ITEMS FROM PAKISTAN

NATIONAL AGRICULTURAL RESEARCH CENTER (NARC), ISLAMABAD WHEAT WIDE CROSSES AND CYTOGENETICS AND COLLABORATING NATIONAL PROGRAMS, ISLAMABAD, PAKISTAN.

Pakistan's wheat scenario: The way forward.

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Pakistan's current national wheat yields are 2.6 tons/ha and annual productivity has approached 24 x 10^6 tons. Increasing productivity in the coming years is necessary to keep pace with population increases and food needs. Our production trends have been erratic and significantly influenced by the environment as the production figures show. They were 23.2 x 10^6 tons in 2006–07, dropped to 20.959 in 2007–08, and have now jumped to near 24 x 10^6 tons in 2008–09. These production measures encompass a wide range of factors that integrate several disciplines within Pakistan and across our country boundaries. The way forward research strengthening emphasis stringently focus upon time-bound, multifaceted integrated activities where the prerequisite factors to determine such goals impinge upon using modern technologies, novel genetic diversity, exploiting molecular tools, and managing an integrated practical breeding program that utilizes top-quality national professionals with a similar blend from international alliances. Our production area from 8.29 x 10^6 ha recently surpassed 9.0 x 10^6, which is disconcerting. Thus, the need to adapt to cultivation area limits and address other production aspects requires a vision that recognizes change and addresses it through integrative technologies. Major attention is being given to the key production constraints covering biotic/abiotic stresses. For food security within Pakistan and the region, the foremost factor is the threat from stem rust that has emanated from the spread of the Ug99 pathogen.

Pakistan wheat research and production areas have a long history of sound progress that has been made through volatile international alliances mostly with centers such as CIMMYT and now ICARDA. Well-spaced, timely capacity building has been effective, and the knowledge tank has been kept in balance reasonably well. Changing times, particularly budgetary constraints within Pakistan and internationally, have imposed restrictions that when prolonged allowed negative aspects to surface. Such a downward trend has dominated the agricultural scene over the past decade and confounding this facet has been emerging environmental problems that pose significant production problems threatening regional coverage. Stem rust around Ug99, and its variants, is one such super pathogenic threat for which satisfactory genetic control measures are being sought. Pakistan is in the thick of this pathogen spread. Confounding the issue is the presence within Pakistan of a local virulent race that holds even greater strength to combine with Ug99 and create major epidemic situations. This is not an issue exclusive Pakistan but has regional ramifications requiring serious scientific interventions.

As a immediate measure, Pakistani scientists are combatting the stem rust problem utilizing the international Kenyan screening site, tapping on the international talent that oversees stem rust directly, or through the BGRI network and work with all regional colleagues to circumvent the imminent danger that confronts the country and the region. At present Ug99 is not present in Pakistan.

The Wheat Wide Cross Program at Islamabad encompasses interlinking areas of research and development that are categorized as basic, strategic, and applied, with all operating in tandem but varying in percentage distribution (Maximum share being given to applied aspects).

The overall program will give durable varietal outputs that can withstand the danger from pathogens that limit wheat yields, such as stem rust. Integration means that leaf and yellow rust also are included. Another major threat, Karnal bunt, also will be addressed. The broad vision of the breeders, aided by pathologists, helps in selecting material that has resistance to minor diseases such as powdery mildew, spot blotch, and barley yellow dwarf, which has aphid involvement. Key abiotic stresses such as drought, salinity, sodicity, and heat are integral to our efforts.

Molecular inputs will elucidate the genetic diversity component of the materials and allow monitored varietal and gene deployment options within the country. The generated data base will help the regional professionals.

The scope of our current wheat-improvement effort requires that all provincial partners are on board. Wheat programs are set up in Sindh at Karachi, Tandojam, and Sakrand for quality and stem rust; lower Punjab at RARI for spot blotch, leaf and stem rust; AARI in Faisalabad for all rusts; BARI at Chakwal for rainfed testing; Islamabad for prebreeding, molecular, Karnal bunt and genetic diversity; NWFP at CCRI for yellow rust and BYDV; Kaghan for stem rust and mildew; and Baluchistan for yellow rust and rainfed conditions. These provinces are rich in sites and talent that also can cover salient abiotic stresses that are crucial for giving a holistic wheat-improvement scenario. The entire above structure is in place. The information is being shared here for global awareness.

Some of the salient objectives of our program are:

- the handling of accquired materials that form the 'adaptive' research category,
- executing a recombination breeding program,
- using efficient tools to shorten the breeding cycle,
- using genetic diversity, including conventional and diverse sources,
- using molecular tools to better understand germ plasm and targeted breeding goals,
- disease screening for of all rusts, mildew, Karnal bunt, spot blotch; *in vivo* and *in vitro*
- exposure to field practices and simple data analytical techniques,
- exposure to some upstream and basic techniques, and
- hands-on training of students, provincial support staff, local support personnel, and internationally supported capacity building to cover regional professionals as well.

The winter crop cycle of 2009–10 marks the conclusion of a four-year effort of the wide crosses program that has addressed three major areas of wheat research and development in Pakistan. This time perios has seen the emergence of a modest but quality infrastructure that covers laboratory research, controlled environment investigations, and a field set-up that assists both basic and applied output. We were able to structure a multidisciplinary research team of young professionals that are involved in knowledge generation aspects that embrace wide crossing, cytology, cytogenetics, biochemical genetics, diversity analyses, marker application, biotic stresses, abiotic stresses, prebreeding/breeding, and, through *in vitro* testing, other related areas of interest that relate to wheat productivity.

The focus of our investigations is high on recombination breeding using the novel diversity of the various gene pools that are underutilized globally and scarcely used within Pakistan, thus, no duplication occurs. Here we report on powdery mildew resistance, quality, general biotic stresses, abiotic stresses, and winter synthetics. The program has benefitted from exceptional support from international peers through their sharing of valuable cytogenetic stocks in which germ plasm provided by Dr. B.S. Gill of Kansas State University occupies a significant place.

Evaluation of wheat A- and B-genome-based amphiploids for powdery mildew resistance: morpho-molecular characterization, diversity, and utilization potential for wheat improvement.

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Hexaploid amphiploids (AAAABB) along with their durum parents (AABB) and AABBBB (or SS) were evaluated for their response to *Erysiphe graminis* f. sp. *tritici* at the seedling stage in the greenhouse at Murree, Pakistan, to identify and characterize resistance. Results indicated that 89 (56%) A-genome synthetic hexaploids (SH, AAAABB) along with their nine durum parents (39%) and nine (60%) B-genome synthetic hexaploids (AABBBB) showed seedling resistance against powdery mildew, indicating a valuable source of major resistance genes. The resistant accessions also were subjected to morphological characterization and molecular diversity analysis. Data on morphological traits showed substantial variation and demonstrated that these synthetic hexaploid wheat germ plasm are an important source of genes for desirable traits and can be utilized in wheat breeding programs. Among the A-genome synthetics, 37 genotypes, one durum parent, and among B-genome synthetics, five genotypes were found to be the best morphologically. To evaluate genetic diversity of the resistant genotypes, 12 SSR markers were used for 89 A-genome synthetics and their nine durum parents and 30 SSR markers for nine B-genome synthetics, with scorable bands (Table 1, p. 133). The average polymorphic loci per primer was 4.25 among the A-genome synthetics and their durum parents and 4.3 among the B-genome synthetics. The average similarity matrix was 0.265 (26.5%) in A-genome-based synthetic hexaploids and their durum

parents and 0.433 (43.3%) in B-genome-based synthetic hexaploids. This study suggested that powdery mildew resistance and morpho-molecularly diverse hexaploid amphiploids are important genetic stocks for future utilization in wheat-improvement programs.

Seedling resistance evaluation. Powdery mildew development was good in greenhouse evaluations and readily identifiable variations in disease reactions between resistant and susceptible seedlings were observed (Table 2). The frequency of genotypes among A-genome SHs and their durum parents and among B-genome SHs showing different infection types is presented (Fig 1). Results indicated that 89 accessions (56%) of A-genome-based synthetic hexaploids (AAAABB) or amphiploids along with their nine durum parents (39%) and nine accessions (60%) of B-genome-based synthetic hexaploids (AABBBB) or amphiploids showed seedling resistance against powdery mildew. Among the A-genome SHs, 15 were completely resistant (immune) to powdery mildew; 74 were resistant, 49 were intermediate, and 18 susceptible. Nine durum cultivars were resistant, eight intermediate, and six susceptible. In the B-genome-based SHs, five accessions were completely resistant (immune), four resistant, two intermediate, and four susceptible. The A-genome-based synthetics, along with their durum wheat parents, and the B-genome-based synthetics that gave resistant reactions (IT < 0–3) are listed in Table 3 (p. 134).

Table 1. A list of SSR primers used for the genetic analysis of A- and B-genome-based synthetic hexaploids and their durum parents.

Locus	Primer	Locus	Primer	Locus	Primer
Primers for A-genome-based synthetic hexaploids					
2A	*Xgwm-311*	3A	*Xgwm-666.2*	4A	*Xgwm-397*
4A	*Xgwm-601*	2A	*Xgwm-372*	2A	*Xgwm-473*
2A	*Xgwm-312*	2A	*Xgwm-382*	2A	*Xgwm-515*
4A	*Xgwm-637*	3A	*Xgwm-391*	2A	*Xgwm-558*
Primers for B-genome-based synthetic hexaploids					
5B	*Xgwm-66*	4B	*Xgwm-66*	6B	*Xgwm-88*
5B	*Xgwm-408*	3B	*Xgwm-112*	4B	*Xgwm-113*
6B	*Xgwm-219*	4B	*Xgwm-165*	4B	*Xgwm-149*
5B	*Xgwm-335*	5B	*Xgwm-213*	2B	*Xgwm-191*
2B	*Xgwm-382*	1B	*Xgwm-124*	2B	*Xgwm-210*
2B	*Xgwm-374*	2B	*Xgwm-16*	5B	*Xgwm-234*
5B	*Xgwm-159*	1B	*Xgwm-18*	3B	*Xgwm-340*
6B	*Xgwm-193*	2B	*Xgwm-55.1*	6B	*Xgwm-361*
2B	*Xgwm-257*	5B	*Xgwm-68*	4B	*Xgwm-368*
4B	*Xgwm-6*	3B	*Xgwm-72*	5B	*Xgwm-371*

Table 2. Evaluation of A-genome synthetics, their durum wheat parents, and B-genome synthetics for seedling resistance to powdery mildew.

Seedling infection type range	Reaction	Number of lines tested		
		A-genome synthetics (AAAABB)	Durum (AABB)	B-genome synthetics (AABBBB)
0–3	Resistant	89	9	9
4–6	Intermediate	49	8	2
7–9	Susceptible	18	6	4

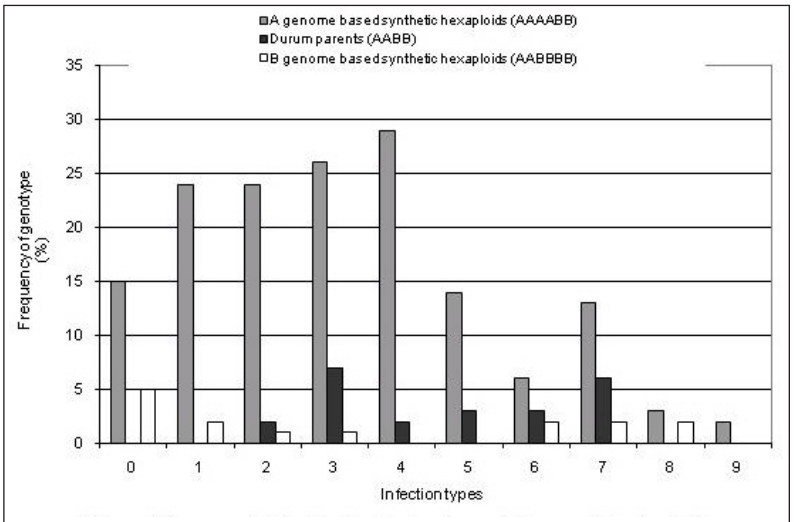

Fig. 1. Frequency distribution of the infection types of powdery mildew resistance of the A-genome-based synthetic hexaploids (AAABB), their durum wheat parents, and the B-genome-based synthetic hexaploids

Phenotypic evaluation. Data on morphological traits of resistant SH wheats, including A-genome-based SHs along with their durum parents and B-genome-based SHs, are given in Table 4 (p. 134-137). On the basis of 1,000-kernel weight and other phenotypic characters, the A-genome-based SH genotypes that were found morphologically good and diverse were 1, 3, 5, 7, 11, 12, 13, 14, 17, 18, 21, 24, 25, 31, 34, 36, 37, 40, 43, 50, 51, 52, 53, 54, 57, 58, 60, 61, 62, 66, 68, 71, 72, 86, and 89, and among durums the genotype D4. Among the B-genome-based SH, genotypes 1, 2, 3, 4, 6, and 7 were found best for the morphological traits.

Table 3. Sources of seedling resistance (IT < 3 on a 0–9 scale) to powdery mildew identified in A-genome-based synthetic hexaploids along with their durum cultivar parents and in B-genome-based synthetic hexaploids

Resistance reaction	Accession numbers		
	AAAABB	AABB	AABBBB
0	27, 30, 31, 39, 40, 49, 51, 52, 53, 54, 56, 59, 69, 86, 89	—	1, 2, 3, 5, 9
1	4, 13, 17, 19, 24, 28, 32, 33, 34, 38, 42, 43, 44, 50, 55, 57, 61, 66, 67, 68, 74, 77, 78, 85	—	4, 7
2	1, 3, 9, 11, 15, 18, 21, 22, 23, 25, 26, 41, 46, 47, 60, 63, 64, 70, 71, 73, 79, 80, 83, 87	D5, D9	6
3	2, 5, 6, 7, 8, 10, 12, 14, 16, 20, 29, 35, 36, 37, 45, 48, 58, 62, 65, 72, 75, 76, 81, 82, 84, 88	D1, D2, D3, D4, D6, D7, D8	8

Table 4. Phenological characterization of 89 powdery mildew resistant, A–genome–based synthetic hexaploids (AAAABB), their nine durum wheat parents (AABB), and nine B–genome–based synthetic hexaploids (AABBBB). * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. GH = growth habit, prostrate (+), erect (–), or medium (M); FLOW = days–to–flowering; PUB = pubescence, absence (–) or prescence (+); HT = plant height at maturity; AWN = awn color, LB = light brown, DB = dark brown, B = black, AW = amery white, and Y = yellow; PMA = days–to–physiological maturity; TKW = 1,000–kernel weight; G/S = number of grains/spike; and SL = spike length (cm).

No.	Parentage/Pedigree	GH	FLOW	PUB	HT	AWN	PMA	TKW	G/S	SL
AAAABB (A-genome-based synthetic hexaploids)										
1	YUK/T.BOEOTICUM (1)*	–	125	+	104	LB	175	46.0	18	13.5
2	STY–US/CELTA//PALS/3/SRN_5/4/ T.BOEOTICUM (54)	–	127	+	133	LB	176	43.8	6	8.0
3	SCA/T.BOEOTICUM (10)	–	116	+	105	LB	180	60.0	15	13.2
4	GARZA/BOY//T.BOEOTICUM (10)	–	134	+	117	LB	174	40.0	30	12.0
5	GARZA/BOY//T.BOEOTICUM (12)	–	133	–	129	LB	184	60.0	40	10.3
6	ALG86/4/FGO/PALES//MEXI_1/3/ RUFF/FGO/5/ENTE/6/ T.BOEOTICUM (14)	–	135	+	124	LB	181	38.0	7	11.1
7	ALG86/4/FGO/PALES//MEXI_1/3/ RUFF/FGO/5/ENTE/6/ T.BOEOTICUM (15)	–	135	+	127	LB	184	48.0	33	14.1
8	ALG86/4/FGO/PALES//MEXI_1/3/ RUFF/FGO/5/ENTE/6/T.BOEOTICUM (74)	–	135	+	115	LB	193	37.8	13	11.1
9	ALG86/4/FGO/PALES//MEXI_1/3/ RUFF/FGO/5/ENTE/6/T.BOEOTICUM (83)	–	140	–	124	LB	180	40.0	33	9.0
10	BOTNO/T.BOEOTICUM (20)	–	154	+	104	LB	186	32.0	7	12.1
11	DOY1/T.URARTU (543)	–	125	+	120	LB	150	46.0	43	13.1
12	DOY1/T.URARTU (560)	–	127	+	145	LB	175	63.0	11	15.0
13	DOY1/T.URARTU (563)	–	119	+	75	LB	171	49.6	20	14.0
14	DOY1/T.URARTU (552)	–	127	+	100	DB	189	45.3	6	8.0
15	DOY1/T.URARTU (559)	–	131	+	131	LB	173	35.8	20	6.0
16	SHAG_22/T.BOEOTICUM (24)	–	133	+	139	DB	180	41.4	7	12.0
17	SHAG_22/T.BOEOTICUM (56)	–	145	+	121	LB	190	60.0	4	13.5
18	SHAG_22/T.BOEOTICUM (68)	–	116	+	106	LB	178	47.5	4	10.0
19	SHAG_22/T.BOEOTICUM (88)	–	131	+	119	DB	187	32.2	21	11.0
20	SCOOP_1/T.BOEOTICUM (40)	–	135	+	104	LB	186	42.6	12	10.1

Table 4 (continued). Phenological characterization of 89 powdery mildew resistant, A–genome–based synthetic hexaploids (AAAABB), their nine durum wheat parents (AABB), and nine B–genome–based synthetic hexaploids (AABBBB). * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. GH = growth habit, prostrate (+), erect (–), or medium (M); FLOW = days–to–flowering; PUB = pubescence, absence (–) or prescence (+); HT = plant height at maturity; AWN = awn color, LB = light brown, DB = dark brown, B = black, AW = amery white, and Y = yellow; PMA = days–to–physiological maturity; TKW = 1,000–kernel weight; G/S = number of grains/spike; and SL = spike length (cm).

No.	Parentage/Pedigree	GH	FLOW	PUB	HT	AWN	PMA	TKW	G/S	SL
21	SCOOP_1/T.BOEOTICUM (46)	–	133	+	101	DB	186	48.2	22	10.1
22	SCOOP_1/T.BOEOTICUM (50)	–	147	+	129	LB	190	27.2	29	11.5
23	SCOOP_1/T.BOEOTICUM (59)	–	149	+	104	LB	190	40.6	24	11.0
24	SCOOP_1/T.BOEOTICUM (60)	–	118	+	102	LB	176	54.2	16	10.0
25	SCOOP_1/T.BOEOTICUM (69)	–	125	–	94	LB	175	45.2	24	9.5
26	SCOOP_1/T.BOEOTICUM (79)	–	126	+	110	Y	182	40.0	33	13.5
27	SCOOP_1/T.BOEOTICUM (87)	–	125	+	123	LB	181	44.4	21	10.0
28	SCOOP_1/T.BOEOTICUM (89)	–	146	+	130	DB	193	37.0	16	11.1
29	D67.2/P66.270//T.BOEOTICUM (35)	–	124	+	130	LB	175	13.7	7	18.0
30	D67.2/P66.270//T.MONOCOCCUM (108)	–	127	–	85	LB	182	24.4	18	10.0
31	D67.2/P66.270//T.URARTU (550)	–	122	+	120	LB	173	45.2	7	13.0
32	D67.2/P66.270//T.URARTU (553)	–	135	+	98	LB	177	25.0	4	7.0
33	AJAIA/T.BOEOTICUM (55)	–	135	+	125	LB	181	42.6	9	11.1
34	AJAIA/T.BOEOTICUM (56)	–	127	+	133	LB	179	56.0	18	15.0
35	68.111/RGB–U//WARD/3/ T.MONOCOCCUM (112)	–	121	–	108	LB	158	24.0	6	12.8
36	68.111/RGB–U//WARD/3/ T.URARTU (554)	–	133	+	108	LB	185	46.0	26	14.6
37	AOS/T.MONOCOCCUM (98)	–	127	–	105	LB	150	55.6	21	11.6
38	AOS/T.MONOCOCCUM (111)	–	127	+	103	LB	151	41.6	16	11.1
39	GAN/T.BOEOTICUM (29)	–	120	+	125	LB	175	37.0	35	10.0
40	DEVERD_2/T.BOEOTICUM (37)	–	122	+	125	LB	173	50.0	11	12.0
41	DEVERD_2/T.BOEOTICUM (43)	–	128	+	115	LB	178	38.0	15	11.0
42	DEVERD_2/T.BOEOTICUM (44)	–	131	+	109	LB	172	31.6	5	9.0
43	DEVERD_2/T.BOEOTICUM (45)	–	134	+	119	LB	175	51.8	25	10.0
44	YAV_2/TEZ//T.BOEOTICUM (25)	–	133	+	123	LB	176	31.0	4	13.0
45	YAV_2/TEZ//T.BOEOTICUM (37)	–	125	–	127	LB	178	41.2	15	13.0
46	YAV_2/TEZ//T.BOEOTICUM (43)	–	128	+	104	LB	183	31.0	6	12.0
47	YAV_2/TEZ//T.BOEOTICUM (45)	–	134	+	118	LB	176	40.0	17	11.0
48	YAV_2/TEZ//T.BOEOTICUM (47)	–	139	+	120	LB	180	40.0	13	13.0
49	YAV_2/TEZ//T.BOEOTICUM (62)	–	124	+	118	LB	179	43.4	6	13.0
50	YAV_2/TEZ//T.BOEOTICUM (64)	–	133	+	108	LB	180	65.6	13	7.0
51	YAV_2/TEZ//T.BOEOTICUM (65)	–	134	+	116	LB	183	56.6	38	12.0
52	YAV_2/TEZ//T.BOEOTICUM (67)	–	133	+	89	LB	181	61.6	14	9.8
53	YAV_2/TEZ//T.BOEOTICUM (83)	–	125	+	105	LB	185	48.0	15	13.0
54	YAV_2/TEZ//T.MONOCOCCUM (113)	–	126	–	117	LB	189	48.0	27	12.6
55	YAV_2/TEZ//T.MONOTICUM (121)	–	143	+	124	LB	191	32.4	8	11.0
56	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM (38)	–	136	+	112	LB	178	40.0	7	8.0
57	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM (41)	–	141	–	110	LB	175	46.0	17	11.0
58	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM (48)	–	134	+	123	LB	184	71.0	6	10.0

Table 4 (continued). Phenological characterization of 89 powdery mildew resistant, A–genome–based synthetic hexaploids (AAAABB), their nine durum wheat parents (AABB), and nine B–genome–based synthetic hexaploids (AABBBB). * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. GH = growth habit, prostrate (+), erect (–), or medium (M); FLOW = days–to–flowering; PUB = pubescence, absence (–) or prescence (+); HT = plant height at maturity; AWN = awn color, LB = light brown, DB = dark brown, B = black, AW = amery white, and Y = yellow; PMA = days–to–physiological maturity; TKW = 1,000–kernel weight; G/S = number of grains/spike; and SL = spike length (cm).

No.	Parentage/Pedigree	GH	FLOW	PUB	HT	AWN	PMA	TKW	G/S	SL
59	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM (76)	–	145	+	81	LB	179	30.8	21	9.0
60	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM (78)	–	145	+	128	LB	186	44.6	21	11.0
61	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM (81)	–	120	+	123	DB	178	46.2	14	10.0
62	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM (4)	–	147	+	113	DB	186	45.2	57	14.0
63	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM (9)	–	143	–	110	LB	176	35.6	5	10.0
64	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM (51)	–	125	+	133	LB	186	42.0	41	12.0
65	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM (63)	–	140	+	123	LB	185	40.0	3	17.0
66	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM (72)	–	140	+	120	LB	179	58.0	16	11.0
67	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM (85)	–	140	+	143	LB	187	41.6	20	13.0
68	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM (53)	–	125	+	108	LB	177	49.6	7	7.0
69	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.MONOCOCCUM (107)	–	137	–	107	LB	182	33.6	12	11.0
70	CPI/GEDIZ/3/GOO//JO//CRA/4/ T. URARTU (548)	–	120	–	86	LB	182	35.6	27	13.0
71	CROC_1/T.URARTU (548)	–	134	+	95	LB	186	59.8	32	14.0
72	ALTAR 84/T.URARTU (558)	–	132	+	100	LB	183	46.2	19	10.0
73	CETA/T.URARTU (558)	–	127	–	120	LB	176	36.6	9	7.0
74	CETA/T.URARTU (562)	–	122	–	119	LB	183	41.8	24	8.0
75	CETA/T.BOEOTICUM((42)	–	140	+	138	LB	185	35.6	9	10.0
76	ARLIN_1/T.BOEOTICUM((32)	–	140	+	137	LB	185	31.6	5	13.0
77	ARLIN_1/T.BOEOTICUM (84)	–	140	+	122	LB	181	27.0	18	12.0
77	ARLIN_1/T.BOEOTICUM (84)	–	140	+	122	LB	181	27.0	18	12.0
78	ARLIN_1/T.BOEOTICUM (86)	–	139	+	135	LB	185	37.6	30	10.1
79	ARLIN_1/T.BOEOTICUM (103)	–	133	–	135	LB	187	30.8	14	10.0
80	ARLIN_1/T.BOEOTICUM (105)	–	141	–	155	Y	188	24.0	13	11.0
81	ARLIN_1/ T.BOEOTICUM (117)	–	128	–	137	LB	185	40.8	29	14.0
82	ARLIN_1/ T.BOEOTICUM (120)	–	127	–	137	LB	187	35.0	24	12.0
83	ARLIN_1/T.MONOCOCCUM (95)	–	141	+	118	LB	183	28.5	4	12.6
84	ARLIN_1/T.MONOCOCCUM (97)	–	140	+	131	LB	180	25.2	34	8.0
85	ARLIN_1/T.MONOCOCCUM (107)	–	129	+	144	LB	187	20.7	25	10.0
86	ARLIN_1/T.MONOCOCCUM (108)	–	136	+	95	LB	180	48.9	12	14.0
87	ARLIN_1/T.MONOCOCCUM (110)	–	133	–	116	LB	186	21.4	52	12.8
88	ARLIN_1T.URARTU (547)	–	141	+	117	LB	187	22.0	29	7.0
89	ARLIN_1/T.URARTU (548)	–	129	+	115	LB	177	44.8	22	8.0

Table 4 (continued). Phenological characterization of 89 powdery mildew resistant, A–genome–based synthetic hexaploids (AAAABB), their nine durum wheat parents (AABB), and nine B–genome–based synthetic hexaploids (AABBBB). * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. GH = growth habit, prostrate (+), erect (–), or medium (M); FLOW = days–to–flowering; PUB = pubescence, absence (–) or prescence (+); HT = plant height at maturity; AWN = awn color, LB = light brown, DB = dark brown, B = black, AW = amery white, and Y = yellow; PMA = days–to–physiological maturity; TKW = 1,000–kernel weight; G/S = number of grains/spike; and SL = spike length (cm).

