). The presence of the powdery mildew resistant genes with

tightly linked and flanking markers identified and reported here should aid in the incorporation of these powdery mildew resistance genes into future cultivars.

The assessment of the screening data and the presence of *Pm* genes permits the suggestion that the reported genes are essentially responsible for the resistance expressed either at seedling or adult stage or both in identified resistant entries. The observation on the involvement of *Pm* genes in APR suggested that among SHs, entry 14 showed the presence of all four *Pm* genes, of which, one or all the genes might be involved in APR. This suggested that all these are minor genes that showed non-race specific resistance and provided APR in the field. In the remaining entries, the absence of genes showed that some other major or minor *Pm* genes were involved in disease resistance. Similarly, among the BW/SHs, APR showed by entry 5 possessed *Pm9* gene as minor gene involved in durable resistance while the other two entries showed the absence of the identified genes. These *Pm* genes involved in APR identified in germplasm entries can be incorporated into high yielding varieties. Also, the pyramiding of all these reported genes resistant against different pathogen races into one variety would provide an alternative way of utilization of these resistance genes resources for breeding new resistant wheat lines and cultivars with more durable disease resistance.

Conclusion

Resistance identified in this study is associated with chromosomes 2AL, 4AL, 7AL and 5B. It opens up doors whereby greater emphasis must be given to target those genes that are located on D genome chromosomes and rigidly screen the bread wheats for their presence before entering them in a crossing program with synthetic wheats that has such a target gene identified. This corrective forward course will also open up doors for conducting precision direct crossing (Gill & Raupp, 1987) options. Integrated different alleles at the SH level will generate derivatives that would be more breeder user-friendly as the swift introgression of simultaneous novel sources would add greater efficiency to wheat breeding. This gene assembly holds future promise and hence is mentioned as a way forward that exploits the SH wheats in a unique manner.

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