No.	Parentage/Pedigree	GH	FLOW	PUB	HT	AWN	PMA	TKW	G/S	SL
Durun wheat parent lines										
D1	ALG86/4/FGO/PALES//MEXI_1/3/ RUFF/FGO/5/ENTE	–	117	–	128	Y	181	32.0	27	13.0
D2	DECOY 1	–	126	–	103	Y	183	28.0	18	8.0
D3	SCOOP_1	–	123	+	124	B	181	37.0	28	9.0
D4	D67.2/P66.270	–	121	+	126	B	180	47.0	34	6.0
D5	LCK59.61	–	122	–	121	Y	184	36.0	15	6.6
D6	FGO/USA2111	–	116	–	128	LB	179	43.0	32	15.0
D7	GAN	–	122	+	144	Y	180	37.0	16	10.0
D8	CPI/GEDIZ/3/GOO//JO/CRA	–	114	+	138	Y	181	40.0	30	7.0
D9	CERCETA	–	115	–	135	B	177	41.0	23	8.0
AABBBB (B-genome-based synthetic hexaploids)										
1	CETA/AE.SPELTOIDES (127)	–	113	–	72	Y	161	52.0	22	10.0
2	CPI/GEDIZ/3/GOO//JO/CRA/4/ AE.SPELTOIDES (133)	–	113	–	75	Y	160	60.3	56	14.0
3	ARLIN_1/AE.SPELTOIDES (134)	–	133	+	90	AW	164	62.7	28	15.0
4	CPI/GEDIZ/3/GOO//JO/CRA/4/ AE.SPELTOIDES (135)	–	112	–	85	AW	163	44.8	60	12.0
5	CETA/AE.SPELTOIDES (135)	–	129	–	98	Y	164	18.0	5	13.0
6	CETA/AE.SPELTOIDES (139)	–	133	+	93	Y	162	44.6	24	14.5
7	ALTAR 84/AE.SPELTOIDES (141)	–	133	+	66	LB	166	60.0	30	13.0
8	ARLIN_1/ AE.SPELTOIDES (130)	–	145	+	74	AW	166	13.2	14	11.0
9	ARLIN_1/ AE.SPELTOIDES (157)	–	118	–	63	AW	161	28.6	46	8.0

Molecular evaluation. ***Genetic diversity estimation and cluster analysis of powdery mildew resistant A- genome based synthetic hexaploids and their durum parents***. Twelve SSR markers produced 51 polymorphic bands in sizes ranging from 50 bp to 400 bp. The average polymorphic loci/primer was 4.25. Primer *Xgwm-558-2A* generated the highest number of polymorphic bands.

The SSR amplification data was used to obtain a similarity matrix and generate a dendrogram. The similarity matrix was calculated using Nei and Li's coefficient analysis and showed genetic distance between individual pairs of all the genotypes. Using the resistant A-genome-based SH accessions along with their resistant durum wheat parents, the genetic diversity ranged from 0 to 100, where 0 represents the minimum genetic distance and 100 represents the maximum genetic distance among the genotypes, thus revealing high variability among accessions. Similarly, the value of the similarity coefficient, based on 12 SSR markers, ranged from 1 (100 percent) to 0. The accessions could be divided into main clusters A and B using UPGMA analysis based on genetic distances (Fig. 2, p. 138).

Cluster A consisted of 38 genotypes (87, 83, 78, D9, D8, 70, 69, 67, 66, 62, 61, 60, 58, 55, 52, 51, 50, 48, 47, 45, 44, 43, D7, 39, 37, 36, 26, 21, 20, 16, 15, 14, 11, 10, 9, 8, 7, and 6). All these genotypes showed the maximum genetic distance of 1 (100%). Cluster B consisted of the remaining 60 genotypes and was subdivided into three groups (1B, 2B, and 3B). Group 1B consisted of six genotypes (13, 79, 59, 68, 42, and 41). Two genotypes (41 and 42) in this group were exactly similar. The most diverse genotype with the highest genetic distance or least similarity in this group was 13, with an average genetic distance of 0.99 (99%) with a group of 59 genotypes. Group 2B consisted of 13 genotypes (89, 30, 74, 73, 53, 77, 72, 3, 57, 46, 71, 28, and 27). The most diverse genotype in this group was 77, with an average genetic distance of 0.633 (63.3%) with a group of two other genotypes. In this group, many genotypes also

showed a minimum genetic distance or 100 % similarity and one grouping included lines 71, 28, and 27. Group 3B was a large group and consisted of the remaining 41 genotypes (23, 40, D6, 88, 76, 56, 75, 12, 65, 64, 63, 54, 80, 49, 38, 33, 84, 85, 82, 86, 81, 29, D4, 32, D3, 22, 25, 24, 35, 34, 31, D1, 18, 17, 19, D2, 4, 2, D5, 5, and 1). Genotype 2 in this group was the most diverse, with an average genetic distance of 0.811 (81.1%) with a group of 16 other genotypes. The dendrogram generated from the SSR data indicated that the most genetically diverse A-genome-based SH wheat genotypes and durum parents were in Cluster A.

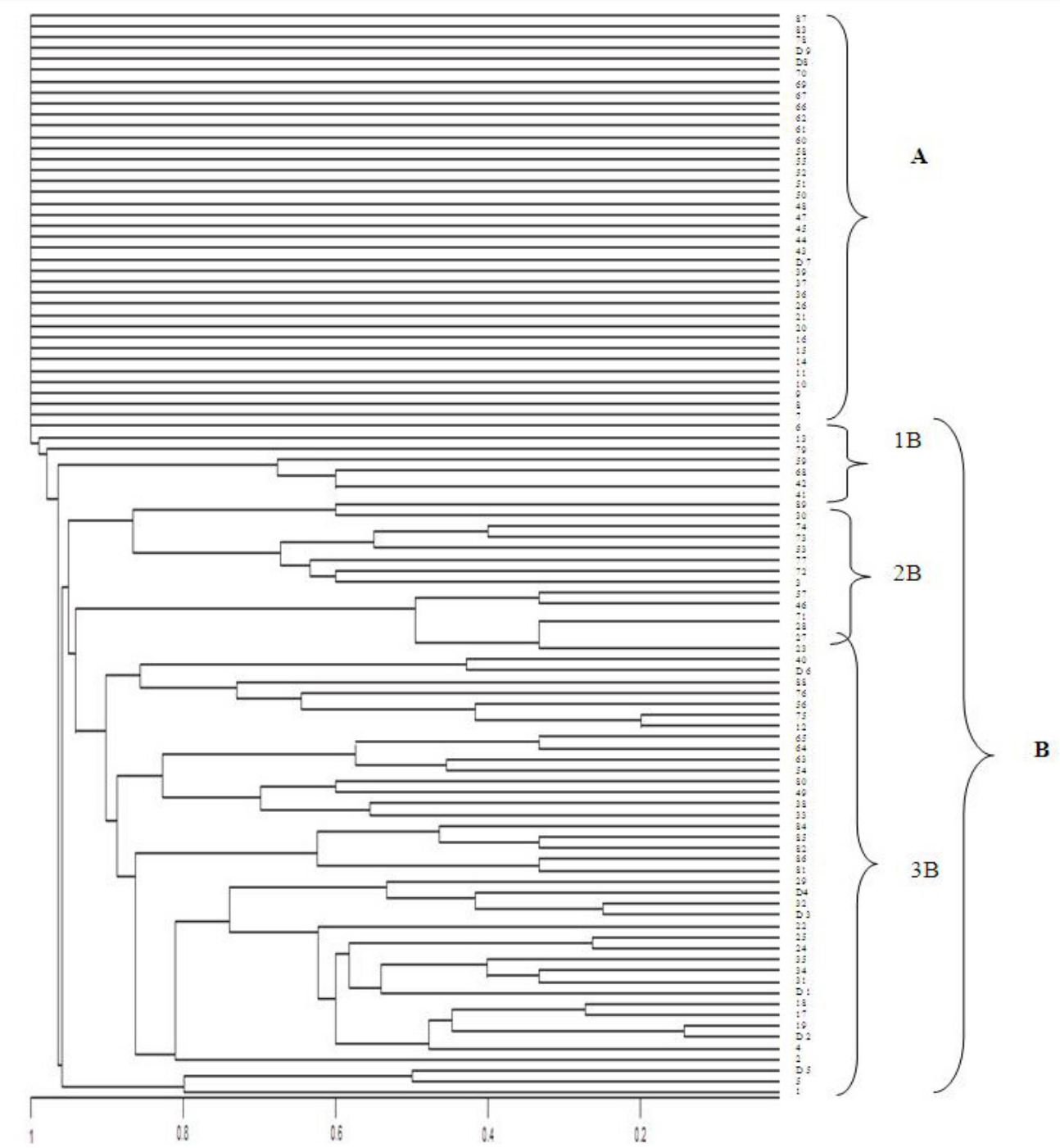

Fig. 2. Grouping of the resistant A-genome-based synthetic hexaploids (2n=6x=42; AAAABB) and their durum wheat parents based on genetic distance

Genetic diversity estimation and cluster analysis of powdery mildew resistant B-genome-based synthetic hexaploids. Thirty SSR markers produced 132 polymorphic bands in size ranging from 50 bp to 1000 bp. The average polymorphic loci/primer were 4.3. Primer *Xgwm-213-5B* generated highest number of polymorphic bands.

The SSR amplification data was used to obtain a similarity matrix and generate a dendrogram. The similarity matrix was calculated using Nei and Li's coefficient analysis and showed genetic distance between individual pairs of all genotypes. Using the resistant B-genome-based SH accessions, the genetic diversity ranged from 0.296 (29.6%) to 0.806 (80.6%), in which 0.296 represents minimum genetic distance and 0.806 represents maximum genetic distance among the genotypes, also revealing considerable variability among the accessions. The value of similarity coefficient based on 30 SSR markers ranged from 0.704 (70.4%) to 0.194 (19.4%). The accessions could be divided into two main clusters A and B using UPGMA analysis based on genetic distances (Fig. 3).

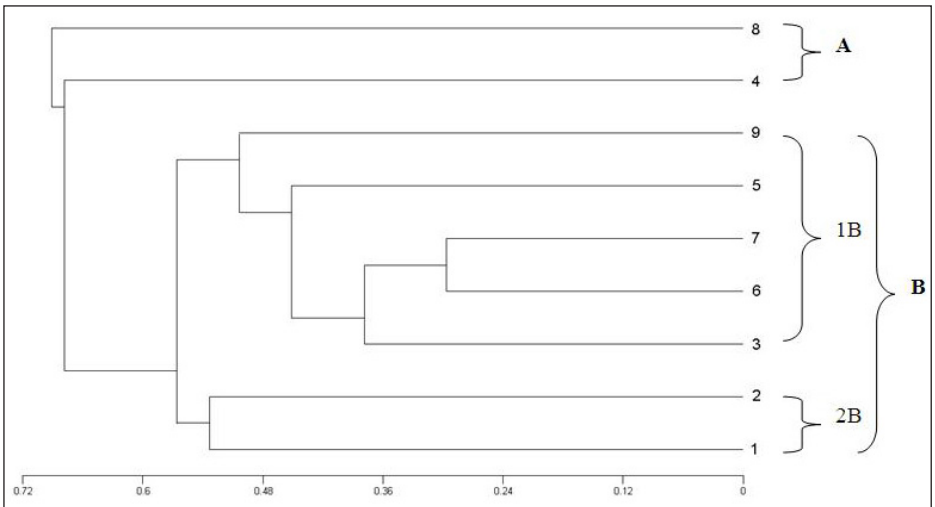

Fig. 3. Grouping of the resistant B-genome-based synthetic hexaploids on the basis of genetic distance.

Cluster A consisted of two genotypes, 8 and 4. These genotypes were found to be highly diverse from all the other genotypes. Compared to all the other gneotypes, the average genetic distance of genotype 8 was 0.691 (69.1%). Similarly, the average genetic distance of genotype 4 with all other genotypes except genotype 8 was 0.679 (67.9%). Cluster B was further subdivided into two groups, 1B and 2B. Group 1B consisted of five genotypes (9, 5, 7, 6 and 3). Among this group, genotype 9 had a maximum genetic distance of 0.503 (50.3%). Group 2B consisted of genotypes 1 and 2, which also were more diverse than the other genotypes in subcluster 1B with an average genetic distance of 0.533 (53.3%). The dendrogram generated from the SSR data indicated that the greatest diversity was observed in genotypes 8 and 4 among B-genome-based SH wheats.

Results. Valuable sources of powdery mildew resistance, good agronomic traits, and novel genetic diversity are available in AAAABB and AABBBB hexaploid amphiploids. The moderate frequency of seedling resistance in the A-genome SHs (56%), the durum wheat parents (39%), and B-genome SHs (60%) could provide diverse sources of resistance. According to seedling resistance evaluations, the A-genome-based genotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 87, 86, 88, 89, and 85; durum wheat genotypes D1, D2, D3, D4, D5, D6, D7, D8, and D9 were resistant to powdery mildew. The A-genome-based SHs were resistant due to alien genes received from their durum parents. The resistance in ccessions 6, 7, 8, and 9 was from their durum parent D1; accessions 11, 12, 13, 14, and 15 from parent D2; accessions 20, 21, 22, 23, 24, 25, 26, and 27 from D3; accessions 29, 30, 31, 32 from D4; line 39 from D7; lines 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 6, 69, and 70 from D8; and accessions 73, 74, and 75 from D9. For the B-genome-based SHs, genotypes 1, 2, 3, 4, 5, 6, 7, 8, and 9 were resistant to powdery mildew. These accessions also are a valuable source of major resistance genes to powdery mildew and, therefore, should be used in future breeding programs for incorporation of powdery mildew resistance.

After morphological examination of the resistant synthetics, 35 A-genome synthetics (1, 3, 5, 7, 11, 12, 13, 14, 17, 18, 21, 24, 25, 31, 34, 36, 37, 40, 43, 50, 51, 52, 53, 54, 57, 58, 60, 61, 62, 66, 68, 71, 72, 86, and 89), one durum wheat genotype (D4), and six B-genome synthetics (1, 2, 3, 4, 6, and 7) were morphologically good and diverse based on grain weight and other phenotypic characters. Among the A-genome synthetics, genotype 31 and its durum parent D4 were morphologically good; this genotype inherited these traits from its durum parent. Variation in morphological traits

exists in these respective lines and, therefore, are important sources of genes for desirable traits in plant breeding. These genotypes also should be used by the breeders in wheat improvement programs.

The genetic diversity estimation and cluster analysis revealed a highly diverse relationship between the hexaploid amphiploids (1.0 and 0.691). Kuleung et al. (2006) also reported moderate polymorphic relationships among the Triticale accessions (0.54). Our results indicate that SH wheatsareis very important in broadening of genetic base in hexaploid wheat. This novel diversity residing in SHs is anticipated to add to the durability and give sustainable output. In this study, SSR marker analysis revealed polymorphism between the genotypes indicating that SSR markers are a valuable diagnostic tool showing considerable genetic diversity. According to Röder et al. (1998), microsatellites or SSRs are an important tool for studies on genetic diversity, population structure, genetic mapping, and crop breeding due to their abundance, co-dominance, level of polymorphism, reliability, and ease of assay. Similarly, Parker et al. (2002) compared AFLP and SSR marker systems across 11 and 124 wheat varieties, respectively. Their results suggested that these markers are most effective in detecting polymorphism. Polymorphisms revealed by PCR amplification are due to variation of the number of repeats in a defined region of the genome (Morgante and Olivieri 1993).

The SSR cluster analysis of the A-genome SHs and durum wheats indicated genotypes 87, 83, 78, D9, D8, 70, 69, 67, 66, 62, 61, 60, 58, 55, 52, 51, 50, 48, 47, 45, 44, 43, D7, 39, 37, 36, 26, 21, 20, 16, 15, 14, 11, 10, 9, 8, 7, and 6 were the most diverse with a maximum genetic distance of 100%. The diversity found in A-genome SHs and their durum parents suggested that the durums are the donor of variability in A genome SHs. The diversity found in A-genome SH genotypes 58, 60, 61, 62, 66, 67, 68, 69, and 70 was from durum parent D8 and that in genotype 39 D7. Among B-genome SHs, genotypes 8 and 4 were found to be diverse.

The diversity generated by SSRs is more accurate and reliable, and this information will be helpful for future breeding programs that can utilize the recommended lines with a broad genetic base for incorporating powdery mildew resistance. These results provide an insight to the genetic diversity of these synthetics that should facilitate efficient utilization and management of these germ plasms or genetic stocks. According to Bretting and Widrlechner (1995), knowledge of diversity patterns allows plant breeders to better understand the evolutionary relationships among accessions, to sample germ plasm in a more systematic fashion, and to develop strategies to incorporate useful diversity in their breeding programs. Information about genetic similarity was helpful to avoid any chance of elite germ plasm becoming genetically uniform and endangering long-term productivity gains (Messmer et al. 1992). The data of genetic diversity among closely related lines along with phenotypic evaluation for various parameters proved very effective in selecting powdery mildew resistant lines that are genetically distant and phenotypically excellent. The A-genome synthetic lines 7, 11, 14, 21, 36, 37, 43, 502, 51, 52, 58, 60, 61, 62, and 66, and the B-genome synthetic line 4, exhibited the best seedling resistance against powdery mildew along with good phenotypic characters with a broader genetic base. These lines are recommended for further exploitation by world's breeders in wheat improvement programs.

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Evaluating D-genome-based synthetic hexaploids and their advanced derivatives for powdery mildew resistance: Morpho-molecular characterization, diversity, and utilization potential for wheat improvement.

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We screened the 93 Elite-I and 32 Elite-II synthetic wheat hexaploids and their advanced derivatives at the seedling stage in greenhouses at Murree, Pakistan, and at the adult-plant stage under field conditions at Kaghan, Pakistan, for powdery mildew resistance. In total, 44 Elite-I synthetics (57%), 20 Elite-II synthetics (62%), and 56 D-genome-derived advanced derivatives (93%) showed resistance at the seedling stage. These Elite-I and Elite-II accessions also had adult-plant resistance (APR). Among the advanced derivatives, 11 (18%) accessions showed only seedling resistance. In both the Elite-I and Elite-II synthetics, APR was 100%, and 80% in the D-genome-derived advanced derivatives. Of these lines, 53% of the Elite I, 37% of Elite II, and 5% of the D-genome-derived advanced derivatives had only APR. Most genotypes resistant at seedling stage also were resistant at the adult-plant stage. Forty-four accessions (57%) of tge Elite I, 20 accessions (62%) of the Elite II, and 45 (75%) of the D-genome-derived advanced derivatives were found to be significantly resistant with the resistance being expressed in both seedlings and adult plants. The resistant synthetic germ plasm found in this study has potential for wheat improvement.

A morphological evaluation of the synthetics resistant at adult and at both stages showed that 76 Elite-I genotypes, 13 Elite-II genotypes, and five D-genome-derived advanced derivative genotypes are good and diverse. These synthetic hexaploid wheats are an important source of genes for desirable traits in plant breeding. The resistant 93 Elite-I synthetics were subjected to SSR analysis for molecular diversity evaluation. We used 13 SSR markers (Table 5) that gave clear bands, and the average polymorphic loci/primer was 6.07 and the average similarity matrix was 0.77 (77%). The resistant Elite-II synthetics and D-genome-derived advanced derivatives were checked for the presence of resistance genes *Pm4b*, *Pm9*, *Pm16*, and *Pm30* using SSR markers. The results indicated that SSR marker *Xgwm-382* flanked the resistance gene *Pm4b* in one Elite-II line and five advanced derivatives, *Xgwm-4* detected *Pm9* in one Elite-II line and 27 advanced derivatives, *Xgwm-332* showed the presence of *Pm9* gene in one Elite-II line and in 13 advanced derivatives, and *Pm16* and *Pm30* genes were present in one Elite-II genotype and in two advanced derivatives indicated by *Xgwm-159*. The SSR analysis proved that the resistant Elite-II and D-genome advanced derivative lines with respective genes can be used in wheat breeding programs against powdery mildew. Incorporating and deploying of these powdery mildew resistance genes will be helpful to provide wheat growers with resistant cultivars.

Table 5. A list of the SSR primers used for genetic analysis of Elite-1 synthetic hexaploids.

Locus	Primer	Locus	Primer	Locus	Primer
1D	*Xgwm-106*	3A	*Xgwm-2*	3D	*Xgwm-383*
2B	*Xgwm-210*	4A	*Xgwm-160*	3B	*Xgwm-112*
1B	*Xgwm-140*	6A	*Xgwm-169*	2B	*Xgwm-47*
1A	*Xgwm-136*	4D	*Xgwm-608*		
2A	*Xgwm-71.1*	1D	*Xgwm-458*		

Molecular diagnostics of the *Pm* resistance genes in powdery mildew-resistant the Elite-II synthetic hexaploid set and in D-genome-derived advanced derivatives. Thirty-two Elite-II accessions and 48 D-genome-derived advanced derivatives were tested with SSR markers for the presence of four genes, *Pm4b*, *Pm9*, *Pm16* and *Pm30* (Table 6). The SSR markers use were *Xgwm-382* for powdery mildew resistant gene *Pm4b* (Yi et al. 2008), *Xgwm-4* and *Xgwm-332* linked to *Pm9* (Srnic et al. 2005), and SSR marker *Xgwm-159* linked to genes *Pm16* and *Pm30* (Chen et al. 2005).

Table 6. Sequence and annealing temperature of the molecular markers linked to Pm genes.

Locus	Marker	*Pm* gene	Primer sequence	Annealing temperature
2A	Xgwm-382$_{-125}$	*Pm4b*	F: GTC AGA TAA CGC CGT CCA AT R: CTA CGT GCA CCA CCA TTT TG	60°C
4A	Xgwm-4$_{-253}$	*Pm9*	F: GCT GAT GCA TAT AAT GCT GT R: CAC TGT CTG TAT CAC TCT GCT	55°C
7A	Xgwm-332$_{-212}$	*Pm9*	F: AGC CAG CAA GTC ACC AAA AC R: AGT GCT GGA AAG AGT AGT TTTG	60°C
5B	Xgwm-159$_{-201}$	*Pm16*, *Pm30*	F: GGG CCA ACA CTG GAA CAC R: GCA GAA GCT TGT TGG TAG GC	60°C

Greenhouse evaluation for seedling resistance. Different infection types (ITs) were recorded within the Elite-I, Elite-II, and D genome-derived advanced derivatives at the seedling stage. At the seedling stage, 57% percent of the Elite-I synthetics and 62% of the Elite-II synthetics were resistant. These lines also also had APR, indicating that none were resistant only at seedling stage. Among the D-genome-derived advanced derivatives, 93% of the accessions resistant at the seedling stage also had APR, and 18% were resistant only at the seedling stage. Most of the SHs were found to possess good resistance at seedling stage to powdery mildew.

Field evaluation for adult plant resistance. Synthetic germ plasm also was tested under field conditions at Kaghan, Pakistan, to evaluate APR (Table 7). At the adult-plant stage, 100% of Elite-I synthetics and Elite-II synthetics and 80% of D-genome-derived advanced derivatives were resistant (Fig 4.). Only adult-plant resistance to powdery mildew was found in 53% of the Elite-I, 37% of the Elite-II lines, and 5% of the D-genome-derived advanced derivatives had (Table 8). Most of the lines resistant at seedling stage also had APR. Significant resistance expressed in both seedling and adult-plant stages was found in 57% of the Elite I, 62% of the Elite II, and 75% of the D-genome-derived advanced derivatives (Table 9, p. 143).

Table 7. Powdery mildew evaluation for adult-plant resistance under field condidtions at Kaghan, Pakistan.

Infection type	Reaction	Number of lines tested		
		Elite-I	Elite-II	Advanced derivatives
0–3	Resistant	49	12	3
4–6	Intermediate	—	—	—
7–9	Susceptible	—	—	—

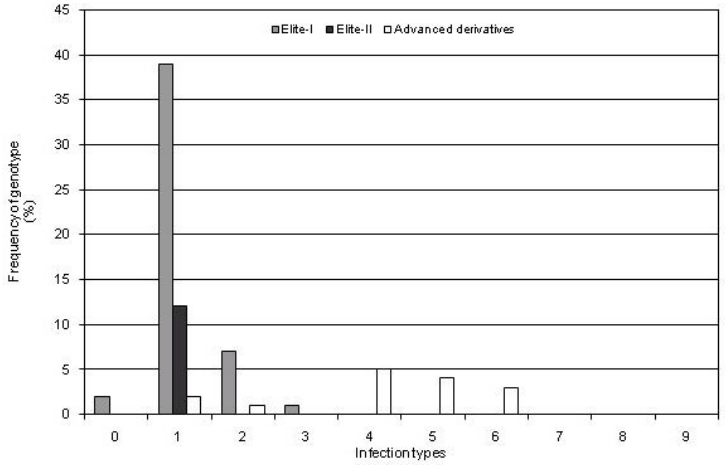

Fig. 4. Frequency distribution for powdery mildew infection type of the Elite-I and Elite-II synthetic hexaploids and the D-genome-derived advanced derivatives at the adult-plant stage.

Phenotypic evaluation. Morphological trait data of the resistant Elite-I and Elite-II SH wheats and the D-genome-derived advanced derivatives are presented in Table 10 (pp. 143-148). On the basis of 1,000-kernel weight and other phenotypic characters, Elite-I SH genotypes 2, 5, 8, 9, 12, 14, 18, 19, 20, 21, 22, 23, 26, 29, 30, 31, 32, 33, 34, 35, 37, 38, 42, 43, 44, 45, 52, 59, 60, 61, 62, 76, 78, 80, 81, 83, 84, 85, 86, 87, 89, and 92; Elite-II SH genotypes 3, 7, 9, 10, 12, 13, 16, 26, 30, 31, and 32; and D-genome-derived advanced derivative genotypes L.R Fusarium-833, M.BCN-7, Dr.MP-2-10, Elite HYDRL-6, and Elite HYDRL-13 were morphologically desirable.

Table 8. Sources of adult-plant resistance (IT < 3 on a 0-9 scale) to powdery mildew identified in Elite-I, Elite-II sets and advanced derivatives tested under field conditions.

Resistant reaction	Accession numbers		
	Elite I	Elite II	Advanced derivatives
0	5, 58	—	—
1	1, 2, 4, 9, 10, 12, 13, 15, 16, 17, 19, 23, 24, 26, 28, 29, 30, 31, 33, 35, 39, 41, 63, 65, 66, 67, 72, 73, 75, 76, 77, 80, 82, 85, 86, 87, 88, 89, 92	1, 4, 13, 14, 15, 20, 21, 23, 24, 25, 27, 28	4, 26
2	3, 20, 21, 22, 46, 61, 74	—	5
3	18	—	—

Molecular evaluation. ***Genetic diversity estimation and cluster analysis of synthetic hexaploid Elite-I set.*** For SSR analysis, 13 primers were used that yielded 79 polymorphic bands, generating 6.07 polymorphic loci/primer. The size range of amplified bands ranged from 50 bp to 1,000 bp. The highest number of polymorphic bands (3) was achieved with primer *Xgwm-383-3D*, 1.96% of the total number of amplified bands.

The SSR amplification data was used to generate similarity matrix and dendrogram. The similarity matrix was calculated using Nei and Li's coefficient analysis and showed genetic distance between individual pairs of all the genotypes. Using all the Elite-I SHs, the genetic diversity using SSR markers ranged from 0.049 (4.9 %) to 0.838 (83.8%), in which 0.049 represents minimum genetic distance and 0.838 represents maximum genetic distance among the genotypes and revealing high variability among the accessions. Similarly, the value of similarity coefficient based on 13 SSR markers ranged from 0.951 (95.1%) to 0.162 (16.2%).

The clustering of the 93 Elite-I SH accessions, based on genetic distances using UPGMA analysis produced, two main clusters A and B (Fig. 5, p. 149). Cluster A was comprised of eight genotypes (80, 75, 76, 44, 31, 18, 17, and 8). Among these genotypes, 80 and 75 were highly diverse, having similarity coefficient of 0.500 (50%). These two genotypes were more diverse than the remaining genotypes with an average genetic distance of 0.68 (68%). The least diverse genotypes in this group were 18 and 17, with a genetic distance of 0.28 (28%). Cluster B was com-

Table 9. Lines of the Elite-I and Elite-II synthetic hexaploids and advanced derivatives resistant (IT = 0–3) to powdery mildew at the seedling and adult-plant stages under field condidtions at Kaghan, Pakistan.

Accession numbers		
Elite-I	**Elite-II**	**Advanced derivatives**
6, 7, 8, 11, 14, 25, 27, 32, 34, 36, 37, 38, 40, 42, 43, 44, 45, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 59, 60, 61, 62, 64, 68, 69, 70, 71, 78, 79, 81, 83, 84, 90, 91, 93	2, 3, 5, 6, 7, 8, 9, 10, 11, 12, 16, 17, 18, 19, 22, 26, 29, 30, 31, 32	1, 2, 3, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48

Table 10. Phenological characterization of 93 Elite-I and 32 Elite-II synthetic hexaploid lines and 48 D-genome-derived advanced derivatives. * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. GH = growth habit, prostrate (+), erect (–), or medium (M); FLOW = days–to–flowering; PUB = pubescence, absence (–) or prescence (+); HT = plant height at maturity; AWN = awn color, LB = light brown, DB = dark brown, B = black, AW = amery white, and Y = yellow; PMA = days–to–physiological maturity; TKW = 1,000–kernel weight; G/S = number of grains/spike; and SL = spike length (cm).

No.	Parentage/Pedigree	GH	FLOW	PUB	HT	AWN	PMA	TKW	G/S	SL
Elite-I synthetic hexaploid lines										
1	ALTAR 84/AE.SQUARROSA (188)*	M	120	+	120	LB	148	48.0	22	12.0
2	DOY1/AE.SQUARROSA (188)	M	121	+	130	LB	160	53.2	43	12.0
3	ALTAR 84/AE.SQUARROSA (192)	M	120	+	125	DB	156	48.0	31	12.0
4	ALTAR 84/AE.SQUARROSA (193)	M	121	+	119	LB	152	49.0	23	8.0
5	ALTAR 84/AE.SQUARROSA (198)	M	130	+	130	LB	161	50.3	38	12.0
6	CROC_1/AE.SQUARROSA (205)	+	130	+	103	LB	160	48.2	20	12.2
7	ALTAR 84/AE.SQUARROSA (205)	M	131	+	96	LB	159	49.5	10	10.2
8	CPI/GEDIZ/3/GOO//JO69/CRA/4/ AE.SQUARROSA (208)	M	132	+	85	LB	157	58.3	25	7.2
9	ALTAR 84/AE.SQUARROSA (211)	M	120	+	85	LB	157	59.5	14	8.0
10	D67.2/P66.270//AE.SQUARROSA (211)	M	122	–	102	LB	151	39.4	9	14.0
11	D67.2/P66.270//AE.SQUARROSA (213)	M	128	+	82	LB	157	39.6	13	12.0
12	ROK/KML//AE.SQUARROSA (214)	+	130	+	125	LB	155	58.5	14	11.0
13	D67.2/P66.270//AE.SQUARROSA (217)	M	132	+	80	LB	163	49.0	48	7.0
14	YUK/AE.SQUARROSA (217)	+	131	+	90	LB	171	64.0	42	14.0
15	D67.2/P66.270//AE.SQUARROSA (218)	M	115	+	135	LB	144	39.5	10	11.0
16	ALTAR 84/AE.SQUARROSA (219)	M	132	+	95	LB	164	47.5	9	9.0
17	ALTAR 84/AE.SQUARROSA (220)	–	129	+	106	LB	157	49.3	54	13.7
18	D67.2/P66.270//AE.SQUARROSA (220)	+	115	–	93	LB	157	56.0	10	13.2
19	DVERD_2/AE.SQUARROSA (221)	M	134	–	93	LB	160	54.7	15	12.0
20	ALTAR 84/AE.SQUARROSA (221)	M	133	+	128	LB	162	53.0	14	10.0
21	D67.2/P66.270//AE.SQUARROSA (221)	M	130	–	105	LB	161	50.5	12	10.5
22	D67.2/P66.270//AE.SQUARROSA (222)	M	125	+	139	LB	157	59.2	15	9.0
23	D67.2/P66.270//AE.SQUARROSA (223)	M	111	–	125	LB	155	58.6	18	12.3

Table 10 (continued). Phenological characterization of 93 Elite–I and 32 Elite–II synthetic hexaploid lines and 48 D–genome–derived advanced derivatives. * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. GH = growth habit, prostrate (+), erect (–), or medium (M); FLOW = days–to–flowering; PUB = pubescence, absence (–) or prescence (+); HT = plant height at maturity; AWN = awn color, LB = light brown, DB = dark brown, B = black, AW = amery white, and Y = yellow; PMA = days–to–physiological maturity; TKW = 1,000–kernel weight; G/S = number of grains/spike; and SL = spike length (cm).

No.	Parentage/Pedigree	GH	FLOW	PUB	HT	AWN	PMA	TKW	G/S	SL
24	CROC_1/AE.SQUARROSA (224)	–	120	+	117	LB	127	48.8	16	12.0
25	ALTAR 84/AE.SQUARROSA (224)	+	128	+	112	LB	164	43.0	12	13.0
26	ACO89/AE.SQUARROSA (309)	+	123	+	89	LB	153	51.0	49	9.2
27	GARZA/BOY//AE.SQUARROSA (311)	–	130	–	78	LB	165	46.8	28	7.3
28	68.111/RGB-U//WARD/3/AE. SQUARROSA (316)	M	129	+	108	LB	158	41.0	11	12.5
29	68.111/RGB-U//WARD/3/AE. SQUARROSA (326)	M	120	+	132	LB	158	56.8	6	11.0
30	68112/WARD//AE.SQUARROSA (369)	M	117	+	139	LB	152	61.0	12	14.0
31	68112/WARD//AE.SQUARROSA (369)	+	113	+	109	LB	139	56.0	23	14.2
32	DOY1/AE.SQUARROSA (447)	+	133	+	102	LB	161	55.0	16	14.0
33	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE. SQUARROSA (498)	–	130	+	106	LB	163	55.0	14	13.0
34	DOY1/AE.SQUARROSA (511)	M	116	+	136	LB	158	50.0	10	14.0
35	68.111/RGB-U//WARD/3/AE. SQUARROSA (511)	M	117	+	109	LB	148	60.3	17	14.3
36	DOY1/AE.SQUARROSA (515)	M	113	–	125	LB	151	49.6	13	14.5
37	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE. SQUARROSA (629)	+	128	+	132	LB	162	53.9	15	12.0
38	FGO/USA2111//AE.SQUARROSA (658)	–	120	+	132	LB	154	54.2	23	14.0
39	CROC_1/AE.SQUARROSA (725)	–	120	–	135	DB	151	49.0	38	14.0
40	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (781)	–	130	+	139	LB	173	49.5	10	15.5
41	68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (783)	M	129	+	129	LB	160	44.5	16	14.2
42	YAR/AE.SQUARROSA (783)	M	132	+	121	LB	176	57.9	9	14.8
43	YUK/AE.SQUARROSA (864)	M	132	+	119	LB	167	52.3	13	11.0
44	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	M	123	+	88	LB	159	54.0	17	12.0
45	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	M	136	+	97	LB	173	53.0	28	9.0
46	CROC_1/AE.SQUARROSA (879)	M	134	–	103	LB	161	49.4	18	12.0
47	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (882)	M	131	+	86	LB	161	46.0	20	12.0
48	SORA/AE.SQUARROSA (884)	–	130	–	140	DB	166	39.4	22	12.0
49	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE. SQUARROSA (890)	–	130	+	135	LB	156	46.9	5	10.0
50	CROC_1/AE.SQUARROSA (518)	+	138	+	92	LB	165	49.0	20	10.0
51	PBW114/AE.SQ	+	136	+	104	LB	165	45.9	6	12.3
52	ALTAR 84/AE.SQUARROSA (JBANGOR)	+	113	–	142	LB	139	53.1	12	14.0
53	YAV_2/TEZ//AE.SQUARROSA (249)	M	130	–	145	LB	155	39.6	8	14.2
54	CETA/AE.SQUARROSA (895)	–	133	+	138	LB	156	43.5	23	14.0
55	D67.2/P66.270//AE.SQUARROSA (257)	M	133	–	99	LB	168	49.0	9	10.0
56	LCK59.61/AE.SQUARROSA (313)	M	135	+	99	LB	164	40.7	5	12.0
57	LCK59.61/AE.SQUARROSA (324)	M	127	+	112	LB	159	44.0	8	12.0

Table 10 (continued). Phenological characterization of 93 Elite-I and 32 Elite-II synthetic hexaploid lines and 48 D-genome-derived advanced derivatives. * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. GH = growth habit, prostrate (+), erect (–), or medium (M); FLOW = days–to–flowering; PUB = pubescence, absence (–) or prescence (+); HT = plant height at maturity; AWN = awn color, LB = light brown, DB = dark brown, B = black, AW = amery white, and Y = yellow; PMA = days–to–physiological maturity; TKW = 1,000–kernel weight; G/S = number of grains/spike; and SL = spike length (cm).

No.	Parentage/Pedigree	GH	FLOW	PUB	HT	AWN	PMA	TKW	G/S	SL
58	SRN/AE.SQUARROSA (358)	+	127	+	132	LB	156	45.0	11	10.2
59	SCOOP_1/AE.SQUARROSA (358)	M	131	+	135	LB	171	54.2	16	14.0
60	GAN/AE.SQUARROSA (408)	M	134	–	126	LB	179	52.6	61	14.2
61	SCA/AE.SQUARROSA (518)	M	134	+	97	LB	179	56.8	46	13.0
62	YAR/AE.SQUARROSA (518)	M	131	–	114	LB	157	52.0	8	11.0
63	BOTNO/AE.SQUARROSA (617)	M	131	+	140	LB	169	46.0	58	9.3
64	BOTNO/AE.SQUARROSA (620)	M	131	+	142	LB	161	49.8	22	15.2
65	BOTNO/AE.SQUARROSA (625)	M	132	–	99	LB	159	41.0	15	14.0
66	SNIPE/YAV79//DACK/TEAL/3/ AE.SQUARROSA (629)	M	134	+	139	LB	158	48.8	14	12.0
67	D67.2/P66.270//AE.SQUARROSA (633)	M	126	+	96	LB	149	49.0	4	8.0
68	D67.2/P66.270//AE.SQUARROSA (659)	M	132	+	98	LB	156	48.0	10	14.0
69	SNIPE/YAV79//DACK/TEAL/3/ AE.SQUARROSA (700)	M	131	+	140	LB	155	47.0	15	10.2
70	TRN/AE.SQUARROSA (700)	M	130	+	132	LB	157	49.0	30	12.0
71	SNIPE/YAV79//DACK/TEAL/3/ AE.SQUARROSA (877)	–	128	+	138	LB	156	48.0	21	14.3
72	GAN/AE.SQUARROSA (897)	M	129	+	98	LB	161	39.3	9	13.3
73	YAV_2/TEZ//AE.SQUARROSA (895)	M	135	+	112	LB	165	48.6	18	11.2
74	ARLIN/AE.SQUARROSA (283)	M	138	+	119	LB	169	47.9	17	14.3
75	FALCIN/AE.SQUARROSA (312)	+	136	+	140	LB	164	45.2	18	14.3
76	RASCON/AE.SQUARROSA (312)	+	136	+	138	LB	165	55.0	5	12.0
77	SCOT/MEXI_1//AE.SQUARROSA (314)	M	133	+	120	LB	166	45.5	15	14.0
78	DOY1/AE.SQUARROSA (333)	M	132	–	115	LB	164	56.5	12	16.0
79	68.111/RGB-U//WARD/3/AE.SQUARROSA (452)	M	131	+	120	LB	160	38.3	18	16.0
80	68.111/RGB-U//WARD/3/AE.SQUARROSA (454)	M	128	+	130	LB	158	52.0	20	16.0
81	DOY1/AE.SQUARROSA (458)	M	130	+	130	LB	161	54.0	14	15.2
82	GREEN/AE.SQUARROSA (458)	M	135	–	130	LB	171	44.4	13	16.0
83	CETA/AE.SQUARROSA (174)	M	135	+	131	LB	174	61.0	19	15.0
84	DOY1/AE.SQUARROSA (372)	M	134	+	123	LB	165	58.4	15	15.0
85	SCA/AE.SQUARROSA (409)	M	134	+	128	LB	166	50.9	16	15.0
86	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE. SQUARROSA (409)	+	134	+	132	LB	163	54.0	18	14.0
87	STY-US/CELTA//PALS/3/SRN_5/4/ AE.SQUARROSA (502)	–	133	+	122	LB	158	52.0	15	14.0
88	ALTAR 84/AE.SQUARROSA (502)	+	132	+	109	LB	164	44.0	16	16.0
89	CROC/AE.SQUARROSA (517)	M	115	+	128	LB	153	55.0	13	15.2
90	CETA/AE.SQUARROSA (1024)	M	115	+	118	LB	152	48.4	14	15.0
91	DVERD_2/AE.SQUARROSA (1027)	+	133	+	127	LB	162	42.0	16	15.0
92	CETA/AE.SQUARROSA (1027)	+	140	+	118	LB	165	55.4	30	15.0
93	DOY1/AE.SQUARROSA (1030)	+	128	+	112	LB	160	49.9	28	15.0

Table 10 (continued). Phenological characterization of 93 Elite-I and 32 Elite-II synthetic hexaploid lines and 48 D-genome-derived advanced derivatives. * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. GH = growth habit, prostrate (+), erect (–), or medium (M); FLOW = days–to–flowering; PUB = pubescence, absence (–) or prescence (+); HT = plant height at maturity; AWN = awn color, LB = light brown, DB = dark brown, B = black, AW = amery white, and Y = yellow; PMA = days–to–physiological maturity; TKW = 1,000–kernel weight; G/S = number of grains/spike; and SL = spike length (cm).

No.	Parentage/Pedigree	GH	FLOW	PUB	HT	AWN	PMA	TKW	G/S	SL
Elite-II synthetic hexaploid lines										
1	SORA/AE.SQUARROSA (192)	–	135	+	131	LB	192	38.0	20	11.5
2	CROC_1/AE.SQUARROSA (210)	M	135	–	120	Y	191	30.8	15	14.6
3	DVERD_2/AE.SQUARROSA (214)	M	128	–	120	B	187	57.4	10	10.5
4	ARLIN_1/AE.SQUARROSA (218)	M	128	+	130	LB	179	31.6	29	10.6
5	GAN/AE.SQUARROSA (236)	M	140	+	140	DB	184	41.6	17	10.6
6	SORA/AE.SQUARROSA (323)	M	135	–	123	DB	178	34.2	28	15.1
7	D67.2/P66.270//AE.SQUARROSA (308)	M	144	–	130	Y	180	50.0	26	15.0
8	STY-US/CELTA//PALS/3/SRN_5/4/ AE.SQUARROSA (431)	+	141	–	128	DB	182	48.0	13	10.3
9	LCK59.61/AE.SQUARROSA (693)	M	139	+	127	LB	178	51.5	25	13.1
10	SKARV_2/AE.SQUARROSA (304)	–	133	–	143	Y	183	54.0	33	12.6
11	CETA/AE.SQUARROSA (1025)	–	124	+	117	DB	184	43.0	30	10.3
12	DOY1/AE.SQUARROSA (1027)	–	126	+	112	LB	183	57.8	26	12.0
13	CETA/AE.SQUARROSA (386)	–	146	+	143	Y	178	56.0	22	12.0
14	CETA/AE.SQUARROSA (392)	–	123	+	116	LB	180	33.3	42	12.0
15	CETA/AE.SQUARROSA (533)	M	129	+	119	LB	182	24.3	30	12.0
16	CPI/GEDIZ/3/GOO//JO/CRA/4/ AE.SQUARROSA (1018)	M	146	+	136	DB	192	58.2	11	10.6
17	CETA/AE.SQUARROSA (1031)	–	133	+	113	Y	195	33.0	20	11.0
18	CETA/AE.SQUARROSA (1038)	M	135	+	138	DB	192	40.0	21	10.0
19	CETA/AE.SQUARROSA (1046)	–	124	+	136	LB	183	43.5	25	10.0
20	CETA/AE.SQUARROSA (1053)	M	143	–	110	B	182	34.0	11	11.5
21	CROC_1/AE.SQUARROSA (212)	M	143	–	110	Y	189	49.2	27	13.0
22	CETA/AE.SQUARROSA (368)	M	139	+	132	LB	195	36.0	18	11.5
23	ARLIN_1/AE.SQUARROSA (430)	M	124	+	115	DB	185	14.0	16	11.0
24	D67.2/P66.270// AE.SQUARROSA (497)	M	126	+	80	DB	182	27.0	9	13.0
25	D67.2/P66.270// AE.SQUARROSA (1015)	M	128	+	115	DB	184	40.4	21	12.8
26	GAN/AE.SQUARROSA (206)	M	146	+	124	LB	194	50.2	16	10.0
27	ARLIN_1/AE.SQUARROSA (335)	M	150	+	102	LB	196	40.2	11	12.5
28	GAN/AE.SQUARROSA (335)	–	143	+	141	DB	181	27.8	20	11.6
29	68.111/RGB-U//WARD RESEL/3/STIL/4/ AE.SQUARROSA (385)	–	143	+	132	LB	182	34.0	14	9.6
30	CETA/AE.SQUARROSA (417)	–	146	–	128	DB	196	57.6	18	11.5
31	68.111/RGB-U//WARD RESEL/3/STIL/4/ AE.SQUARROSA (431)	M	146	–	134	B	185	64.0	31	14.0
32	DOY1/ AE.SQUARROSA (534)	M	146	+	131	DB	179	50.0	23	11.0
D-genome-derived advanced lines										
1	BCN//CETA/AE. SQUARROSA (895)	–	107	–	101	DB	146	26.6	57	12.0
2	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE. SQUARROSA (409) CIGM93.388	–	120	+	106	DB	153	34.0	13	14.0
3	ALTAR 84/ AE. SQUARROSA (224)	–	120	–	82	DB	152	31.8	27	11.0
4	68.111/RGB-U//WARD/3/FGO/4/RABI/5/ AE. SQUARROSA (809)	–	95	+	112	AW	149	26.8	19	10.0

Table 10 (continued). Phenological characterization of 93 Elite-I and 32 Elite-II synthetic hexaploid lines and 48 D-genome-derived advanced derivatives. * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. GH = growth habit, prostrate (+), erect (–), or medium (M); FLOW = days–to–flowering; PUB = pubescence, absence (–) or prescence (+); HT = plant height at maturity; AWN = awn color, LB = light brown, DB = dark brown, B = black, AW = amery white, and Y = yellow; PMA = days–to–physiological maturity; TKW = 1,000–kernel weight; G/S = number of grains/spike; and SL = spike length (cm).

No.	Parentage/Pedigree	GH	FLOW	PUB	HT	AWN	PMA	TKW	G/S	SL
5	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE. SQUARROSA (878)	–	114	+	92	LB	152	32.6	57	11.0
6	BOTNO	–	124	+	121	LB	152	24.0	32	11.0
7	AJAIA_9	–	114	+	82	LB	155	43.3	45	10.0
8	-	–	107	–	102	AW	148	45.0	62	13.0
9	CETA/AE.SQUARROSA (533)	–	119	+	115	LB	151	20.1	15	14.0
10	CETA/AE.SQUARROSA (1038)	–	111	+	109	AW	151	23.0	21	12.0
11	YS/PASTOR	–	112	+	104	AW	149	26.6	46	13.0
12	YS/PASTOR	–	105	–	109	AW	149	28.0	65	12.0
13	YS/PASTOR	–	107	–	113	AW	150	40.4	63	13.0
14	YS/PASTOR	–	108	–	113	AW	150	33.4	49	14.0
15	YS/PASTOR	–	111	+	113	AW	144	40.9	40	13.0
16	YS/PASTOR	–	110	+	105	AW	151	40.2	59	13.5
17	YS/PASTOR	–	110	–	109	Y	151	32.6	66	12.8
18	MAYOOR//TK SN1081/AE. SQUARROSA (222)/3/FCT	–	119	–	101	LB	154	33.8	36	14.3
19	MAYOOR//TK SN1081/AE. SQUARROSA (222)/3/BCN	–	105	–	111	LB	151	34.8	56	12.0
20	MAYOOR//TK SN1081/AE. SQUARROSA (222)/3/BCN	–	113	–	99	LB	150	44.9	40	13.3
21	TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE. SQUARROSA (205)/3/3*BUC/6/OPATA	–	108	–	95	AW	150	38.8	74	13.3
22	TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE. SQUARROSA (205)/3/3*BUC/6/CNO	–	108	–	90	AW	151	35.8	72	13.0
23	TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE. SQUARROSA (205)/3/3*BUC/6/BCN	–	109	–	96	AW	151	36.6	47	12.1
24	TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE. SQUARROSA (205)/3/3*BUC/6/BCN	–	109	–	89	AW	152	41.8	58	13.5
25	SABUF/3/BCN//CETA/AE. SQUARROSA (895)/4/BCN	–	106	–	99	LB	154	37.4	29	14.6
26	SABUF/3/BCN//CETA/AE. SQUARROSA (895)/4/BCN	–	110	–	100	LB	152	35.8	65	11.3
27	SABUF/3/BCN//CETA/AE. SQUARROSA (895)/4/FCT	–	112	–	82	Y	151	39.2	65	12.4
28	SABUF/3/BCN//CETA/AE. SQUARROSA (895)/4/FCT	–	110	–	104	LB	154	23.7	29	12.6
29	YAV_3/SCO//JO69/CRA/3/YAV79/4/AE. SQUARROSA (498)/5/OPATA	–	90	+	74	Y	152	53.0	24	12.0
30	GAN/AE. SQUARROSA (897)//OPATA	–	90	–	107	Y	152	30.4	22	11.6
31	Not Available	–	100	–	90	Y	149	31.8	52	11.0
32	Not Available	–	110	–	108	LB	149	39.0	64	12.9
33	Not Available	–	113	–	109	Y	156	29.4	77	15.3
34	Not Available	–	111	–	94	Y	156	43.6	62	14.0
35	Not Available	–	110	–	93	LB	154	33.2	58	12.6
36	Not Available	–	107	–	102	AW	155	34.6	42	12.3

Table 10 (continued). Phenological characterization of 93 Elite-I and 32 Elite-II synthetic hexaploid lines and 48 D-genome-derived advanced derivatives. * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. GH = growth habit, prostrate (+), erect (–), or medium (M); FLOW = days–to–flowering; PUB = pubescence, absence (–) or prescence (+); HT = plant height at maturity; AWN = awn color, LB = light brown, DB = dark brown, B = black, AW = amery white, and Y = yellow; PMA = days–to–physiological maturity; TKW = 1,000–kernel weight; G/S = number of grains/spike; and SL = spike length (cm).

No.	Parentage/Pedigree	GH	FLOW	PUB	HT	AWN	PMA	TKW	G/S	SL
37	Not Available	–	108	–	94	AW	154	36.0	64	11.6
38	Not Available	–	109	–	94	AW	154	24.4	42	13.0
39	Not Available	–	110	–	95	AW	152	18.2	49	13.0
40	Not Available	–	112	–	94	AW	156	41.8	67	13.0
41	Not Available	–	109	+	90	Y	156	36.6	62	12.0
42	Not Available	–	110	–	93	AW	156	26.4	51	12.6
43	Not Available	–	112	–	89	Y	155	36.4	50	11.6
44	Not Available	–	112	–	94	Y	154	40.8	80	13.0
45	Not Available	–	116	–	100	Y	154	36.4	77	12.0
46	Not Available	–	109	–	96	AW	154	40.2	60	13.6
47	Not Available	–	101	–	104	Y	153	52.2	51	12.0
48	Not Available	–	105	–	98	Y	150	47.2	56	11.0

posed of remaining 85 genotypes and was subdivided into three groups, 1B, 2B, and 3B. Group 1B consisted of seven genotypes (45, 28, 93, 74, 73, 72, and 43). The most diverse genotype with the highest genetic distance or least similarity in this group was 45, with a genetic distance of 0.479 (47.9%) with genotype 28. The least diverse group included 73 and 74 with a genetic distance of 0.102 (10.2%). Group 2B consisted of 56 genotypes (89, 92, 90, 88, 87, 91, 84, 82, 81, 79, 78, 77, 30, 85, 83, 56, 53, 38, 37, 36, 35, 20, 71, 64, 60, 59, 68, 70, 65, 67, 66, 62, 61, 32, 86, 19, 16, 63, 69, 58, 57, 55, 49, 54, 52, 51, 39, 6, 50, 47, 46, 42, 41, 40, 48, and 21). Among this group, the most diverse genotype was 30, having a genetic distance of 0.334 (33.4%) with a group of 35 genotypes. Genotype 20 also was diverse with respect to the others, having a genetic distance of 0.319 (31.9%) with four other genotypes. Genotype 16 had a genetic distance of 0.247 (24.7%) with genotypes 19 and 86. Group 3B consisted of the remaining 23 genotypes (3, 33, 29, 27, 25, 26, 24, 23, 22, 34, 14, 5, 12, 15, 9, 13, 11, 10, 7, 4, 2, and 1). Among this group, the most diverse genotype was 3, with a genetic distance of 0.289 (28.9%) with a group of 21 genotypes. The least diverse genotypes, 10 and 11, also fall in this group, which have a genetic distance of 0.049 (4.9%). By analyzing dendrogram generated on SSR data, the most genetically diverse Elite-1 SH wheat genotypes were 80 and 75.

Molecular diagnostics of Pm resistant genes using SSR markers. A number of genes are responsible for powdery mildew resistance in wheat. In this study, four SSR markers were used to detect the presence of *Pm* genes *Pm4b*, *Pm9*, *Pm16*, and *Pm30* in the Elite-II accessions and the D-genome-derived advanced derivatives. The gene *Pm9* is linked to *Xgwm-4* with a fragment size of 253 bp in the D-genome-derived advanced derivatives (Fig. 6, Tables 11 and 12, p. 150). These results demonstrate that novel sources of powdery mildew resistance are available in the Elite-I and Elite-II sets and D-genome-derived advanced derivatives. Lines resistant at the seedling stage also gave a good level of field or APR in 57% of the Elite-I, 62% of the Elite-II, and 75% of the derivatives. All these lines had excellent resistance to powdery mildew at both stages. Most genes that confer mildew resistance at seedling stage also confer a good level of APR.

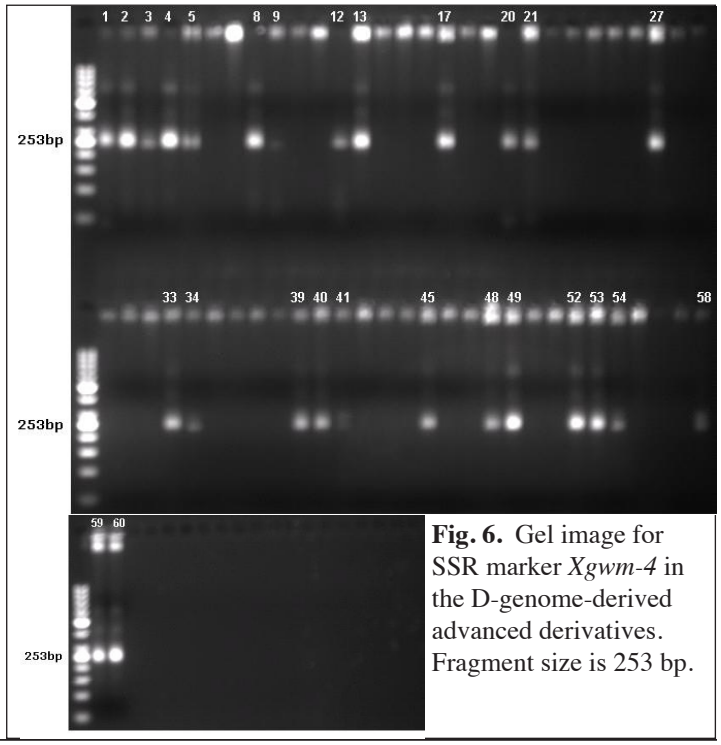

Fig. 6. Gel image for SSR marker *Xgwm-4* in the D-genome-derived advanced derivatives. Fragment size is 253 bp.

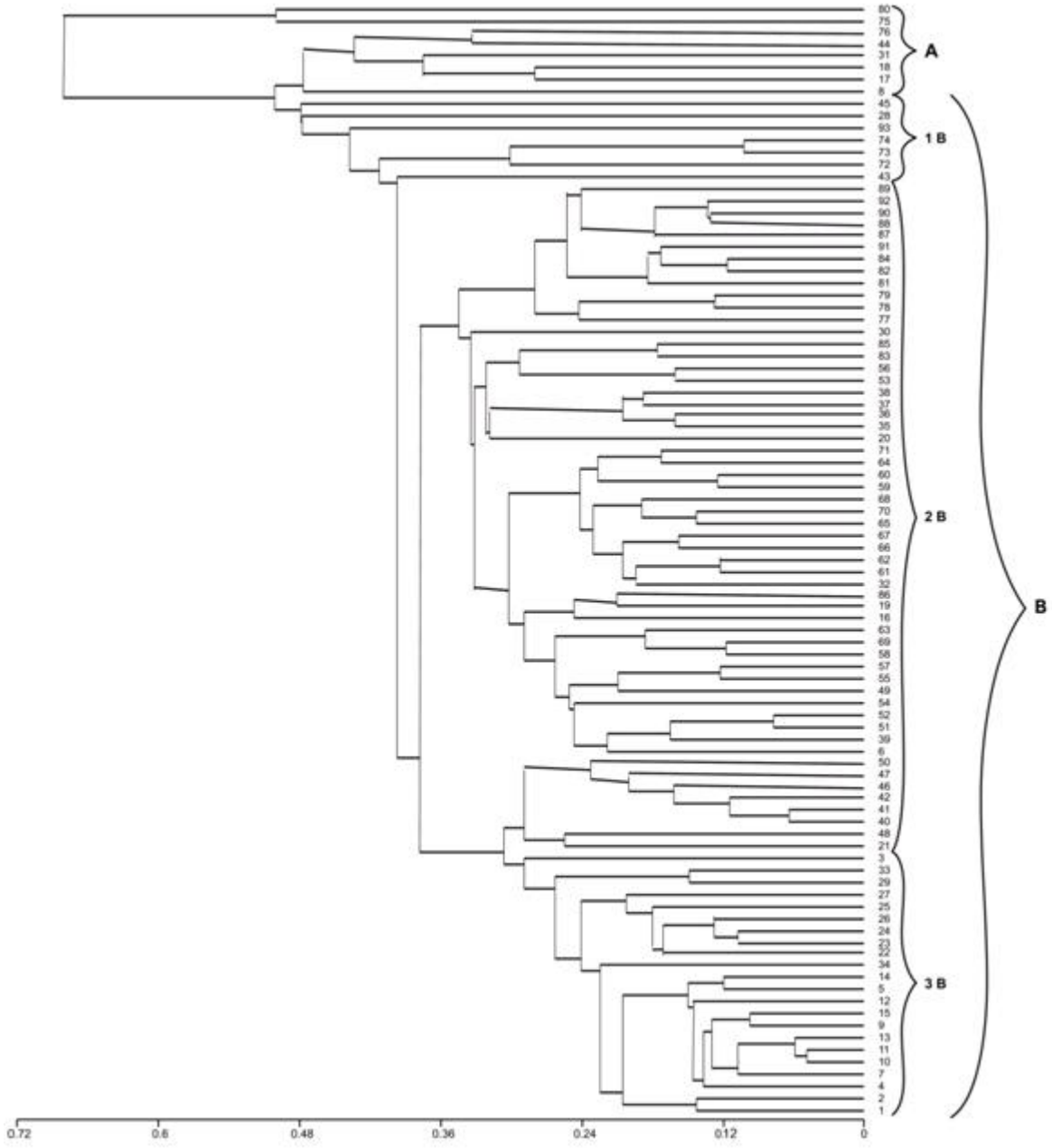

Fig. 5. Grouping of the resistant Elite-I synthetic hexaploids (2n=6x=42; AABBDD) on the basis of genetic distance.

Thus, identifying APR in these lines provides new sources of resistance that could be durable. Synthetics with both seedling and APR are preferred for utilization in wheat breeding programs. These lines can be used in direct crossing with elite commercial cultivars of wheat for further exploitation as source of resistance against powdery mildew.

The morphological trait data showed that the resistant synthetic lines possessed good phenotypic characters that will be an important source of genes for plant breeding. For 1,000-kermel weight and other phenotypic characters, Elite-I SH genotypes 2, 5, 8, 9, 12, 14, 18, 19, 20, 21, 22, 23, 26, 29, 30, 31, 32, 33, 34, 35, 37, 38, 42, 43, 44, 45, 52, 59, 60, 61, 62, 76, 78, 80, 81, 83, 84, 85, 86, 87, 89, and 92; Elite-II SH genotypes 3, 7, 9, 10, 12, 13, 16, 26, 30, 31, and 32; and D-genome-derived advanced derivatives 8, 20, 29, 47, and 48 were found to be morphologically good and desirable. The SH wheats have a considerable variability for these morphological characteristics that can be utilized in wheat improvement programs. Similar results were found by Villareal et al. (1994) among the synthetic hexaploid wheats derived from the cross '*T. turgidum*/*Ae. tauschii*', and they proposed that these synthetic lines possess substantial variation among their morphological traits can be utilized in hexaploid wheat for broadening of genetic base.

Table 11. Powdery mildew genes detected in the 32 resistant Elite-II synthetic hexaploid lines. **+** indicates presence and – absence of the *Pm* gene.

Sample No.	*Pm* Gene			
	Pm4b	*Pm9*	*Pm16*	*Pm30*
1	–	–	–	–
2	–	–	–	–
3	–	–	–	–
4	–	–	–	–
5	–	–	–	–
6	–	–	–	–
7	–	–	–	–
8	–	–	–	–
9	–	–	–	–
10	–	–	–	–
11	–	–	–	–
12	–	–	–	–
13	–	–	–	–
14	+	+	+	+
15	–	–	–	–
16	–	–	–	–
17	–	–	–	–
18	–	–	–	–
19	–	–	–	–
20	–	–	–	–
21	–	–	–	–
22	–	–	–	–
23	–	–	–	–
24	–	–	–	–
25	–	–	–	–
26	–	–	–	–
27	–	–	–	–
28	–	–	–	–
29	–	–	–	–
30	–	–	–	–
31	–	–	–	–
32	–	–	–	–

Evaluating genetic diversity and cluster analysis of the Elite-I SH revealed a diverse relationship between accessions. The molecular diversity generated in the present study would be useful in future breeding programs that can employ the recommended synthetic lines with powdery mildew resistance incorporation and a broad genetic base. The novel diversity residing in SH lines is anticipated to add the durability and give sustainable outputs (Mujeeb-Kazi and Rajaram 2002). These results indicated that SSR analysis could be successfully used to estimate genetic diversity among wheat cultivars. Thus, it could serve as an efficient tool for the selection of genetically diverse genotypes. The polymorphism revealed by SSR primers between the genotypes clearly

Table 12. Powdery mildew genes detected in the 48 resistant D-genome-derived advanced derivative. **+** indicates presence and – absence of the *Pm* gene.

Sample No.	*Pm* Gene			
	Pm4b	*Pm9*	*Pm16*	*Pm30*
1	–	+	–	–
2	–	+	–	–
3	–	+	–	–
4	–	+	–	–
5	–	–	–	–
6	–	–	–	–
7	–	+	–	–
8	–	+	–	–
9	–	–	–	–
10	–	–	–	–
11	+	+	–	–
12	–	+	–	–
13	–	–	–	–
14	–	–	–	–
15	–	–	–	–
16	–	+	–	–
17	–	–	–	–
18	–	+	–	–
19	–	+	+	+
20	–	–	–	–
21	–	–	–	–
22	–	–	–	–
23	–	–	–	–
24	–	–	–	–
25	–	+	–	–
26	–	–	–	–
27	–	–	–	–
28	–	–	–	–
29	–	–	–	–
30	–	–	–	–
31	–	+	–	–
32	–	+	–	–
33	–	–	–	–
34	–	–	–	–
35	–	–	–	–
36	–	–	–	–
37	–	+	–	–
38	–	+	–	–
39	–	+	–	–
40	–	–	–	–
41	–	–	–	–
42	–	–	–	–
43	–	+	+	+
44	–	–	–	–
45	–	+	–	–
46	–	+	–	–
47	+	+	–	–
48	–	+	–	–

demonstrated that SSRs are a valuable diagnostic tool showing considerable genetic diversity. According to Röder et al. (1998), microsatellites or SSRs represent an important tool for genetic diversity studies, population structure, genetic mapping, and crop breeding because of their abundance, co-dominance nature, polymorphism level, reliability, and ease of assay. Information on genetic similarity helped avoid any chance of elite germ plasm becoming genetically uniform and endangering long-term productivity gains (Messmer et al. 1992). The data on genetic diversity among closely related lines, along with the morphological evaluation for various parameters, proved very effective in the selection of powdery mildew resistant lines that are genetically distant and morphologically excellent. Among the Elite-1 SHs, genotypes 80 and 75 were found most genetically variable and also exhibit best resistance against powdery mildew and good phenotypic characters and are recommended for future breeding efforts.

Molecular markers are powerful tools to identify gene of interest and have been used to genetically and physically locate *Pm* genes in the wheat genome. Using the Elite II and D-genome-derived advanced derivatives, four powdery mildew resistance genes (*Pm4b*, *Pm9*, *Pm16*, and *Pm30*) were found to be present and linked to four SSR markers and were used to identify powdery mildew resistant lines carrying these *Pm* genes. In our study, the SSR marker *Xgwm-382* amplified the PCR fragment in one Elite II and five D-genome-derived advanced derivatives with a size of 125 bp for *Pm4b*. Yi et al. (2008) confirmed that a 125-bp allele indicates the presence of *Pm4b* gene located on the chromosome 2AL . Two SSR markers were employed to detect the presence of *Pm9* resistance gene located on the long arm of chromosome 7AL. Polymorphism between the Elite II and the advanced derivatives were observed at the *Xgwm-4* and *Xgwm-332* SSR loci. A 253-bp fragment was observed at the *Xgwm-4* locus in only one Elite-II genotype but in 27 of the D-genome-derived advanced derivatives. A 212-bp fragment was observed at the *Xgwm-332* locus in one Elite-II genotype of and in 13 of the D-genome-derived advanced derivatives. Srnic et al. (2005) reported that *Pm9* was linked with the SSR locus *Xgwm-4* at 253 bp and *Xgwm-332* at 212 bp on chromosome 4AL and 7AL, respectively. Powdery mildew resistance genes *Pm16* and *Pm30* share common origin and chromosome location, short arm of chromosome 5B linked to SSR locus *Xgwm-159* at 201 bp (Chen et al. 2005). The result indicates that both genes were present in one Elite-II and two advanced derivative genotypes at 201 bp. We assume that the presence of these *Pm* genes in these respective genotypes that were resistant at both seedling and adult-plant stages indicates that all these genes are major genes.

Powdery mildew resistance conferred by the synthetic germ plasm lines utilized in this study with desirable morphological traits and molecular diversity should have utility in cultivar-development programs. The powdery mildew resistant genes with tightly linked and flanking markers identified and reported in this manuscript should aid in the incorporation of these powdery mildew resistance genes into future cultivars.

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Genetic diversity for some yield and quality traits in selected lines of the 4th Early Bread Wheat Yield Trial (4EBWYT).

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A randomized complete block design with three replications was used to characterize 20 4EBWYT genotypes for yield and quality characteristics. Plot size was 5 x 1.2 m^2. The end-use quality and utilization of wheat is highly dependent on the traits such as kernel texture, protein content, ash content, wet gluten content, and α-amylase activity. A significant genotypic effect was shown for all the characters studied except days-to-heading, plant height, and test weight.

Plant height ranged from 99.27 to 110.27 cm with an average of 105.70 cm. Days-to-heading ranged from 116.67 to 128 days with an average of 121.78. Most of the genotypes were not significantly different from the mean. Grain yield ranged from 1,034 to 3,025 g/plot. The genotypic effect was significant at $F_{0.05}$. Ten genotypes were significantly different from the mean, and the rest were non-significantly different (Table 13, p. 153).

Moisture content ranged from 8.57–9.9% with an average of 9.34%. The co-efficient of variation for moisture content was 3.36%, indicating lower variability among genotypes for this trait compared to other traits. Moisture content is greatly influenced by variation in the processing of the grain, the method of grinding, and variation in the climatic conditions and temperature during harvest (FAO 1999).

The maximum protein content was in 4EBWYT-520 (12.57%) and the minimum in 4EBWYT-527 (10.63%). All genotypes were significantly different at $CD_{0.05}$ except 4EBWYT-503. Mean protein content was 11.72% with a CV of 5.87%, indicating better variability among genotypes for this character. The protein content in Pakistani wheat cultivars ranged from 10.32–15.42% (Ahmad et al. 2001). Finney and Bolte (1985) recorded protein in the range of 9.0–14.6% in different wheat cultivars. The strong negative association between protein content and grain yield makes it difficult to breed for both traits. However, finding lines with high yield and high protein is of prime importance, and the nurseries consisting of higher-yielding genotypes can be used to identify lines to screen for higher protein content.

Ash content of these genotypes ranged from 2.77% (4EBWYT-506) to 1.50% (4EBWYT-512, 513, 514) with an average of 1.81%. All the genotypes were significantly different from the mean at $CD_{0.05}$. Ash content is the inorganic material left after flour is burned and is an important determinant of extraction rate and influences flour color and quality. None of these genotypes meet the criteria for good ash content, which ideally should be 0.39–0.42% (Li and Posner 1987). Zahoor (2003) reported that the ash content ranged from 0.30–0.53% in Pakistani wheat cultivars. The higher ash content found in these genotypes indicates the presence of a higher proportion of bran than for endosperm flour.

Maximum wet gluten was observed in 4EBWYT-507 (29.46%) and the minimum in 4EBWYT-525. The average wet gluten was 24.66%. Both replications and genotypes were significantly different (P = 0.05). Wet gluten has a strong effect on dough rheology and baking performance. Wet forms are more quickly incorporated into low protein flour than dry form (Czuchajowska and Paszczynska 1996) and also affects dough strength, gas retention and controlled expansion, structural enhancement, water absorption and retention, and natural flavor (Grausgruber et al. 2000; William 1997).

Thousand-kernel weight ranged from 29.80 (4EBWYT-504) to 47.17 g (4EBWYT-506) with an average of 39.79 g. The coefficient of variation (12.74%) was sufficient for this trait among genotypes. All genotypes were significantly different from the mean at $CD_{0.05}$. Both the replication and genotypic effects were significant at 0.05. This trait is a function of grain size and density. Wheat kernels can be classified according to grain weight; 15–25 g (very small), 26–35 g (small), 36–45 g (medium), 46–55 g (large), and over 55 g (very large) (Williams et al. 1986). According to this scale, most of the genotypes are of medium grain. Zanetti et al. (2001) reported 1,000-kernel weight in the range of 42.4–48.7 g in 128 wheat cultivars, whereas Anjum et al. (2002) reported a range of 31.43–37.28 g in Pakistani wheat cultivars.

The test weight (kg/hl) of these genotypes ranged from 49.92–77.43 kg/hl with an average of 72.60 kg/hl. Both replications and genotypes were not significantly different, however, genotype 4EBWYT-503 was significantly different from the mean at $CD_{0.05}$. Test weight is an important criterion in all wheat grading systems because it is a rough index of flour yield. Milling yield decreases rapidly with decreasing test weight. Previous studies indicated that this trait is

Table 13. Means and ANOVA for quality traits of 20 4th Early Bread Wheat Yield Trial genotypes. Numbers with an * are significant at CD $_{0.05}$; ** at 0.01 probability level, and NS indicates not significant.

Name	Pedigree	Days-to-heading	Plant height (cm)	Grain yield (g/plot)	Moisture (%)	Protein (%)	Ash content (%)	Wet gluten (%)	1,000-kernel weight (gm)	Test weight (kg/hl)	Falling number (Sec)	Hardness score
4EBWYT-502	WAXWING*2/KIRITATI	121.67	106.73	1,749.97*	9.20	12.17*	2.12*	25.52*	35.40*	73.33	334.33*	51.33*
4EBWYT-503	KIRITATI/4/2*SERI.1B*2/3/ KAUZ*2/BOW//KAUZ	120.33	110.27*	3,025.84*	9.60*	11.77	1.70*	24.22*	39.67*	49.92*	452.00*	52.33
4EBWYT-504	SERI/RAYON*2//PFAU/WEAVER	118.00	108.80	1,268.86*	9.10*	12.33*	2.13*	26.29*	29.80*	70.42	419.67*	54.33*
4EBWYT-505	SAAR/2*WAXWING	121.33	102.27	1,924.83	9.13*	11.93*	2.19*	25.57*	31.90*	72.62	326.67*	49.33*
4EBWYT-506	SERI.1B*2/3/KAUZ*2/BOW// KAUZ*2/5/CNO79//…	116.67*	113.67*	2,186.68	9.90*	11.33*	2.77*	23.24*	47.17*	76.18	303.33*	52.67
4EBWYT-507	PBW343*2/KUKUNA/3/PASTOR// CHIL/PRL/4/…	121.00	102.33	2,747.28*	9.40	12.33*	2.29*	29.46*	44.82*	74.03	430.00*	52.33
4EBWYT-508	WHEAR//INQLAB91*2/TUKURU	119.33	105.47	3,373.96*	9.20	12.37*	2.51*	27.45*	43.03*	77.18	478.67*	55.67*
4EBWYT-509	PBW343*2/KUKUNA//PBW343*2/ KUKUNA	123.00	98.60*	2,651.82*	9.40	11.53*	1.61*	25.29*	43.00*	69.68	483.00*	52.33
4EBWYT-512	CNDO/R134//ENTE/MEXI_2/3/…..	121.67	106.33	2,360.51	9.60*	11.30*	1.50*	23.07*	41.03*	74.60	417.00*	54.33*
4EBWYT-513	MINO/898.97	123.33	107.00	1,888.64	9.47	11.30*	1.50*	24.12*	44.60*	74.75	399.67	54.00*
4EBWYT-514	KIRITATI//SERI/RAYON	124.67	99.27*	1,589.59*	9.27	11.67	1.50*	23.20*	42.80*	77.43	399.00	50.33*
4EBWYT-515	WBLL1*2/BRAMBLING	128.00*	107.67	2,496.25	9.30	10.77*	1.48*	24.54	45.27*	74.42	395.00	51.67
4EBWYT-517	WBLL1*2/KIRITATI	121.33	108.87	2,091.83	8.87*	11.63*	1.62*	23.49*	36.00*	70.53	398.00	52.00
4EBWYT-518	WBLL1*2/KIRITATI	122.00	109.40	2,374.90	9.27	10.87*	1.60*	22.37*	42.40*	73.62	393.33	51.67
4EBWYT-519	PRL/2*PASTOR//PBW343*2/ KUKUNA	121.00	101.47*	2,563.86	9.20	12.37*	1.58*	26.22*	47.00*	73.92	356.00*	55.33*
4EBWYT-520	PBW343/HUITES/4/YAR/ AE.SQUAROSSA(783)//	121.00	107.13	2,048.26	9.87*	12.57*	1.54*	24.58	40.59*	74.63	376.00*	51.33*
4EBWYT-524	PFAU/SERI.1B//AMAD*2/3/ PBW343*2/KUKUNA	123.67	103.20	1,034.39*	9.87*	12.53*	1.67*	28.18*	31.60*	69.47	366.67*	52.00
4EBWYT-525	PFAU/SERI.1B//AMAD*2/3/ PBW343*2/KUKUNA	121.00	107.33	2,341.16	9.27	11.20*	1.70*	28.96*	37.23*	73.13	448.67*	53.33
4EBWYT-527	WAXWING*2//PBW343*2/KUKU-NA	124.33	105.20	1,299.60*	8.57*	10.63*	1.49*	20.57*	34.20*	74.13	425.33*	54.67*
4EBWYT-530	WHEAR//2*PRL/2*PASTOR	122.33	103.07	2,966.33*	9.13*	12.03*	1.71*	23.60*	42.37*	72.10	384.00	51.00*
	Mean	121.78	105.70	2,199.23	9.33	11.73	1.81	25.00	39.99	72.22	399.32	52.60
	SED	2.84	2.73	303.6	0.11	0.08	0.03	0.18	0.05	7.96	11.00	0.68
	CV (%)	2.85	3.16	16.88	1.45	0.81	2.20	0.87	0.16	13.50	3.37	1.57
	ANOVA											
	Replication	NS	NS	NS	**	**	**	**	**	NS	NS	NS
	Genotype	NS	NS	**	**	**	**	**	**	NS	**	**
	C.D $_{0.05}$	5.70	5.48	609.16	0.26	0.16	0.07	0.36	0.11	16.00	22.11	1.37

controlled not only genetically, but environmental conditions also affect this trait (Halverson and Zeleny 1988). Pushman and Bingham (1975) reported that test weight provides a useful guide to flour yield but is likely to be misleading for comparison between cultivars.

Falling number of the genotypes ranged from 303.33–483 sec with an average of 397.71. Replications were not significant, whereas genotypes were significant at $P_{0.05}$. Fourteen genotypes were found significant at $CD_{0.05}$. Falling number is an important determinant of α-amylase activity and an indicator of sprout damage and set up ability of the flour. Mailhott and Patton (1988) reported that all types of bread flour should have falling number values in between 200–300. Wheat flour with a falling number higher than 400 has very low or no α-amylase activity. In this study, nine genotypes have a falling number greater than 400; 4EBWYT-506 has the lowest value for this trait at 303.3 sec.

Grain texture is the most important trait which determines hardness or softness of wheat. Hardness scores ranged from 49.33–55.67 with an average of 52.54. Replications were not significant, whereas genotypes were statistically significant. Eleven genotypes were significantly different from mean, and the others were not significant at $CD_{0.05}$. Grain hardness is the key determinant for the classification and end -product quality in wheat (Campbell et al. 1999). Grain hardness primarily influences rheological properties of dough (Martinant et al. 1998). The most important physical difference between the endosperm of hard and soft wheat lies in the adhesion between the starch granules and the surrounding protein matrix (Simmonds et al. 1973). All these wheat genotypes fall into the category of soft wheat according to NIR hardness scale of Williams et al. (1986). Some authors also report that kernel size exerts an effect on grain hardness, however, differ in their opinion about the extent of the effect. Williams et al. (1987) emphasized that kernel size exert a small effect, whereas Pomeranz et al. (1988) reported direct effect of kernel size on grain hardness.

These genotypes were found promising for better yield in Pakistan. Most had good characteristics for useful quality traits and offered good variability. A detailed analysis of quality traits is required in order to exploit these genotypes further via use in recombination breeding programs.

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Physio-chemical characterization in relation to bread-making quality of some candidate wheat genotypes.

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Eight candidate genotypes and the cultivar C-591 were characterized for different physio-chemical characteristics, test weight, wet gluten, dry gluten, falling number, protein content, 1,000-kernel weight, and grain hardness score, at the Grain Quality Testing Laboratory, Karachi. The allelic variations at the *Glu-1* and *Glu-3* loci were determined by SDS–PAGE at the Wheat Wide Crosses and Cytogenetics, Laboratory, NARC, Islamabad. C-591 is cultivar of the pre-Green Revolution Era and is considered excellent for bread-making quality in Pakistan.

High-molecular-weight glutenins subunits (HMW–GS) resolved by SDS–PAGE determined that at locus *Glu-A1*, all the genotypes possessed either allele *Glu-A1a* or *Glu-A1b* encoding subunits 1Dx1 and 1Dx2*, respectively (Table 14). The null allele at this locus was absent. The two subunits found at this locus impart better quality characteristics in terms of bread-making qual (Liang et al. 2010). At the Glu-B1 locus, Kazi-1, and Kazi-2 had allele Glu-B1i encoding subunits 1Bx17+1By18. All other candidate lines had the Glu-B1b allele encoding subunit 1Bx7+1By18. These two subunits at the Glu-B1 locus impart better quality characteristics than do other other subunits at this locus (Payne et al. 1980). At the Glu-D1 locus, all the candidate lines possessed the alleles for Glu-D1d encoding subunits 1Dx5+1Dy10. Gupta and MacRitchie (1994) ity established that 1Dx5+1Dy10 had a stronger effect on dough strength by producing a greater proportion of larger-size glutenin polymers.

Low-molecular-weight glutenin subunits (LMW–GS) were determined by SDS–PAGE and revealed that at the *Glu-A3* locus, the two candidate lines Kazi-1 and Kazi-2 possessed *Glu-A3b*; three lines Kazi-6, Kazi-7, and Kazi-8 had *Glu-A3d*; and the remaining three lines had *Glu-A3e*. Genotypes with *Glu-A3b* are known to possess better quality parameters, such as sedimentation volume, mixing time, and dough development time (Luo et al. 2001; Liang et al. 2010). At the *Glu-B3* locus, Kazi-3 possessed *Glu-B3b*, Kazi-1 and Kazi-4 had *Glu-B3g*, and the remaining lines possessed *Glu-B3d*. *Glu-B3j*, associated with the T1BL·1RS translocation, was absent in these genotypes predicting the absence of a rye chromosome arm in these candidate lines. Previous studies indicated that *Glu-B3b* and *Glu-B3g* are the desirable alleles and gave high quality values (Luo et al. 2001).

Characteristics such as test weight, moisture content, and protein contents were more consistent than other characteristics (Table 14, p. 156). Test weight is measure of the bulk density of grains, expresses soundness and maturity of the grains (Donelson et al. 2002), and is positively correlated with milling yield (Halverson and Zeleny 1988). The test weight ranged from 72.6 to 74.9 kg/hl and is within the desired range reported by Paliwal and Singh (1985). The cultivar C-591 showed the maximum test weight and was significantly different from all the other genotypes (Table 14, p. 156).

Thousand-kernel weight is an important character determining grain yield and grain quality and also reflects soundness of the grains. This trait ranged from 34.4–42.8 g. C-591 had a very low 1,000-kernel weight (36.4g). Improvement in 1,000-kernel weight not only improves yield but also improves milling yield. The grain weight of these candidate lines is higher than average, which is desirable.

The moisture content of these candidate lines ranged from 10.5–11.0% and for C-591 was 9.9%. All candidate lines did not differ significantly from each other or from C-591 (Table 14, p. 156). Many genetic and nongenetic factors

are known to influence moisture content but is mainly influenced by the environmental factors and storage conditions due to hygroscopic nature of the grain (Whiteley 1970). These findings agree with the range of reported by the Anjum and Walker (2000) and Ijaz et al. (2001), indicating that these genotypes can be stored easily due to low moisture content and will be less prone to microbial attack (Zeleny 1991).

Wet gluten ranged from 18.4 to 22.8% with the maximum exhibited by Kazi-1 (Table 14). C-591 had 27.1% more wet gluten than all the candidate lines and was significantly different from all the other genotypes. Gluten content has a significant impact on bread-baking potential of wheat flour (Kent and Evers 1994). Wheat cultivars with higher wet gluten content are suitable for breadmaking, and those with low gluten content can be exploited for other bakery products. Crop year and other environmental factors also influence wet gluten content (Anjum and Walker 2000; Ijaz et al. 2001). The wet gluten content of these lines fell into the desirable category.

Falling number is an indirect method to determine α-amylase activity. The enzyme α-amylase degrades starch to a mixture of glucose and maltose. Screening for α-amylase activity has a high priority in most wheat breeding programs, because the great majority of wheat products are adversely affected by this enzyme (Blackman and Payne 1990). The falling number of our candidate lines ranged from 316–422 sec. The cultivar C-591 had a value of 344 sec. This character varied significantly compared to all other characters (Table 14). A falling number value range from 200–400 sec is considered ideal for bread-making quality (Mailhot and Patton 1988) and values ranging from 350–400 possess a very low α-amylase activity. From these results, these candidate lines have desirable a-amylase activity values.

The protein content of these lines ranged from 9.9–11.0% and did not vary significantly (Table 14). C-591 had a protein content of 12.5%. Protein content is considered to be an important quality criterion governing end-use quality. Protein content not only is an inherited character but also depends on environmental factors (Bushuk et al. 1969). Kent (1983) reported that a protein content between 6–21% among different wheat genotypes is mainly influenced by edaphic factors such as soil, climatic conditions, and fertilizer use.

The grain hardness of the candidate lines ranged from 45–48, and C-591 had a hardness score of 45. Genotypes with grain hardness ranging from 40–50 have ideal bread-making quality. Hard wheat flour is suitable for bread and pasta products use very hard flour. Grain hardness also is negatively correlated with cookie diameter, because damaged starch increases water absorption capacity and viscosity, which hinders cookie spread (Monsalve-Gonzalez and Pomeranz 1993) . These candidate lines had the desirable allelic variation at the *Glu-1* and *Glu-3* loci, and their other physio-chemical characteristics make them acceptable for bread and chapatti making.

Table 14. Physio-chemcial characteristics of candidate wheat lines and C-591 (P = 0.05).

Line	*Glu-1* subunits	*Glu-3* (*Glu-A3*, *Glu-B3* alleles)	Test weight (kg/hl)	1,000-kernel weight (gm)	Moisture content (%)	Wet gluten (%)	Dry gluten (%)	Water binding in wet gluten (%)	Falling number (sec)	Protein (%)	Hardness score
KAZI-1	2*, 17+18, 5+10	b, g	73.9 abc	41.4 ab	10.5 a	22.8 b	10.2 abc	12.6 ab	377 e	10.6 ab	45 bc
KAZI-2	2*, 17+18, 5+10	b, d	73.9 bc	43.0 a	10.7 a	20.2 bcd	8.5 bc	11.7 b	372 f	10.7 ab	45 bc
KAZI-3	1, 7+8, 5+10	e, b	73.8 bc	42.2 a	11.0 a	19.6 cd	8.2 c	11.4 b	362 g	11 ab	45 c
KAZI-4	1, 7+8, 5+10	e, g	72.6 c	39.6 b	10.8 a	21.1 bc	9.5 bc	11.6 b	422 a	10.5 b	47 ab
KAZI-5	2*, 7+8, 5+10	e, d	74.7 ab	43.0 a	10.8 a	21.8 b	12.1 ab	9.7 b	401 c	10.6 b	47 ab
KAZI-6	2*, 7+8, 5+10	d, d	74.9 ab	42.4 a	11.0 a	22.0 b	10.6 abc	11.4 b	419 b	9.9 b	46 bc
KAZI-7	2*, 7+8, 5+10	d, d	74.9 ab	42.8 a	10.6 a	18.4 d	9.7 bc	8.7 b	394 d	9.9 b	45 c
KAZI-8	2*, 7+8, 5+10	d, d	72.6 bc	34.4 d	10.7 a	22.6 b	12.6 a	10.0 b	316 i	10.9 ab	48 a
C-591	1, 13+16, 5+10	b, b	76.6 a	36.4 c	9.9 a	27.1 a	10.8 abc	16.3 a	344 h	12.5 a	45 c

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Allelic variation and composition of HMW–GS in bread wheat/synthetic derivatives.

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Improving the bread-making quality of wheat by utilizing allelic variation and composition of the high-molecular-weight glutenin subunit *Glu-1* (HMW–GS) loci in novel genetic resources is actively going on in the Wheat Wide Crosses Laboratories in Islamabad, Pakistan. The HMW–GS composition of 202 F_7 advanced lines obtained by crosses between 135 different types of bread wheat with synthetic hexaploid wheats was studied using SDS–PAGE. A total of 23 allelic variants and 61 HMW–GS combinations were observed at the *Glu-A1*, *Glu-B1*, and *Glu-D1* loci. In breadwheat, the *Glu-D1* locus usually is characterized by subunits 1Dx2+1Dy12 and 1Dx5+1Dy10 with the latter having a stronger effect on bread-making quality and is observed predominantly in these advanced lines. The inferior subunit 1Dx2+1Dy12 was successfully replaced by other, better allelic variants at the *Glu-D1* locus inherited by these SH wheats from *Ae. tauschii*.

The HMW–GS were analyzed through horizontal SDS–PAGE following the Laemmli (1970) using Pavon, Chinese Spring, and Pak-17951 wheat as standards for identifying and comparing generated bands. The allelic classification at the *Glu-A1* and *Glu-B1* loci and the numbering of HMW–GS were based on the classification of Payne and Lawrence (1983). Alleles at the *Glu-D^{t}1* locus were identified according to William et. al. (1993). The quality score was calculated according to Payne et. al (1980) by adding together the scores of individual subunits.

The genetic diversity at each locus was calculated using Nei's index (Nei 1973), $H=1-\Sigma Pi^2$, with H and Pi denoting the genetic variation index and the frequency of the number of alleles at the locus, respectively. Allelic frequencies were determined by summing the frequencies of alleles in the individual accessions, irrespective of whether the HMW–GS composition was homogeneous or heterogeneous, and then dividing this total by the number of accessions.

Distribution of HMW–GS. Twenty-three HMW-GS allelic variants were detected in the 202 F_7 advanced lines (Table 15). At the *Glu-A1* locus, the composition of alleles were only contributed by x-type subunits, 1Ax1, 1Ax2*, and null, which are controlled by alleles *Glu-A1a*, *Glu-A1b*, and *Glu-A1c*, respectively. The null allele the most frequent, in oOver

62% of the F_7 advanced lines, followed by 1Ax2* (24.26%) and 1Ax1 (12.87%) (Table 16). The predominance of the null allele at this locus was previously reported by several workers. A higher proportion of the null allele in SHs was reported by Pena et. al (1995) and in the world collection of wheat cultivars by Payne and Lawrence (1983) supports the predominant presence of the null allele in these F_7 advanced lines derived from the 'synthetic/bread wheat' crosses. Recently, Fang et. al (2009) and Li et. al (2009) also reported a higher frequency of null alleles in Chinese genotypes. However, the frequency in the European spelt wheat genotypes was reported as minimal (An et al. 2005). Apart from the null allele, 75 (37.13%) of the 202 advanced lines had either 1Ax1 or 1Ax2*, which impart better quality to wheat flour and are associated with higher extensibility plus better dough strength (Branlard and Dardevet 1985; Khan et al. 1989).

Table 15. Number of alleles and combinations of *Glu-1* loci in F_7 advanced lines from 'bread wheat/ synthetic hexaploid' crosses

Locus	Number of alleles	x-type	y-type
Glu-A1	3	3	—
Glu-B1	8	5	5
Glu-D1	12	5	4
Glu-1 combinations	61	—	—

At the *Glu-B1* locus, five x-type subunits, 7, 6, 20, 13, and 17; five y-type subunits, 8, 9, 16, 18, and 15; and their eight combinations were detected (Table 15). Subunit 1Bx7 was found in 18 (8.91%) advanced lines and its combination with 1By8, 1By9, and 1By8 were found in 49 (24.25%) advanced lines (Table 16). The most frequent subunit, 1Bx17+1By18, encoded by *Glu-B1i*, was found in 75 (37.13%) advanced lines followed by 1Bx13+1By16, encoded by *Glu-B1f* in 36 (18.81%) genotypes. The other subunits found at this locus are 1Bx7+1By8 (8.91%), 1Bx7+1By9 (17.82%), 1Bx6+1By8 (4.45%), 1Bx20 (6.43%), and 1Bx7+1By15 (0.99%) encoded by alleles *GluB1b, Glu-B1c, Glu-B1d, GLu-B1e*, and *Glu-B1z*, respectively. The genetic diversity calculated by Nei's index at this locus was the maximum at 0.78. The durum parents of synthetic hexaploids (Pena et al. 1994) and SHs (Pena et al. 1995; Hsam et al. 2001) were known to possess subunits 1Bx7+1By8, 1Bx20, and 1Bx6+1By8 at this locus. Rashid et al. (2003), Masood et al. (2004), and Tayyaba et al. (2007) reported a higher proportion of 1Bx17+1By18 and 1Bx7+1By9 in land races and locally adapted cultivars of Pakistan. Because the studied genotypes consisted of derivatives from SHs and locally adapted germ plasm, a higher proportion (71.28%) of these subunits was observed in these advanced lines. The comparatively higher level of allelic diversity (H) at this locus is attributed in part to the allelic richness and to the diverse parental lines from different genetic backgrounds. Earlier, An et al. (2005), Li et al. (2009), Fang et al. (2009), and Moragues et al. (2006) also reported higher H at the *Glu-B1* locus.

Table 16. Allelic frequency and diversity at the *Glu-1* locus in F_7 advanced lines derived from 'bread wheat/synthetic hexaploid' crosses.

Locus	Allele	Subunit	Number of accessions	Frequency (%)	H (Nei's index)
Glu-A1	a	1	26	12.87	0.53
	b	2*	49	24.26	
	c	null	127	62.87	
Glu-B1	a	7	18	8.91	0.78
	b	7+8	11	5.44	
	c	7+9	36	17.82	
	d	6+8	9	4.45	
	e	20	13	6.43	
	f	13+16	38	18.81	
	i	17+18	75	37.13	
	z	7+15	2	0.99	
Glu-D1	a	2+12	54	26.73	0.70
	b	3+12	4	1.98	
	d	5+10	95	47.02	
	e	2+10	6	2.97	
	h	5+12	3	1.48	
	n	2.1+10	2	0.99	
	x	2+T2	5	2.47	
	z	3+10	3	1.48	
	ae	2.1+T2	4	1.98	
	ah	1.5+10	13	6.43	
	ai	2.1+10.5	7	3.46	
	aj	1.5+12	6	2.97	

The *Glu-D1* locus in these advanced lines contributed 12 alleles having the combination of five x-type and four y-type subunits (Tables 15 and 16). The x-type subunits include 2, 3, 5, 1.5, and 2.1 and y-type subunits include 10, 12, 10.5, and T_2. The most frequent subunit 1Dx5+1Dy10, encoded by allele *Glu-D1d*, was observed in 95 (47.02%) advanced lines followed by 1Dx2+1Dy12, encoded by allele *Glu-D1a*, in 54 (26.73%) advanced lines. The frequency of the other alleles at this locus was less than 3%, except for 1Dx1.5+1Dy10, which had a frequency of 6.43%. The genetic diversity according to Nei's index (H) was 0.70. The cultivars characterized by subunit pair 5+10, a superior and favorable allele imparting greater viscoelasticity and dough characteristics

(Popineau et al. 1994; Redaelli et al. 1997) are considered to be the best. This subunit combinationwas correlated with good bread-making quality characteristics in commercial wheat cultivars grown in Canada (Bushuk 1998), Germany (Wieser and Zimmermann 2000), the U.K. (Payne et al. 1987), Norway (Ulhen 1990), Syria (Mir ali et al. 1999), the United States (Dong et al. 1991), and New Zealand (Luo et al. 2001), and also in SH wheats (Peña et al. 1995). The allelic richness at *Glu-D1* is higher than *Glu-B1* but genetic diversity is slightly lower because two subunit pairs, 2+12 and 5+10, have a greater proportion in these advanced lines. The *Glu-D1* alleles h, n, x, ae, ah, ai, and aj, encoding subunits 1Dx5+1Dy12, 1Dx2.1+1Dy10, 1Dx2+1DyT2, 1Dx2.1+1DyT2, 1Dx1.5+1Dy10, 1Dx2.1+1Dy10.5, and 1Dx1.5+1Dy12, respectively, were incorporated into SHs from *Ae. tauschii* during the course of wide hybridization. These subunit pairs also were observed in these advanced lines, and their association with bread-making quality parameters was previously determined by Pena et al. (1995).

Composition of HMW-GS and *Glu-1* quality score. The HMW–GS compositionsand *Glu-1* quality scores are given in Table 17 (p. 159-165). The HMW-GS allelic composition found most frequently is null, 17+18, 5+10 in 19 out of 202 advanced lines. The other allelic compositions are 1, 17+18, 5+10 (15); null, 7+9, 2+12 (11); null, 7, 5+10; null, 13+16, 5+10; and null, 17+18, 2+12 (9). Twenty-five combinations appeared once in these lines. The *Glu-1* quality score ranged from 4–10 with an average of 7.4. The quality score of these lines is higher than those in German (Rogers et al. 1989) U.K. (Lukow et al. 1989), Danish (Payne et al. 1987), Chinese (Zhong-Hu et al. 1992), and Spanish wheats (Payne et al. 1988) and very close to those of Canadian, Australian, U.S, and Russian cultivars (Graybosch et al. 1990; Khan et al. 1989; Lawrence 1986; Lukow et al. 1989; Morgunov et al. 1990; Ng et al. 1989). The quality score of 54 advanced lines could not be determined because of the presence of rare alleles from *Ae. tauschi*i. However, Pena et al. (1995) determined the different quality characteristics of SHs having these subunits. They reported that groups with subunit pairs 2.1+10 or 1.5+10 had higher flour protein than groups with 2.1+T2. Groups with subunit pairs 1.5+10, 2.1+T2, 5+12, and 3+10 had larger bread loaf volume than those with pair 2+12, and no differences among these subunit pairs were observed in relation to sedimentation volume.

Table 17. High-molecular-weight glutenin subunit allele composition of F_7 advanced lines derived from 'bread wheat/ synthetic hexaploid' crosses (* indicates the *Ae. tauschii* accession number in Wide Crosses Program working collection, CIMMYT, Mexico).

S. No.	Pedigree	*Glu-1* subunits		
		Glu-A1	*Glu-B1*	*Glu-D1*
1	GAN/AE.SQUARROSA (897)//OPATA x D67.2/P66.270//AE.SQUARROSA (223)*	N	7	2+12
2	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)/6/CETA/… x 68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (783)	N	7	2+12
3	Opata x DOY1/AE.SQUARROSA (458)	N	7	2+12
4	MAYOOR/TK SN1081/AE.SQUARROSA (222)/3/PASTOR x CROC_1/ AE.SQUARROSA (444)	N	7	5+10
5	Opata x CROC_1/ AE.SQUARROSA (886)	N	7	5+10
6	Opata x GAN/ AE.SQUARROSA (408)	N	7	5+10
7	Opata x DOY1/AE.SQUARROSA (458)	N	7	5+10
8	Opata x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (629)	N	7	5+10
9	Not Available	N	7	5+10
10	Not Available	N	7	5+10
11	Not Available	N	7	5+10
12	GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA (895)/3/MAIZ/4/IN-QALAB 91 x BKH-94	N	7	5+10
13	M. OPATA-108 x CETA/AE.SQUARROSA (895)	N	7	1.5+10
14	Opata x CETA/AE.SQUARROSA) (895)	N	7+8	2+12
15	MAYOOR//TK SN1081/AE.SQUARROSA) (222)/3/CBRD x ARLIN_1/T. MONOCOCCUM (95)	N	7+8	2+12
16	Opata x ALTAR 84.AE.SQUARROSA (J BANGOR)	N	7+8	2+12
17	Opata x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	N	7+8	1.5+10
18	M.Opata 164 x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	N	7+8	1.5+10

Table 17 (continued). High-molecular-weight glutenin subunit allele composition of F_7 advanced lines derived from 'bread wheat/synthetic hexaploid' crosses (* indicates the *Ae. tauschii* accession number in Wide Crosses Program working collection, CIMMYT, Mexico).

S. No.	Pedigree	*Glu-1* subunits		
		Glu-A1	*Glu-B1*	*Glu-D1*
19	Opata x DOY 1/AE.SQUARROSA (517)	N	7+8	2.1+10.5
20	Opata x 68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (783)	N	7+8	1.5+12
21	Not Available	N	7+8	1.5+12
22	Opata x DOY1/AE.SQUARROSA (372)	N	7+9	2+12
23	ALTAR 84/AE.SQUARROSA (221)//YACO x 68.111/RGB-U//WARD/3/ FGO/4/RABI/5/AE.SQUARROSA (878)	N	7+9	2+12
24	Opata x CETA/AE.SQUARROSA (1031)	N	7+9	2+12
25	Opata x DOY1/AE.SQUARROSA (372)	N	7+9	2+12
26	Opata x CPI/GEDIZ/3/GOO//JO/CRA/4/AE.SQUARROSA (227)	N	7+9	2+12
27	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)/5/OAPTA x 68.111/ RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (783)	N	7+9	2+12
28	Opata x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	N	7+9	2+12
29	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)/5/OAPTA x 68.111/ RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (783)	N	7+9	2+12
30	MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/PASTOR x SARSABZ	N	7+9	2+12
31	TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/6/ FCT x Opata	N	7+9	2+12
32	Opata x 68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (1038)	N	7+9	2+12
33	Opata x 68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (1038)	N	7+9	5+10
34	ALTAR 84/AE.SQUARROSA (224)//2*YACO/3/MAYOOR/TK SN1081/ AE.SQUARROSA/) (222)/4/KUKUN x GAN/AE.SQUARROSA (248)	N	7+9	5+10
35	CNDO/R143//ENTE/MEXI_2/3/AE.SQUARROSA (TAUS)/4/ WEAVER/5/2*KAUZ x DOY1/AE.SQUARROSA (458)	N	7+9	5+10
36	Opata x ALTAR 84.AE.SQUARROSA (J BANGOR)	N	7+9	5+10
37	Opata x ALTAR 84/AE.SQUARROSA (205)	N	7+9	5+10
38	Opata x 74 INQALAB 91/TSAPKI	N	7+9	5+10
39	URES/PRL//BAV92 x YAV_2/TEZ//AE.SQUARROSA (249)	N	7+9	2+10
40	GAN/AE.SQUARROSA(236)//CETA/AE.SQUARROSA (895)/3/MAIZ/4/IN-QALAB 91 x BKH-94	N	7+9	5+12
41	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)/6/CETA/… x CETA/AE.SQUARROSA (895)	N	7+9	2+T2
42	Opata x CPI/GEDIZ/3/GOO//JO/CRA/4/AE.SQUARROSA (273)	N	7+9	2.1+T2
43	CROC-1/AE.SQUARROSA) (224)//KAUZ x CETA/AE.SQUARROSA (895)	N	7+9	2.1+10.5
44	Not Available	N	7+9	1.5+12
45	MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/PASTOR x GAN/ AE.SQUARROSA (259)	N	6+8	2+12
46	162 CHAPIO/ INQALAB 91 x 68.111/RGB-U//WARD/3/AE.SQUARROSA (452)	N	6+8	2+12
47	Opata x CETA/AE.SQUARROSA (1027)	N	6+8	2+T2
48	Opata x CETA/AE.SQUARROSA) (895)	N	6+8	2+10.5
49	Opata x DOY1/AE.SQUARROSA (1024)	N	6+8	2.1+10.5
50	MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT x	N	20	2+12
51	Not Available	N	20	5+10
52	CHIR3/CBRD x GAN/AE.SQUARROSA (897)//OPATA	N	20	5+10
53	Opata x BORLOUG M95	N	20	5+10
54	SERI x 68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (392)	N	20	5+10

Table 17 (continued). High-molecular-weight glutenin subunit allele composition of F_7 advanced lines derived from 'bread wheat/synthetic hexaploid' crosses (* indicates the *Ae. tauschii* accession number in Wide Crosses Program working collection, CIMMYT, Mexico).

S. No.	Pedigree	*Glu-1* subunits		
		Glu-A1	*Glu-B1*	*Glu-D1*
55	Opata x SCA/AE.SQUARROSA (518)	N	20	5+10
56	139 CHAPIO/INQALAB 91 x PICUS/3/KAUZ*2/BOW//KAUZ	N	13+16	2+12
57	182 SAAR/INQALAB 91 x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/ CBRD	N	13+16	2+12
58	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)/5/OAPTA x 68.111/ RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (783)	N	13+16	2+12
59	Not Available	N	13+16	2+12
60	Not Available	N	13+16	2+12
61	Not Available	N	13+16	2+12
62	Not Available	N	13+16	2+12
63	ALTAR 84/AE.SQUARROSA (193) x PASTOR	N	13+16	2+12
64	MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/PASTOR x SARSABZ	N	13+16	3+12
65	MAYOOR//TK SN1081/AE.SQUARROSA) (222)/3/CBRD x KAMBARA	N	13+16	3+12
66	182 SAAR/INQALAB 91 x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/ CBRD	N	13+16	5+10
67	Not Available	N	13+16	5+10
68	BAV x (MAYOOR//TK SN1081/AE.SQUARROSA) (222)/3/CBRD	N	13+16	5+10
69	Opata x D67.2//P66.270//AE.SQUARROSA (257)/3/OPATA	N	13+16	5+10
70	CROC-1/AE.SQUARROSA) (205)//BORL95 x	N	13+16	5+10
71	KAMBARA x Opata	N	13+16	5+10
72	Not Available	N	13+16	5+10
73	ALTAR 84/AE.SQUARROSA (193) x PASTOR	N	13+16	5+10
74	GAN/AE.SQUARROSA (236)//CETA/AE.SQUARROSA (895)/3/MAIZ/4/IN-QALAB 91 x BKH-94	N	13+16	5+10
75	M.Opata 164 x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	N	13+16	2.1+10
76	D67.2/P66.270//AE.SQUARROSA (223) x ARLIN_1/T.MONOCOCCUM (95)	N	13+16	1.5+10
77	149 CHAPIO/INQALAB 91 x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/ AE.SQUARROSA (878)	N	13+16	1.5+10
78	M.Opata 164 x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	N	13+16	1.5+10
79	M. OPATA-164 x /4/RABI/5/AE.SQUARROSA (878) 68.111/RGB U//WARD/3/ FGO	N	13+16	1.5+10
80	CHIR3/CBRD x Opata	N	13+16	1.5+10
81	M.Opata 164 x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	N	13+16	1.5+12
82	SARSABZ x CHIR3/CBRD	N	13+16	1.5+12
83	Opata x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	N	17+18	2+12
84	MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/PASTOR x GAN/ AE.SQUARROSA (259)	N	17+18	2+12
85	BAKHTAWAR 94 x 68.111/RGB-U//WARD RESEL/3/STIL/4/ AE.SQUARROSA (431)	N	17+18	2+12
86	162 CHAPIO/ INQALAB 91 x 68.111/RGB-U//WARD/3/AE.SQUARROSA (452)	N	17+18	2+12
87	Opata x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (629)	N	17+18	2+12
88	MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT x YAV_3/SCO//JO69/ CRA/3/YAV/79/4/AE.SQUARROSA (498)/5/OPATA	N	17+18	2+12
89	Not Available	N	17+18	2+12

Table 17 (continued). High-molecular-weight glutenin subunit allele composition of F_7 advanced lines derived from 'bread wheat/synthetic hexaploid' crosses (* indicates the *Ae. tauschii* accession number in Wide Crosses Program working collection, CIMMYT, Mexico).

S. No.	Pedigree	*Glu-1* subunits		
		Glu-A1	*Glu-B1*	*Glu-D1*
90	Not Available	N	17+18	2+12
91	Opata x DVERD_2/AE.SQUARROSA (333)	N	17+18	2+12
92	Not Available	N	17+18	3+12
93	Opata x CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)/5/OAPTA	N	17+18	5+10
94	Opata x YAV_3/SCO//JO69/CRA/3/YAV/79/4/AE.SQUARROSA (498)/5/ OPATA	N	17+18	5+10
95	MAYOOR//TK SN1081/AE.SQUARROSA) (222)/3/CBRD x	N	17+18	5+10
96	Opata x CPI/GEDIZ/3/GOO//JO/CRA/4/AE.SQUARROSA (227)	N	17+18	5+10
97	Opata x CETA/AE.SQUARROSA (895)	N	17+18	5+10
98	Opata x 0 INQALAB 91/AC8528	N	17+18	5+10
99	Opata x ALTAR 84/AE.SQUARROSA (205)	N	17+18	5+10
100	Opata x 74 INQALAB 91/TSAPKI	N	17+18	5+10
101	Opata x DOY 1/AE.SQUARROSA (1026)	N	17+18	5+10
102	MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/PASTOR x MH-97	N	17+18	5+10
103	Not Available	N	17+18	5+10
104	Not Available	N	17+18	5+10
105	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	N	17+18	5+10
106	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	N	17+18	5+10
107	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	N	17+18	5+10
108	Opata x YAV_3/SCO//JO69/CRA/3/YAV/79/4/AE.SQUARROSA (498)/5/ OPATA	N	17+18	5+10
109	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	N	17+18	5+10
110	Not Available	N	17+18	5+10
111	INQILAB 91 (RABI) x	N	17+18	5+10
112	Not Available	N	17+18	2+10
113	Opata x CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)/5/OAPTA	N	17+18	2+10
114	Opata x D67.2/P66.270//T.BOEOTICUM (66)	N	17+18	5+12
115	MAYOOR//TK SN1081/AE.SQUARROSA (222)/4/SABUF/3/BCN//CETA/ AE.SQUARROSA (895) x GAN/AE.SQUARROSA (897)//OPATA	N	17+18	2.1+10
116	Opata x YAV_3/SCO//JO69/CRA/3/YAV/79/4/AE.SQUARROSA (498)/5/ OPATA	N	17+18	2+T2
117	M. OPATA-108 x DOY1/AE.SQUARROSA (372)	N	17+18	3+10
118	Opata x ALTAR 84.AE.SQUARROSA (J BANGOR)	N	17+18	3+10
119	KAUZ x MAYOOR//TK SN1081/AE.SQUARROSA) (222)/3/CBRD	N	17+18	1.5+10
120	MAYOOR//TK SN1081/AE.SQUARROSA) (222)/3/CBRD x CPI/GEDIZ/3/ GOO//JO/CRA/4/T.MONOCOCCUM (101)	N	17+18	1.5+10
121	DOYI/AE.SQUARROSA (1018) x CPI/GEDIZ/3/GOO//JO69/CRA/4/ AE.SQUARROSA (208)/5/OAPTA	N	17+18	1.5+10
122	Not Available	N	17+18	1.5+10
123	Not Available	N	17+18	1.5+10
124	M.Opata 164 x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	N	17+18	2.1+10.5
125	D67.2/P66.270//AE.SQUARROSA (223) x ARLIN_1/T.MONOCOCCUM (95)	N	17+18	2.1+10.5
126	D67.2//P66.270//AE.SQUARROSA (257)/3/OPATA x STY-US/CELTA//PALS/3/ SRN_5/4/AE.SQUARROSA (418)	N	7+15	2+12
127	CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)/5/OAPTA x 68.111/ RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	N	7+15	2+12

Table 17 (continued). High-molecular-weight glutenin subunit allele composition of F_7 advanced lines derived from 'bread wheat/synthetic hexaploid' crosses (* indicates the *Ae. tauschii* accession number in Wide Crosses Program working collection, CIMMYT, Mexico).

S. No.	Pedigree	*Glu-1* subunits		
		Glu-A1	*Glu-B1*	*Glu-D1*
128	BACANORA x 68.111/RGB-U//WARD	1	7	5+10
129	Not Available	1	7	5+10
130	Not Available	1	7	5+10
131	Inqilab x BORLOUG M95	1	7+9	5+10
132	144 ALTAR 84/ AE.SQUARROSA) (221)//YACO/3/ INQALAB 91 x D67.2/ P66.270//T.BOEOTICUM (66)	1	7+9	5+10
133	TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/6/ FCT x DOY1/AE.SQUARROSA (458)	1	7+9	3+10
134	Opata x BORLOUG M95	1	20	5+10
135	PBW-343*2/CHAPIO x D67.2/P66.270//T.BOEOTICUM (66)	1	20	5+10
136	CHIR3/CBRD x GAN/AE.SQUARROSA (897)//OPATA	1	20	5+T2
137	MAYOOR//TK SN1081/AE.SQUARROSA (222)/4/SABUF/3/BCN//CETA/ AE.SQUARROSA (895) x GAN/AE.SQUARROSA (897)//OPATA	1	20	5+T2
138	Not Available	1	20	2+10.5
139	TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/6/ FCT x DOY1/AE.SQUARROSA (458)	1	17+18	5+10
140	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	1	17+18	5+10
141	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	1	17+18	5+10
142	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	1	17+18	5+10
143	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	1	17+18	5+10
144	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	1	17+18	5+10
145	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	1	17+18	5+10
146	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	1	17+18	5+10
147	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	1	17+18	5+10
148	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	1	17+18	5+10
149	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	1	17+18	5+10
150	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	1	17+18	5+10
151	PASTOR x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	1	17+18	5+10
152	139 CHAPIO/INQALAB 91 x PICUS/3/KAUZ*2/BOW//KAUZ	1	17+18	5+10
153	TURACO/5/CHIR3/4/SIREN//ALTAR 84/AE.SQUARROSA (205)/3/3*BUC/6/ FCT x Opata	1	17+18	5+10
154	ALTAR 84/AE.SQUARROSA (224)//2*YACO/3/MAYOOR/TK SN1081/ AE.SQUARROSA/) (222)/4/KUKUN x GAN/AE.SQUARROSA (248)	2*	7	5+10
155	Not Available	2*	7	5+10
156	Opata x SCA/AE.SQUARROSA (518)	2*	7+8	2+12
157	Opata x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	2*	7+8	5+10
158	DOYI/AE.SQUARROSA (1018) x Opata CPI/GEDIZ/3/GOO//JO69/CRA/4/ AE.SQUARROSA (208)/5/OAPTA	2*	7+8	5+10
159	Opata x GAN/AE.SQUARROSA (248)	2*	7+9	2+12
160	RABE/2*MO88 x Opata	2*	7+9	2+12
161	Opata x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)	2*	7+9	2+12
162	KAUZ x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	2*	7+9	2+12
163	Opata x CETA/AE.SQUARROSA) (895)	2*	7+9	5+10
164	MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD x CPI/GEDIZ/3/ GOO//JO/CRA/4/T.MONOCOCCUM (101)	2*	7+9	5+10
165	Not Available	2*	7+9	5+10
166	Opata x DOY1/AE.SQUARROSA (1024)	2*	7+9	2+10

Table 17 (continued). High-molecular-weight glutenin subunit allele composition of F_7 advanced lines derived from 'bread wheat/synthetic hexaploid' crosses (* indicates the *Ae. tauschii* accession number in Wide Crosses Program working collection, CIMMYT, Mexico).

S. No.	Pedigree	*Glu-1* subunits		
		Glu-A1	*Glu-B1*	*Glu-D1*
167	Opata x DOY1/AE.SQUARROSA (515)	2*	7+9	2+10
168	DOYI/AE.SQUARROSA (1018) x Opata CPI/GEDIZ/3/GOO//JO69/CRA/4/ AE.SQUARROSA (208)/5/OAPTA	2*	7+9	2+10
169	Opata x CPI/GEDIZ/3/GOO//JO69/CRA/4/AE.SQUARROSA (208)/5/OAPTA	2*	6+8	5+10
170	Not Available	2*	6+8	5+10
171	MAYOOR//TK SN1081/AE.SQUARROSA) (222)/3/CBRD x CPI/GEDIZ/3/ GOO//JO/CRA/4/T.MONOCOCCUM (101)	2*	6+8	5+10
172	ALTAR 84/AE.SQUARROSA (224)//2*YACO/7/OPATA/6/68.111RGB-U// WARD/3/FGO/4/… x 162 SAAR/INQALAB 91	2*	20	5+10
173	MAYOOR//TK SN1081/AE.SQUARROSA) (222)/3/CBRD x	2*	20	5+T2
174	Opata x DOY1/AE.SQUARROSA (458)	2*	13+16	2+12
175	CNDO/R143//ENTE/MEXI_2/3/AE.SQUARROSA (TAUS)/4/ WEAVER/5/2*KAUZ x DOY1/AE.SQUARROSA (458)	2*	13+16	2+12
176	SAT-5/PBW-343 x DOY1/AE.SQUARROSA (188)	2*	13+16	2+12
177	Not Available	2*	13+16	2+12
178	Opata x DOY 1/AE.SQUARROSA (255)	2*	13+16	5+10
179	Opata x 0 INQALAB 91/FISCAL	2*	13+16	5+10
180	Opata x CETA/AE.SQUARROSA (1031)	2*	13+16	5+10
181	Opata x ROK/KML// AE.SQUARROSA (214)	2*	13+16	5+10
182	182 SAAR/INQALAB 91 x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/ CBRD	2*	13+16	5+10
183	MAYOOR//TK SN1081/AE.SQUARROSA) (222)/3/CBRD x KAMBARA	2*	13+16	5+10
184	ALTAR 84/AE.SQUARROSA (224)//2*YACO/3/MAYOOR//TK SN1081/ AE.SQUARROSA (222)/4/KUKUN x ALTAR 84/AE.SQUARROSA (221)// YACO	2*	13+16	5+10
185	Opata x AE.SQUARROSA (1026)/DOY 1	2*	17+18	2+12
186	87 INQALAB 91/TSAPKI x SCA/AE.SQUARROSA (518)	2*	17+18	2+12
187	MAYOOR//TK SN1081/AE.SQUARROSA) (222)/3/CBRD x ARLIN_1/T. MONOCOCCUM (95)	2*	17+18	2+12
188	DOYI/AE.SQUARROSA (1018) x CPI/GEDIZ/3/GOO//JO69/CRA/4/ AE.SQUARROSA (208)/5/OAPTA	2*	17+18	2+12
189	KAUZ x MAYOOR//TK SN1081/AE.SQUARROSA (222)/3/CBRD	2*	17+18	2+12
190	SARSABZ x CHIR3/CBRD	2*	17+18	2+12
191	MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT x Opata	2*	17+18	3+12
192	Opata x CROC_1/AE.SQUARROSA (444)	2*	17+18	5+10
193	M. OPATA-108 x DOY1/AE.SQUARROSA (372)	2*	17+18	5+10
194	Opata x 68.112/WARD//AE.SQUARROSA (369)	2*	17+18	5+10
195	ALTAR 84/AE.SQUARROSA (224)//2*YACO/7/OPATA/6/68.111RGB-U// WARD/3/FGO/4/… x 162 SAAR/INQALAB 91	2*	17+18	5+10
196	Opata x 68.111/RGB-U//WARD RESEL/3/STIL/4/AE.SQUARROSA (1038)	2*	17+18	5+10
197	139 CHAPIO/INQALAB 91 x PICUS/3/KAUZ*2/BOW//KAUZ	2*	17+18	5+10
198	GAN/AE.SQUARROSA (236)//CETA/AE.SQUARROSA (895)/3/MAIZ/4/IN-QALAB 91 x PBW-343	2*	17+18	5+10
199	MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/FCT x Opata YAV_3/SCO// JO69/CRA/3/YAV/79/4/AE.SQUARROSA (498)/5/OPATA	2*	17+18	5+12
200	D67.2/P66.270//AE.SQUARROSA (223) x ARLIN_1/T.MONOCOCCUM (95)	2*	17+18	2+T2
201	139 CHAPIO/INQALAB 91 x PICUS/3/KAUZ*2/BOW//KAUZ	2*	17+18	1.5+12

Table 17 (continued). High-molecular-weight glutenin subunit allele composition of F_7 advanced lines derived from 'bread wheat/synthetic hexaploid' crosses (* indicates the *Ae. tauschii* accession number in Wide Crosses Program working collection, CIMMYT, Mexico).

S. No.	Pedigree	*Glu-1* subunits		
		Glu-A1	*Glu-B1*	*Glu-D1*
202	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (878)/6/CETA/… x CETA/AE.SQUARROSA (895)	2*	6+9	5+T2

After establishing the importance of *Glu-D^{1}1* encoded proteins, many attempts have been made to improve bread-making quality by increasing their genetic variability (Lagudah et al. 1987; William et al. 1993). Our data describes the end-use quality of the newly developed breeding material by combining the HMW–GS from different genetic backgrounds and selecting superior combinations. Most of this material has superior HMW–GS from *Glu-B1* (7+8, 7+9 17+18 and 13+16) and *Glu-D^{1}1* (5+10). In the conventional existing germ plasm, the *Glu-D1* locus is usually present as 1Dx5+1Dy10 or 1Dx2+1Dy12, however, the proportion of 1Dx2+1Dy12 is comparatively very high.

Our most important finding is that the subunit pair 1Dx2+1Dy12 from the *Glu-D1* locus, which has the most negative association with protein quality, weak gluten, and low sedimentation value (Ulhen 1990) is replaced by other variants at this locus using the D-genome SHs, and these subunits are inherited from *Ae. tauschii*. Hsam et al. (2001) also reported while conducting a microbaking test that rheological properties of the gluten as well as bread volume in synthetic wheats depends on the inherent properties of *Ae. tauschii* accessions.

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Potential of A-genome amphiploids (2n=6x=42, AAAABB) to improve bread-making quality: allelic variation at the Glu-1 and Glu-3 loci.

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In durum and common wheat, the HMW-GS *1Ay* is always absent but may be expressed in wild diploid and tetraploid wheats (Waines and Payne 1987). Ciaffi et al. (1995) indicated that the presence of active *Ay* genes could have a clear effect on bread-making quality. The evolution and domestication hexaploid wheat yielded a high degree of genetic erosion, assessed by studing these proteins, which might result in a reduced potential for successful breeding for wheat quality. Consequently, the search for new alleles, especially the active *Ay* gene, is very important. The diploid wild wheat species could be interesting candidates (Caballero et al. 2008). At the diploid level, the three main species *T. monococcum* subsp. *monococcum*, *T. monococcum* subsp. *aegilopoides*, and *T. urartu* (2n=2x=14) (Johnson 1975; Miller 1987) may be important sources of seed-storage proteins for enlarging the gene pool of cultivated wheats. Later studies at molecular level suggested that *T. monococcum* subsp. *aegilopoides* was the species from which einkorn wheat (*T.*

monococcum L. subsp. *monococum*) was domesticated with the A-genome of the polyploid wheats (durum and common wheat) being derived from *T. urartu* (Dvorak et al. 1988).

Accessions of these wild diploids were utilized by bridge crosses, where AAAABB amphiploids produced by '*T. turgidum*/A-genome diploid species' accession hybridization were exploited. Allelic variation exhibited by 193 AAAABB amphiploids at various *Glu-1* and *Glu-3* loci were studied using SDS–PAGE method with the objective of exploring their potential for use as a novel genetic resource to improve durum wheat quality.

In these A-genome amphiploids, 120 out of 193 were derived from *T. monococcum* subsp. *aegilopoides* utilizing 93 different accessions in combination with 20 durum wheat genotypes. At the *Glu-A^b1* locus, 14 allelic variants were observed (Table 18). *Glu-A^b1-I* was found most frequent; in 22 (18.33%) genotypes. Out of these 14 alleles, six have an active *Ay* gene at the *Glu-A^b1* locus. The presence of an active *Ay* gene is very important, because it always is absent in hexaploid bread wheat. The impact of this active *Ay* gene on bread-making quality needs to be explored, and these A-genome amphiploids or their derivatives will be important resources for such studies. Previously, Xu et al. (2009) identified five *Ay* alleles while analyzing 113 diploid wheat accessions. At *Glu-B1*, six allelic variants were observed (Table 18). *Glu-B1b, Glu-B1i*, and *Glu-B1f* were the most common. These alleles were inherited in these genotypes directly from the durum parents. In ours and previous studies, the most frequent alleles at the *Glu-B1* locus were mainly *Glu-B1b*, *Glu-B1d*, and *Glu-B1e* (Branlard et al. 1989; Vallega 1988; Moregues et al. 2006). At the *Glu-3* locus, 18 alleles at *Glu-A^b3* and six alleles at *Glu-B3* locus were observed (Table 18). Allelic variation at *Glu-A^b3* was more than that at *Glu-A^b1*. At the *Glu-B3* locus, allele *Glu-B3g* was most frequent and was identified in 31 genotypes. At this locus five alleles were rare and two alleles were extremely rare.

Table 18. Allelic frequencies at the *Glu-1* and *Glu-3* loci in A-genome amphiploids (2n=6x=42; AAAABB) derived from *Triticum monococcum* subsp. *aegilopoides*.

Allele (*Glu-1*)	Frequency (%)	Population (N)	Allele (*Glu-3*)	Frequency (%)	Population (N)
Glu-A^b1-I	18.33	22	*Glu-$A3^b$-I*	16.67	20
Glu-A^b1-II	15.83	19	*Glu-$A3^b$-II*	14.17	17
Glu-A^b1-III	12.50	15	*Glu-$A3^b$-III*	12.50	15
Glu-A^b1-IV	10.00	12	*Glu-$A3^b$-IV*	12.50	15
Glu-A^b1-V	7.50	9	*Glu-$A3^b$-V*	10.00	12
Glu-A^b1-VI	5.83	7	*Glu-$A3^b$-VI*	5.83	7
Glu-A^b1-VII	5.83	7	*Glu-$A3^b$-VII*	4.17	5
Glu-A^b1-VIII	5.00	6	*Glu-$A3^b$-VIII*	4.17	5
Glu-A^b1-IX	5.00	6	*Glu-$A3^b$-IX*	4.17	5
Glu-A^b1-X	5.00	6	*Glu-$A3^b$-X*	2.50	3
Glu-A^b1-XI	4.17	5	*Glu-$A3^b$-XI*	2.50	3
Glu-A^b1-XII	1.67	2	*Glu-$A3^b$-XII*	2.50	3
Glu-A^b1-XIII	1.67	2	*Glu-$A3^b$-XIII*	1.67	2
Glu-A^b1-XIV	1.67	2	*Glu-$A3^b$-XIV*	1.67	2
			Glu-$A3^b$-XV	1.67	2
Glu-B1a	9.17	11	*Glu-$A3^b$-XVI*	1.67	2
Glu-B1b	29.17	35	*Glu-$A3^b$-XVII*	0.83	1
Glu-B1f	20.00	24	*Glu-$A3^b$-XVIII*	0.83	1
Glu-B1d	7.50	9			
Glu-B1e	10.83	13	*Glu-B3b*	18.33	22
Glu-B1i	23.33	28	*Glu-B3c*	23.33	28
			Glu-B3d	25.83	31
			Glu-B3g	10	12
			Glu-B3h	8.33	10
			Glu-B3i	14.17	17

Of the 193 amphiploids, 35 were derived from *T. urartu* utilizing 20 different accessions. In these genotypes, allelic variation was less compared to genotypes derived from *T. monococcum* subsp. *aegilopoides*. Eight alleles were found at the *Glu-A^u1* locus and five alleles at *Glu-B1* (Table 19, p. 168). At the *Glu-3* locus, nine alleles were found at *Glu-A^u3* and five alleles at *Glu-B3*. Three alleles at the *Glu-B^u3* locus were very rare; three alleles were rare at *Glu-Au1* (Table 19). Caballero et al. (2008) found 17 alleles at the *Glu-A^u1* locus and 24 alleles at the *Glu-A^u3* locus while analyzing 169 different accessions of *T. urartu*. At the *Glu-A^u1* locus, all the alleles expressed the *Ax* subunit and four expressed the *Ay* subunit. These findings agreed with that of Caballero et al. (2008) who found eight *Ay* active subunits

in *T. urartu* accessions. The variability found at this locus was comparatively greater than described for the homologous locus in durum wheat, where only 10 allelic variants are described (McIntosh et al 1998; 2007). The *Glu-B3* locus is traditionally associated with gluten strength in durum wheat. Previously, Dvorak et al. (1988) reported the equivalence of the A genome of polyploid wheat to that of *T. urartu*, thus strengthening the use of these amphiploids to improve end-use quality in durum wheat.

Table 19. Allelic frequencies at the *Glu-1* and *Glu-3* loci in A-genome amphiploids (2n=6x=42; AAAABB) derived from *Triticum urart*u.

Allele (Glu-1)	Frequency (%)	Population (N)	Allele (*Glu-3*)	Frequency (%)	Population (N)
Glu-A^{u}1-I	25.71	9	*Glu-A3^u-I*	28.57	10
Glu-A^{u}1-II	20.00	7	*Glu-A3^u-II*	17.14	6
Glu-A^{u}1-III	14.29	5	*Glu-A3^u-III*	14.29	5
Glu-A^{u}1-IV	11.43	4	*Glu-A3^u-IV*	14.29	5
Glu-A^{u}1-V	11.43	4	*Glu-A3^u-V*	8.57	3
Glu-A^{u}1-VI	5.71	2	*Glu-A3^u-VI*	8.57	3
Glu-A^{u}1-VII	5.71	2	*Glu-A3^u-VII*	2.86	1
Glu-A^{u}1-VIII	5.71	2	*Glu-A3^u-VIII*	2.86	1
			Glu-A3^u-IX	2.86	1
Glu-B1b	34.29	12			
Glu-B1c	14.29	5	*Glu-B3b*	14.29	5
Glu-B1d	17.14	5	*Glu-B3c*	28.57	10
Glu-B1f	14.29	6	*Glu-B3d*	34.29	12
Glu-B1i	20.00	7	*Glu-B3g*	14.13	4
			Glu-B3h	14.13	4

Table 20. Allelic frequencies at the *Glu-1* and *Glu-3* loci in A-genome amphiploids (2n=6x=42; AAAABB) derived from *Triticum monococcum* subsp. *monococcum*.

Allele (Glu-1)	Frequency (%)	Population (N)	Allele (Glu-3)	Frequency (%)	Population (N)
Glu-A^{m}1-I	31.58	12	*Glu-A3^m-I*	23.68	9
Glu-A^{m}1-II	13.16	5	*Glu-A3^m-II*	15.79	6
Glu-A^{m}1-III	13.16	5	*Glu-A3^m-III*	15.79	6
Glu-A^{m}1-IV	10.53	4	*Glu-A3^m-IV*	13.16	5
Glu-A^{m}1-V	10.53	4	*Glu-A3^m-V*	10.53	4
Glu-A^{m}1-VI	10.53	4	*Glu-A3^m-VI*	5.26	2
Glu-A^{m}1-VII	5.26	2	*Glu-A3^m-VII*	5.26	2
Glu-A^{m}1-VIII	5.26	2	*Glu-A3^m-VIII*	5.26	2
			Glu-A3^m-IX	2.63	1
Glu-B1b	21.05	8	*Glu-A3^m-X*	2.63	1
Glu-B1c	26.31	10			
Glu-B1d	15.78	6	*Glu-B3b*	10.53	4
Glu-B1f	26.31	10	*Glu-B3c*	18.42	7
Glu-B1i	10.52	4	*Glu-B3d*	28.95	11
			Glu-B3g	13.16	5
			Glu-B3h	15.79	6
			Glu-B3i	13.16	5

The 38 genotypes of *T. monococcum* subsp. *monococcum* were derived from 28 different accessions and 12 durum wheat genotypes. Eight allelic variants at the *Glu-A^{m}1* and five variants at the *Glu-B1* locus were identified (Table 20). *Glu-A^{m}1-I* was most frequent and two alleles were very rare. At the *Glu-B1* locus, *Glu-B1c* and *Glu-B1f* were most frequent. Saponaro et al. (1995) analyzed 56 accessions of *T. monococcum* subsp. *monococcum* and found 30 *Glu-A^{m}1* allelic variants at the *Glu-A^{m}1* locus. Most of the genotypes contained both x- and y-type subunits, but two accessions were null for both. In our study, five alleles encoded both x- and y-type subunits. These A-genome amphiploids are rich in allelic variation at the *Glu-1* and *Glu-3* loci and most important is their active *Ay* subunit, which can be introduced into durum wheat genotypes to enhance end-use quality.

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Characterization of the HMW–GS in synthetic hexaploid wheats and their durum parents.

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High-molecular-weight glutenin subunit composition and variation in 93 synthetic hexaploid (SH) wheats of an Elite-1 SH subset (Mujeeb-Kazi 2003) and their 31 durum wheat parents were determined by SDS–PAGE. Eighteen different alleles at the *Glu-1* locus in the SHs and nine alleles in durum wheat lines were observed. Forty-nine different patterns of HMW–GS in the SHs and 15 different subunit compositions in durum wheats were found. Genetic variability at the *Glu-D^{t}1* locus was greater than that at other loci. The relatively high frequency of superior alleles, *Glu-B1b* and *Glu-D^{t}1d*, indicated the superior bread-making quality attributes in these SH wheats.

Allelic variation at the *Glu-1* locus for HMW–GS in synthetic hexaploid wheats. The results obtained from this study categorized the HMW–GS composition and allelic frequencies in 93 synthetic hexaploid wheats (Table 21, p. 170, and Table 22, p. 171). Eighteen different *Glu-1* alleles were found, three at *Glu-A1*, six at *Glu-B1*, and nine at *Glu-D^{t}1* (Table 22, p. 171). At the *Glu-A1* locus, three x-type subunits were found, 1, 2*, and null, encoded by alleles *Glu-A1a, Glu-A1b*, and *Glu-A1c*, respectively. The null allele was the most frequent, in 65 (69.89%) of the genotypes, followed by subunit 2* in 27 (17.20%), and subunit 1 was observed in 16 (12.90%). These results are different from those of Peña et al. (1995), who reported the presence of null allele in all SH wheats studied. An et al. (2005) reported the genetic diversity at this locus depicted by different codominant alleles at *Glu-A1* locus to be 0.19, which was very low compared to our findings, i.e., 0.47. The frequency of allele *Glu-A1a* encoding subunit 1 in Chinese, Japanese, and a world collection of 300 cultivars was 5.2%, 12.2%, and 28%, respectively. The frequency of the null allele in Chinese (70%), Japanese (80.4%), and in the world collection (74.1%) also was higher than other subunits at this locus (Payne and Lawrance 1983). The quality characteristics of cultivars with subunit 1 are better than those with 2* and the null allele (Li et al. 2009). However, the quality characteristics of cultivars with subunit 2* and null were not significant.

Six different codominant alleles were found at the *Glu-B1* locus. The *Glu-B1g* allele controlling the subunit 13+19 was less frequent (2.15%) among all the subunits at this locus. The most frequent allele was *Glu-B1i* controlling subunit 17+18 found in 26 (27.95%) genotypes followed by *Glu-B1b* and *Glu-B1f* controlling the subunits 7+8 and 13+16 in 24 (25.80%) and 20 (21.50%) genotypes, respectively. The other HMW-GS alleles found at this locus, *Glu-B1c* encoding 7+9 subunit and *Glu-B1d* encoding subunit 6+8, were observed in 15 (16.12%) and six (6.45%) accessions, respectively. The genetic diversity at this locus by these alleles was 0.78. Peña et al. (1995) reported that subunit 7+8 was most frequent in a studied group of SHs. Chinese (71.9%) and Japanese cultivars (83.2%), in which the subunit 7+8 was most frequent, are similar (Nakamura 2000). For 60 (64.51%) of the synthetics in our study, subunits 7+8, 13+16,

or 17+18 are found, which have a superior impact on bread-making quality. These subunits were considered to have the same quality score at *Glu-B1* locus (Gianibelli et al. 2002). The effect of subunit 7 on quality characteristics was found to be the lowest at this locus (Li et al. 2009), and this subunit was not found alone in these accessions. The frequent subunit 7+8 also is associated with extensibility in bread wheat doughs (Uhlen 1990; Peña et al. 1995). So the higher the frequency of the important alleles *Glu-1Bb, Glu-1Bf*, and *Glu-b1i* increases the inherent potential of these SHs for bread-making quality.

Table 21. Allelic composition, frequency, and quality score of 93 accessions of synthetic hexaploid wheats.

Line No.	Subunit combination	Alleles	No. of accessions	Accessions
1	1, 17+18, 2.1+10	a, i, n	1	E-52
2	1, 17+18, 5+10	a, i, d	2	E-11, E-55
3	1, 7+8, 2+12	a, b, a	2	E-35, E-85
4	1, 7+9, 1.5+10	a, c, ah	1	E-93
5	1, 7+9, 5+10	a, c, d	2	E-53, E-69
6	1, 13+16, 1.5+10	a, f, ah	1	E-71
7	1, 13+16, 2+12	a, f, a	2	E-37, E-82
8	1, 13+16, 5+10	a, f, d	1	E-41
9	2*, 17+18, 1.5+12	b, i, aj	1	E-46
10	2*, 17+18, 2.1+10	b, i, n	2	E-50, E-66
11	2*, 17+18, 2+12	b, i, a	1	E-74
12	2*, 17+18, 5+10	b, i, d	1	E-1
13	2*, 7+8, 2.1+10	b, b, n	1	E-26
14	2*, 7+8, 2.1+12	b, b	1	E-28
15	2*, 7+8, 2+12	b, b, a	1	E-44
16	2*, 7+8, 5+10	b, b, d	1	E-57
17	2*, 13+16, 2.1+12	b, f	2	E-21, E-24
18	2*, 13+16, 2+12	b, f, a	3	E-27, E-33, E-45
19	2*, 13+16, 1.5+T1T2	b, f, ag	1	E-59
20	2*, 6+8, 1.5+12	b, d, aj	1	E-43
21	null, 17+18, 1.5+T1T2	c, I, ag	4	E-16, E-23, E-70, E-84
22	null, 17+18, 2.1+10	c, I, n	3	E-39, E-60, E-83
23	null, 17+18, 2.1+12	c, I,	2	E-54, E-58
24	null 17+18, 2+12	c, I, a	2	E-47, E-87
25	null, 17+18, 2+T1T2	c, I, x	2	E-31, E-79
26	null, 17+18, 5+10	c, I, d	5	E-14, E-15, E-40, E-42, E-81
27	null, 7+8, 1.5+10	c, b, ah	4	E-61, E-62, E-64, E-65
28	null, 7+8, 1.5+12	c, b, aj	3	E-18, E-89, E-9
29	null, 7+8, 1.5+T1T2	c, b, ag	2	E-72, E-77
30	null, 7+8, 2.1+10	c, b, n	2	E-6, E-80
31	null, 7+8, 2.1+12	c, b	3	E-19, E-67, E-92
32	null, 7+8, 2+12	c, b, a	4	E-2, E-4, E-29, E-25
33	null, 7+8, 3+10	c, b, z	1	E-7
34	null, 7+9, 1.5+10	c, c, ah	1	E-34
35	null, 7+9, 1.5+12	c, c, aj	2	E-3, E-5
36	null, 7+9, 1.5+T1T2	c, c, ag	2	E-32, E-78
37	null, 7+9, 2.1+10	c, c, n	1	E-76
38	null, 7+9, 2.1+12	c, c	3	E-30, E-88, E-90
39	null, 7+9, 2+12	c, c, a	2	E-10, E-73
40	null, 13+16, 1.5+T1T2	c, f, ag	1	E-22
41	null, 13+16, 2.1+10	c, f, n	1	E-12
42	null, 13+16, 2.1+12	c	1	E-63
43	null, 13+16, 2+12	c, f, a	1	E-20
44	null, 13+16, 5+10	c, f, d	6	E-13, E-36, E-56, E-68, E-86, E-91
45	null, 13+19, 1.5+12	c, g, aj	1	E-38
46	null, 13+19, 1.5+T1T2	c, g, ag	1	E-17
47	null, 6+8, 2.1+10	c, d, n	1	E-51
48	null, 6+8, 2+12	c, d, a	1	E-49
49	null, 6+8, 5+10	c, d, d	3	E-8, E-48, E-75

Valuable genetic variability (0.85) was found at the *Glu-D^{1}1* locus in these synthetics and also justifies their development. The allelic variation of the HMW–GS strongly influences the variability in bread-making quality and the D-genome strongly influences bread-making quality (Pfluger et al. 2001; William et al. 1993). A higher level of genetic variability at this locus is a valuable genetic reservoir to improve bread-making quality. At the *Glu-D^{1}1* locus, five x-type subunits, 1.5, 2, 2.1, 5, and 3, three y-type subunits, 10, 12, and T2, constituting nine different co-dominant allelic combinations were found. The *Glu-D1d* allele, controlling the subunit 5+10, is the most important and superior bread-making quality subunit, was most frequent (22.58%) among all the subunits at this locus. Li et al. (2009) reported the superiority of this allele among all the other alleles at *Glu-1* loci. Luo et al. (2001) reported the association of the 5+10 subunit with sedimentation volume

Table 22. Allelic frequencies of the high-molecular-weight glutenin subunits at the *Glu-1* loci in 93 synthetic hexaploid wheats.

Locus	Allele	Subunit	Number of accessions	Frequency (%)	H (Nei's index)
Glu-A1	a	1	12	0.13	0.47
	b	2*	16	0.17	
	c	null	65	0.70	
Glu-B1	b	7+8	24	0.26	0.78
	c	7+9	15	0.16	
	f	13+16	20	0.22	
	i	17+18	26	0.28	
	d	6+8	6	0.06	
	g	13+19	2	0.02	
Glu-D1	ah	1.5+10	7	0.07	0.85
	aj	1.5+12	8	0.08	
	ag	1.5+T1T2	11	0.12	
	n	2.1+10	12	0.13	
		2.1+12	12	0.13	
	a	2+12	19	0.20	
	d	5+10	21	0.23	
	x	2+T1T2	2	0.02	
	z	3+10	1	0.01	

and longer Pelshenke time. They also reported that the 5+10 subunit in a genotype also results in greater whole-meal flour protein. Payne et al. (1981) established that the 5+10 subunit has a superior quality affect over 2+12 and all other alleles at *Glu-D1*. Other subunit at this locus included 2+12, encoded by allele *Glu-D1a* and found in 19 (20.43%) genotypes. Some rare subunits, 2+T2 and 1.5+T2, also were observed. The allele *Glu-D^{t}1-2l*, which controls subunit T1+T2, was first reported by William et al. (1993) in *Ae. tauschii*, and they concluded that T1 and T2 occur together and their presence is usually designated as T2. The occurrence of these rare combinations in synthetic wheats is due to the utilization of *Ae. tauschii* accessions with diverse subunit combinations, which also was reported by Peña et al. (1995). Other important subunits at this locus were 2.1+10 and 2.1+12 found in 12 (12.90%) accessions. The subunit pair 3+10 was found in only one genotype. This subunit is associated with extensible gluten type and had larger bread loaf volume than 2+10 (Peña et al. 1995). The subunit 1.5+10 was present in seven of the 93 synthetics, and this subunit had better overall quality characteristics than genotypes with other subunits. Peña et al. (1995) concluded that genotypes with the 1.5+10 subunit possess the best bread-making quality.

HMW–GS composition in synthetic hexaploid wheats. Forty-nine different HMW–GS compositions were observed in synthetic wheats (Table 21, p. 170). Peña et al. (1995) reported 36 different allelic compositions in SH wheats. Six (6.45%) genotypes had the subunit combination null, 13+16, 5+10. Five (5.37%) genotypes had subunit composition of null, 17+18, 5+10. Thirteen synthetics had the rare allele 1DyT2 at *Glu-D^{t}1* with either subunit 1.5 or 2. The quality effects of genotypes with 1DyT2 subunits were not determined, because these are rare and their quality effects are yet to be determined. Durum cultivars with either subunit 1 or 2* at *Glu-A1*, along with *Glu-B1*-encoding subunits 7+8, 17+18, or 13+16, can enhance the bread-making quality of these genotypes. Twenty-four synthetics have either subunit 1 or 2* at the *Glu-A1* locus along with superior (7+8, 17+18, or 13+16) subunits at the *Glu-B1* locus. The variation in the patterns of HMW–GS among the different SH accessions described here is nearly similar to those of Peña et al. (1995), Galili and Feldman (1983), and Lawrence and Shepherd (1980), although there are some discrepancies. From these results, it is evident that synthetics have good potential towards bread-making quality and their exploitation in breeding programs becomes important to the breeder when using SH wheats as diversity sources for wheat improvement.

Allelic variation at the *Glu-1* loci for HMW–GS in durum parental lines. In the durum wheat parents, nine different codominant alleles were found at the *Glu-A1 and Glu-B1* loci (Tables 23 and 24, p. 172). At the *Glu-A1* locus, the x-type subunits 1, 2* and null are encoded by the alleles *Glu-A1a, Glu-A1b*, and *Glu-A1c*, respectively. The null allele was the most frequent, appearing in 14 (45%) lines, followed by subunit 2* in nine (32%) and subunit 1 in seven (23%) genotypes. Branlard et al. (1989) reported the frequency of the *Glu-A1c* (null) allele in 83% of the 502 durum wheat cultivars. The extent of variability exhibited by the *Glu-A1* alleles was 66%, which is higher compared to that at this locus in the synthetics.

At the *Glu-B1* locus, six alleles were found, *Glu-B1b, Glu-B1c, Glu-B1f, Glu-B1i, Glu-B1g*, and *Glu-B1d*, control the subunits 7+8, 7+9, 13+16, 17+18, 13+19, and 6+8, respectively. Aghai et al. (1996) reported seven alleles at this locus in landraces from Turkey, Trucheta et al. (1995) reported ten in landraces from Iran, and Branlard et al. (1989) and Kaan et al. (1993) described 12 alleles in a world collection of durum wheats. The frequency of subunit 7+8 and 13+16

was highest, found in nine (29%) genotypes. The results agree with Pfluger et al. (2000). Kaan et al. (1993), Branlard et al. (1989), and Carrillo (1995) also determined that the most frequent subunit was 7+8. Subunit 17+18 was found in six (6.45%) genotypes. The diversity of alleles at this locus was recorded 77% according to Nei's index. Moragues et al. (2006) reported genetic diversity of 80% at this locus in a study of 63 durum land races from diverse geographic origins. Alleles *Glu-B1c* and *Glu-B1i*, encoding the subunits 7+9 and 13+16 and generally absent in tetraploid wheats, were found in these durums. The same alleles were also reported by Xu et al. (2009). Some subunits, such as 6+8 (10%), 7+9 (10%), and 13+19 (3%), were found with very less frequency.

Table 23. Allelic composition, frequency, and quality score of 31 durum parents of D-genome synthetic hexaploid wheats.

Line No.	Subunit combination	Alleles	No. of accessions	Quality score	Accessions
1	1, 7+8	a, b	1	6	D-16
2	1, 17+18	a, i	1	6	D-22
3	1, 7+9	a, c	1	5	D-27
4	1, 13+16	a, f	3	6	D-20, D-28, D-30
5	1, 6+8	a, d	1	5	D-24
6	2* 7+8	b, b	4	6	D-2, D-19, D-23, D-25
7	2*, 17+18	b, i	3	6	D-11, D-18, D-26
8	2*, 13+16	b, f	2	6	D-4, D-31
9	2*, 6+8	b, d	1	5	D-29
10	null, 7+8	c, b	4	4	D-1, D-3, D-5, D-7
11	null, 17+18	c, i	2	4	D-9, D-17
12	null, 7+9	c, c	2	3	D-13, D-21
13	null, 13+16	c, f	4	4	D-6, D-8, D-10, D-12
14	null, 13+19	c, g	1	4	D-14
15	null, 6+8	c, d	1	3	D-15

HMW–GS composition and quality score in durum parental lines of synthetic wheats. Fifteen different combinations of HMW–GS were found at the *Glu-1* loci in the durum wheat genotypes. Three combinations, 2*, 7+8; null, 7+8; and null, 13+16; were found in four (12.90%) lines (Table 24). Branlard and Le Blanc (1985) and Ponga et al. (1985) reported that commercial durum cultivars generally possess subunits 7+8 and 13+16. The quality score ranged from 3–6, and 14 genotypes exhibited the quality score of 6, which was considered the best.

Table 24. Allelic frequencies of the high-molecular-weight glutenin subunits at the *Glu-1* loci in 31 durum wheat parental lines.

Locus	Allele	Subunit	Number of accessions	Frequency (%)	H (Nei's index)
Glu-A1	a	1	7	0.23	0.47
	b	2*	9	0.32	
	c	null	14	0.45	
Glu-B1	b	7+8	24	0.29	0.78
	c	7+9	9	0.10	
	i	17+18	3	0.19	
	f	13+16	9	0.29	
	g	13+19	1	0.03	
	d	6+8	3	0.10	

These results reveal that higher variability at the *Glu-1* loci is associated with SH wheats. This variability could be effectively utilized as a source for the improvement of bread-making quality in breeding programs. The higher crossability of SH wheats with bread wheat will increase their utilization for introducing new *Glu-D*^{t}*1* allelic variations into bread wheat. Undesirable, qualitative effects associated with the *Glu-B1* locus can be avoided by utilizing satisfactory quality durum cultivars in SH wheat production.

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A comprehensive report on the attack of foliar blight on wheat in Pakistan in the 2008–09 wheat crop.

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The 2008–09 crop season was different. Early on, a shortage of urea and the high price of DAP fertilizer remained a burning problem for the farming community of Pakistan. Temperature fluctuations also were unpredictable for the wheat crop. Timely rain and normal sowing were good signs for a bumper wheat crop. Up to mid February, 2009, farmers expected a good harvest, but some unexpected problems changed the situation in the Sindh and Southern Punjab. The spread of a new disease in the region became a new threat to the wheat crop in Pakistan, especially in the upper Sindh and Southern Punjab, which account for nearly 50% of the wheat production area in Pakistan.

Favorable environmental conditions increase the chances of disease in any field. For the first time in the history of Pakistan, a foliar disease had covered such a vast area of million acres in Sindh and Punjab. The disease spread in Sindh at early stages of growth caused a significant loss in wheat production. The crop was found to be susceptible

in the Kacha and Pucca areas of the Khairpur District of Sindh, Rahim Yar Khan, Rajan Pur, Bahawalpur District, Dera Ghazi Khan, Multan, Khanewal District, Lodhran District, Bahawalnagar, and Vehari district.

Spot blotch or Helminthosporium leaf blight (HLB, Fig. 7) is a major disease of wheat, particularly in warmer growing areas where the average temperature is above 17°C in the coolest months. In past 20 years, HLB has been recognized as a major disease in plains of south Asia and is a major problem in India, Bangladesh, and Nepal. This disease is seed and soil borne.

Fig. 7. Spot blotch or Helminthosporium leaf blight cused by *Cochiobolus sativus*.

Varietal resistance. The disease was found with different intensity in fields on all wheat cultivars. Bhakkar-02, covering more than 50% area in the Upper Sindh and Southern Punjab, was badly affected. Mehran 89/ Pak 81 was also found to be susceptible in Sindh. TJ-83 and TD-1 of Sindh were resistant. Two wheat cultivars of Punjab, Seher-06 and Fareed-06, were resistant both in Punjab and Sindh. A new wheat strain 032862 was found to be resistant under high pressure at P.S.C Khanewal. The resistant sources can be used as good sources for breeding purposes in the future.

Direction of disease spread. The disease epidemic was first thought to have begun in the river-side area of Moro, Dadu, and then spread south. The direction of spread from south to north indicated its southern origin. The spread near the Indus River shows greater prevalence in hot and humid areas. The temperature of South Punjab and Sindh remained high during the 2008–09 wheat crop season. Although spot blotch is seed borne, the spread also shows an airborne trend. If airborne, spore shift is expected from the coastal areas of Southern India and Bangladesh.

Estimating losses. Losses in Kacha area of the Upper Sindh may range between 15–20%, whereas 10–15% losses were found in the Pucca area of Sindh. In Punjab, losses in crops near the Indus River ranged from 10–15%. Losses may be 5–10% in areas away from the river. Crop yield was expected to be 26 x 10^6 tons with losses close to 8% (2 x 10^6 tons). Expected losses in monitory terms may reach up to one-billion rupees (1 USD = Rs. 85 Pak).

Control of disease. Genetic control is cheap, easy, and effective. Recommended chemicals can be used at the proper growth stage to control the disease but are expensive. Susceptible wheat cultivars such as Bhakkar-02 should be banned in the Upper Sindh and Southern Punjab. Seed of two Punjabi wheat cultivars, Seher-06 and Fareed-06, should be allowed for sowing in the Upper Sindh. Two new wheat cultivars, Mairaj-08 and Faisalabad-08, which may have better resistance, can be spread in Punjab. Awareness in this respect should be created in the farming community and extension workers through the press and electronic media.

Future threat. The susceptible wheat Bhakkar-02 covers about 50% of the area and will be too difficult to replace in one year because of the lack of seed availability. In the case of disease reoccurrence, 5–10% losses are expected in wheat production during 2009–10 due to HLB. A three-year, continuous, high disease pressure in the field can create an uncontrollable disease complex, thus, it is important to control it at the proper time.

Stripe rust resistance and genetic diversity of some A-genome, diploid progenitor resources of wheat.

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Resistance to stripe rust in 194 A-genome-based SH lines (2n=6x=42; AAAABB) was evaluated at the seedling stage in the greenhouse using bulk inoculum. Twenty-four (15%) A-genome amphiploids were found resistant at the seedling stage. The remaining accessions were either moderately resistant (IT 4-6) or susceptible (IT 7–9). Seventeen (12.93%) genotypes resistant at the seedling stage were also resistant as adult plants, indicating the presence of major resistance genes. Sixty-two (54.31%) genotypes that were susceptible as seedlings were resistant as adult plants indicated the presence of minor resistance genes. The resistant A-genome-based SHs also were molecularly evaluated for DNA-based diversity using RAPD and SSR primers. Five hundred twenty RAPD primers of the Operon series were screened; 107 generated bands and only 50 of these were subsequently selected for further analysis. Ninty-two A-genome-specific SSR primers also were tested. Only 35 primers generated bands, and 13 of these were subsequently selected for further analysis. The 13 SSR primers produced a total of 58 polymorphic bands, whereas 216 polymorphic bands were generated using the 50 RAPD primers. Genetic similarities among the entries were estimated using Nei and Li's coefficient and cluster analysis was performed using the UPGMA clustering method. The average similarity matrix for RAPDs and SSRa were 7.05 and 1.39, respectively. Two known markers for *Yr* resistance genes, namely *Yr* 155 and *Yr* 501, were also applied on resistant entries. The A-genome-based SHs found resistant in this study provide a useful genetic resource for stripe rust resistance, which can be transferred to *T. turgidum* and also be used for bread wheat improvement.

Phenotypic evaluation. The evaluation was across the following categories: pubescence, days-to-heading, plant height, awn color, days-to-physiological maturity, 1,000-kernel weight, number of grains/spike, and spike length. Resistant genotypes that showed good agronomic traits were 2, 29, 35, 36, 39, 40, 49, and 53 (Table 25, pp. 175-177).

Table 25. Phenotypic evaluation and stripe rust infection-type data of A-genome-based synthetic hexaploids. * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. PUB = pubescence, absence (–) or prescence (+); FLOW = days–to–flowering; HT = plant height at maturity; AWN = awn color, LB = light brown, DB = dark brown, B = black, AW = amery white, and Y = yellow; PMA = days–to–physiological maturity; TKW = 1,000–kernel weight; G/S = number of grains/spike; and SL = spike length (cm); S IT = seedling infection type; and AP IT = adult-plant infection type.

Line No.	PEDIGREE	PUB	FLOW	HT (cm)	AWN	PMA	TKW (g)	G/S	SL (cm)	S IT	AP IT
1	YUK/T.BOEOTICUM(1)*	+	125	104	LB	175	46	18	14	9	0
2	GARZA/BOY// T.BOEOTICUM(12)	–	133	129	LB	184	60	40	10	6	0
3	SCA/ T.BOEOTICUM(14)	+	125	125	LB	185	38	5	14	7	0
4	ALG86/4/FGO/PALES//MEXI_1/3/ RUFF/FGO/5/ENTE/6/T.BOEOTICUM(14)	+	135	124	LB	181	38	7	11	9	0
5	BOTNO/ T.BOEOTICUM(20)	+	124	104	LB	176	32	7	12	8	0
6	GARZA/BOY// T.BOEOTICUM(21)	–	143	104	LB	186	23	24	12	1	0
7	DOY1/ T.BOEOTICUM(23)	+	131	128	LB	187	14	2	12	9	0
8	DOY1/ T.BOEOTICUM(26)	+	133	125	LB	186	41	13	12	0	0
9	DOY1/ T.BOEOTICUM(27)	+	135	135	LB	186	16	4	12	1	0
10	SCA// T.BOEOTICUM(28)	+	143	125	LB	185	44	18	13	9	0
11	SCA/ T.BOEOTICUM(31)	–	142	92	LB	181	50	11	11	0	0
12	SCA/ T.BOEOTICUM(33)	+	140	121	LB	187	50	5	12	4	0
13	SCOOP_1/ T.BOEOTICUM(33)	+	135	133	LB	187	44	9	13	8	0
14	SCA/ T.BOEOTICUM(39)	+	108	119	LB	176	43	9	12	0	0
15	SCA/ T.BOEOTICUM(40)	+	133	100	LB	181	44	2	11	8	0
16	SCOOP_1/ T.BOEOTICUM(40)	+	135	104	LB	186	43	12	10	0	0
17	SCOOP_1/ T.BOEOTICUM(46)	+	133	101	DB	186	48	22	10	8	0
18	SCOOP_1/ T.BOEOTICUM(50)	+	147	129	LB	190	27	29	12	9	0
19	LCK59.61/ T.BOEOTICUM(52)	+	135	118	LB	187	48	6	10	2	0
20	AJAIA/ T.BOEOTICUM(55)	+	135	125	LB	181	43	9	11	9	0

Table 25. Phenotypic evaluation and stripe rust infection-type data of A-genome-based synthetic hexaploids. * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. PUB = pubescence, absence (–) or prescence (+); FLOW = days–to–flowering; HT = plant height at maturity; AWN = awn color, LB = light brown, DB = dark brown, B = black, AW = amery white, and Y = yellow; PMA = days–to–physiological maturity; TKW = 1,000–kernel weight; G/S = number of grains/spike; and SL = spike length (cm); S IT = seedling infection type; and AP IT = adult-plant infection type.

Line No.	PEDIGREE	PUB	FLOW	HT (cm)	AWN	PMA	TKW (g)	G/S	SL (cm)	S IT	AP IT
21	SHAG_22/ T.BOEOTICUM(56)	+	145	121	LB	190	60	4	14	9	0
22	SCOOP_1/ T.BOEOTICUM(59)	+	149	104	LB	190	41	24	11	8	0
23	SCOOP_1/ T.BOEOTICUM(69)	–	125	94	LB	175	45	24	9	9	0
24	SCOOP_1/ T.BOEOTICUM(71)	–	133	102	AW	189	33	4	13	9	0
25	BOTNO/ T.BOEOTICUM(75)	+	145	80	LB	191	29	32	12	9	0
26	D67.2/P66.270// T.BOEOTICUM(75)	+	126	110	AW	182	40	33	14	9	0
27	SCOOP_1/ T.BOEOTICUM(79)	+	146	130	DB	193	37	16	11	89	0
28	SCOOP_1/ T.BOEOTICUM(89)	–	136	116	LB	189	56	27	11	1	0
29	SCOOP_1/ T.BOEOTICUM(91)	–	131	117	LB	171	56	37	12	1	0
30	SCOOP_1/ T.MONOCOCCUM(98)	–	127	105	LB	150	56	21	12	0	0
31	AOS/ T.MONOCOCCUM(98)	+	127	103	LB	151	42	16	11	0	0
32	AOS/ T.MONOCOCCUM(111)	–	121	108	LB	158	24	6	13	9	0
33	68.111/RGB-U//WARD/3/ T.MONOCOCCUM(112)	+	125	120	LB	150	42	43	12	9	2R
34	DOY1/ T. URARTU (550)	+	126	144	LB	179	46	31	13	2	3R
35	DOY1/ T. URARTU (560)	+	127	145	LB	175	63	11	15	9	2R
36	DOY1/ T. URARTU (563)	+	119	75	LB	171	59	20	14	9	5R
37	DOY1/ T. URARTU (543)	+	128	104	LB	183	31	6	12	9	5R
38	YAV_2/TEZ// T.BOEOTICUM(44)	+	131	114	LB	180	52	19	11	9	8R
39	YAV_2/TEZ// T.BOEOTICUM(43)	+	148	114	LB	191	59	6	13	6	0
40	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM(49)	+	134	116	LB	183	57	38	12	9	4R
41	YAV_2/TEZ// T.BOEOTICUM(65)	+	133	89	LB	181	62	14	9	9	0
42	YAV_2/TEZ// T.BOEOTICUM(67)	–	133	85	LB	179	50	51	10	9	0
43	YAV_2/TEZ// T.BOEOTICUM(73)	–	135	79	LB	178	72	36	9	1	0
44	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM(77)	+	145	128	LB	186	45	21	11	0	0
45	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM(78)	–	135	100	LB	187	48	20	13	9	0
46	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM(93)	+	135	102	LB	187	44	9	11	9	0
47	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.MONOCOCCUM (99)	+	145	114	LB	188	45	26	8	9	0
48	STN/ T.MONOCOCCUM (111)	+	143	107	LB	188	38	7	14	9	0
49	STN/ T.MONOCOCCUM (112)	–	141	117	LB	189	33	34	12	2	0
50	YAV_2/TEZ// T.MONOTICUM (112)	–	126	117	LB	189	54	27	13	9	0
51	YAV_2/TEZ// T.MONOCOCCUM (113)	+	139	87	LB	187	48	7	13	9	0
52	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.MONOCOCCUM (114)	–	143	137	LB	191	44	10	12	9	0
53	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.MONOCOCCUM (115)	+	143	124	LB	191	46	8	11	9	0
54	YAV_2/TEZ// T.MONOCOCCUM(121)	+	127	100	DB	189	60	6	8	9	0
55	DOY1/T. URARTU (552)	+	132	100	LB	183	45	19	10	9	0
56	ALTAR 84/T. URARTU (558)	–	127	120	LB	176	46	9	7	8	4R
57	CETA/T. URARTU (558)	+	131	131	LB	173	36	20	6	1	5R
58	DOY1/T. URARTU (559)	–	140	115	LB	175	36	9	13	9	0
59	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM(30)	+	140	137	LB	185	32	5	13	9	0
60	ARLIN_1/T.BOEOTICUM((32)	+	140	138	LB	185	36	9	10	9	5R

Table 25 (continued). Phenotypic evaluation and stripe rust infection-type data of A-genome-based synthetic hexaploids. * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. PUB = pubescence, absence (–) or prescence (+); FLOW = days–to–flowering; HT = plant height at maturity; AWN = awn color, LB = light brown, DB = dark brown, B = black, AW = amery white, and Y = yellow; PMA = days–to–physiological maturity; TKW = 1,000–kernel weight; G/S = number of grains/spike; and SL = spike length (cm); S IT = seedling infection type; and AP IT = adult-plant infection type.

Line No.	PEDIGREE	PUB	FLOW	HT (cm)	AWN	PMA	TKW (g)	G/S	SL (cm)	S IT	AP IT
61	CETA/T.BOEOTICUM((42)	+	125	133	LB	186	42	41	12	8	0
62	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM(51)	+	140	122	LB	181	27	18	12	9	6R
63	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM(63)	+	140	143	LB	187	48	20	13	9	8R
64	ARLIN_1/T.BOEOTICUM(84)	+	139	135	LB	185	38	30	10	7	0
65	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM(100)	+	140	135	LB	180	25	6	11	7	0
66	CPI/GEDIZ/3/GOO//JO//CRA/4/ T.BOEOTICUM(85)	–	133	135	LB	187	31	14	10	7	0
67	ARLIN_1/T.BOEOTICUM(86)	–	135	111	LB	186	38	11	8	9	5R
68	ARLIN_1/T.BOEOTICUM(103)	–	144	130	LB	187	30	28	12	9	0
69	ARLIN_1/ T.BOEOTICUM(109)	+	141	131	LB	186	20	19	12	9	0
70	ARLIN_1/T.BOEOTICUM(11)	+	141	117	LB	187	22	29	7	9	0
71	ARLIN_1T. URARTU (547)	+	133	119	LB	181	40	33	12	9	0
72	ARLIN_1/T.BOEOTICUM(66	+	133	100	LB	182	33	9	14	9	0
73	D67.2/P66.270//T.BOEOTICUM(66)	+	128	108	LB	182	34	16	13	9	0
74	ARLIN_1/T.MONOCOCCUM(97)	+	140	131	LB	180	35	34	8	9	5 R
75	ARLIN_1/T.MONOCOCCUM(110)	–	133	116	LB	186	21	52	13	8	0
76	D67.2/P66.270//T. URARTU (542)	+	128	112	LB	176	42	32	13	8	5 R
77	D67.2/P66.270//T. URARTU(543)	+	124	113	LB	175	43	25	10	8	5 R
78	ARLIN_1/T. URARTU (548)	+	129	115	LB	177	44	22	8	9	5R
79	D67.2/P66.270//T. URARTU(550)	+	122	120	LB	173	46	7	13	9	0

Rust resistance. ***Greenhouse evaluation for seedling resistance.*** Different infection types were recorded in A-genome amphiploids. Among the 194 SHs tested at the seedling stage, 25 (20.83%) were classified as resistant (IT 0–3), nine (5.42%) as intermediate (IT 4-6), and 132 (79%) as susceptible (IT 7-9) (Table 26). Those showing consistently low ITs are listed in Table 25 (pp. 175-177).

Table 26. Seedling stripe rust resistance evaluation of the A-genome-based synthetic hexaploids under greenhouse conditions.

Infection type	Rating	Number of A-genome synthetic hexaploids
0–3	Resistant	25
4–6	Moderately resistant	9
7–9	Susceptible	132

Adult-plant screening. Of the 194 A-genome-based synthetic hexaploids, only 17 (12.93%) genotypes resistant at seedling stage were resistant at the adult-plant stage. These genotypes are 6, 8, 9, 11, 12, 14, 16, 19, 28, 29, 30, 31, 34, 43, 44, 49, and 57. Sixty-two (54.31%) genotypes susceptible at seedling stage were resistant at the adult-plant stage. These genotypes have the high APR needed by breeders and agronomists. Disease control is most efficient using different minor genes working independently or a group of genes working together, so that overcomming the resistance by new races of the pathogen will be difficult. Genotypes in this group are 1, 2, 3, 4, 5, 7, 10, 13, 15, 17, 18, 20, 21, 22, 23, 24, 25, 26, 27,32, 33, 34, 35, 36, 37, 38, 40,41, 42, 45, 46, 47, 18, 50,51, 52, 53, 54, 55, 56, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 77, 78, and 79. These APR in these accessions was either from their durum parents or the A-genome accessions (Table 27). These accessions provide novel durable resistance genes against new emerging races of stripe rust. They can be screened separately and used in direct crosses to enrich the A-genome of durum in bread wheat cultivars.

Table 27. Evaluation of adult-plant resistance in the A-genome-based synthetic hexaploids and their durum parents under field conditions.

Rating	Number of A-genome synthetic hexaploids
Resistant	79
Moderately resistant	11
Susceptible	31

Molecular fingerprinting. Fifty RAPD primers amplified a total of 216 polymorphic bands and 13 SSR primers produced a total of 58 polymorphic bands (Table 28). Genetic similarity calculated using Nei and Li's coefficient gave comparable values. Both similarity matrices, when clustered by UPGMA, produced major clustering differences among the genotypes.

Table 28. RAPD and SSR primers used for genetic analysis of A-genome-based synthetic hexaploid lines.

RAPD primers				
OPA-07	OP E-14	OP F-20	OP H-04	OP I-06
OPD-20	OP E-15	OP G-02	OP H-05	OP I-07
OPE-01	OP E-16	OP G-03	OP H-11	OP I-09
OPE-02	OP E-19	OP G-08	OP H-12	OP I-10
OP E-03	OP F-10	OP G-10	OP H-13	OP I-12
OP E-04	OP F-12	OP G-17	OP H-15	OP I-14
OP E-05	OP F-13	OP G-18	OP H-17	OP I-16
OP E-06	OP F-14	OP G-19	OP H-19	OP I-17
OP E-07	OP F-15	OP H-01	OP I-02	OP I-18
OP E-12	OP F-16	OP H-02	OP I-04	OP I-20
SSR primers				
Xgwm5-3A	*Xgwm47.1-2A*	*Xgwm71.2-2A*	*Xgwm162-3A*	*Xgwm249-2A*
Xgwm10-2A	*Xgwm47.2-2A*	*Xgwm95-2A*	*Xgwm265-2A*	*Xgwm501-1B*
Xgwm30-3A	*Xgwm71.1-2A*	*Xgwm122-2A*	*Xgwm296-2A*	*Xgwm155-3A*

From the RAPD and SSR analyses, the minimum genetic distance shown by genotypes was 0.3 for the RAPDs and 0 for the SSRs. The maximum genetic distance for both was 1. The average similarity matrix for the RAPDs and SSRs was 1.39 and 7.05, respectively. The two dendrograms showed clustering differences (Fig. 8, p. 179, and Fig. 9, p. 180). In the RAPDs, 79 A-genome-based SHs were clustered in three clusters, A, B, and C (Fig. 8, p. 179). Cluster A consists of five genotypes with a maximum genetic distance with each other of 15. These genotypes are 48, 63, 67, 77, and 79. Cluster B consists of 29 genotypes. Genotype 78 is highly diverse with an average genetic distance of 98% with 73 other genotypes. Genotypes 71 and 41 are exactly similar. Genotype 71 has an average genetic distance of 76% with 72 other genotypes and genotype 41 has an average genetic distance of 97% with 71 other genotypes. Genotype 26 is highly diverse with an average genetic distance of 89%. In this cluster, genotypes 23 and 39 appear to be the least diverse with genetic distance of 41% with between them. Cluster C consists of 45 genotypes. In this cluster, genotype 5 and 25 are highly diverse with an average genetic distance of 60%, and genotypes 33 and 34 appear to be the least diverse with average genetic distance of 23%

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In the SSRs (Fig. 9, p. 180), cluster A consists of 20 genotypes with a maximum genetic distance of 1. These genotypes are 2, 6, 9, 13, 17, 27, 26, 35, 40, 41, 47, 48, 51, 63, 64, 66, 73, 74, and 78. Cluster B consists of 27 genotypes. Genotype 42 is highly diverse and has an average genetic distance of 96%with two other genotypes. Highly diverse genotypes in this group are 52 and 127. Cluster C consists of 32 genotypes in which genotype 50 is highly diverse and has a genetic distance of 84% with three other genotypes. The level of genetic diversity is high among the genotypes. A few genotypes amplified bands for stripe rust resistance marker *Xgwm-501* at 195 bp/160 bp. Genotypes 11, 13, and 77 generated bands of 195 bp and genotypes 34, 35, and 36 generated bands of 160 bp. Genotypes that amplified the bands for marker *Xgwm-155* at 147 bp are 38, 65, 69, 74, and 75 (Table 29).

Table 29. Application of simple sequence repeat markers for stripe rust genes *Yr5* and *YrSp* (+ = present; – = absent).

Accession	***Yr5***	***YrSp***	**Accession**	***Yr5***	***YrSp***
11	+	–	65	–	+
13	+	–	69	–	+
34	+	–	74	–	+
35	+	–	75	–	+
36	+	–	77	+	–
38	–	+			

Resistance to rust diseases may comprise genes effective at both the seedling and adult-pant stages. The germ plasm showed a wide range of variability for disease response in both greenhouse and field tests, indicating the presence of major and minor genes. Seventeen genotypes with seedling resistance were found to be resistant at the adult-plant stage, whereas 62 genotypes, which were susceptible or intermediate as seedlings, were found to be resistant at adult plants.

We also studied different morphological parameters to select the best agronomic types. Eight genotypes proved to be best for spike length, grains/spike, and 1,000-kernel weight and can be used in wheat yield improvement programs in Pakistan.

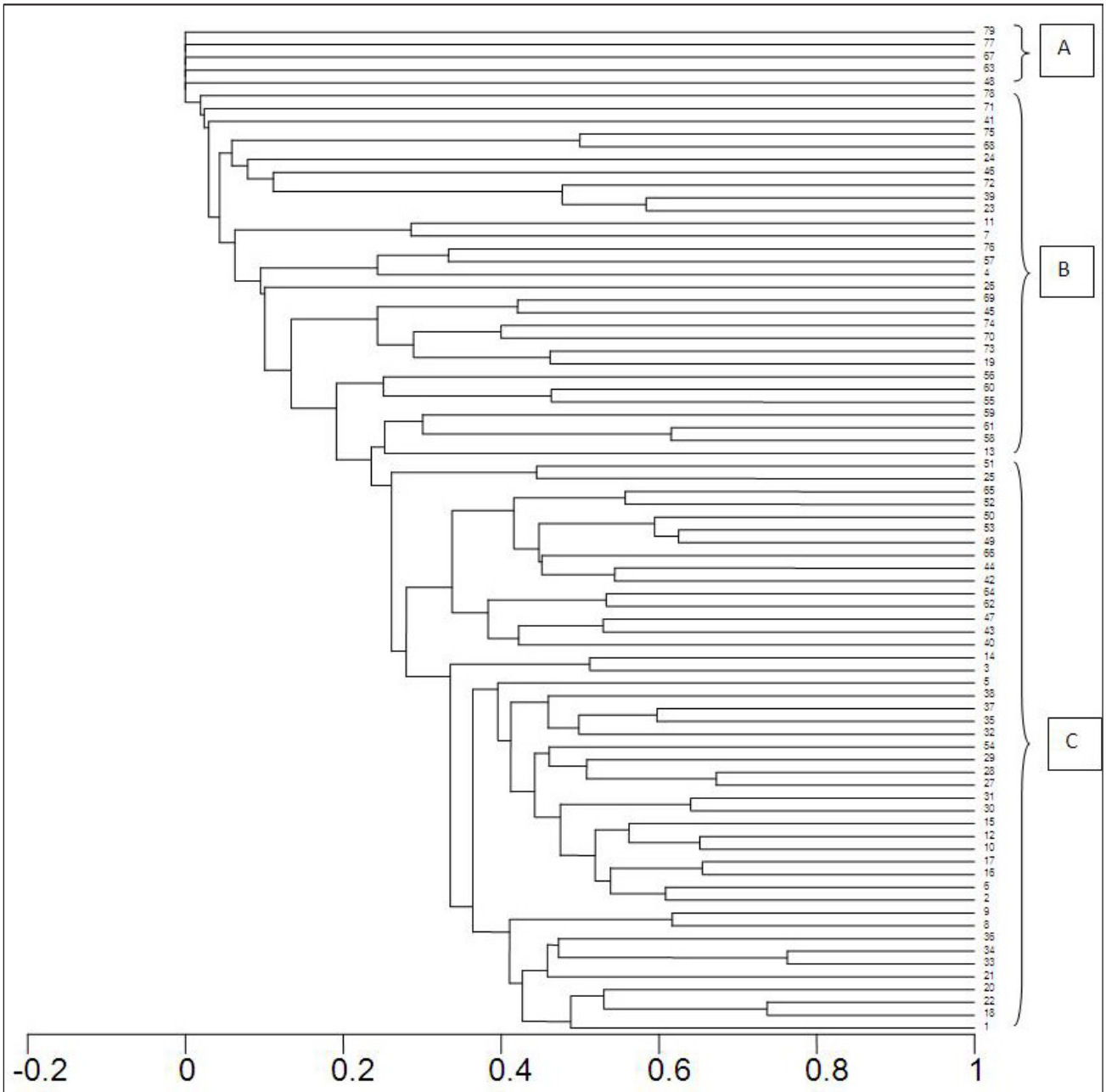

Fig. 8. Grouping of A-genome-based synthetic hexaploids (2n=6x=42; AAAABB) on the basis of genetic distance.

DNA marker technology is a powerful tool to study the genetic diversity among genotypes. In RAPD markers, the maximum number of polymorphic loci were produced by primers OPE-1 and OPE-2. In the SSR markers, the maximum number of polymorphic loci were produced by primer *Xgwm-249*. The average number of polymorphic loci produced by RAPDs is 4.32 and in SSR is 4.46.

Our study has indicated that wild wheat relatives are genetically more diverse than modern cultivars. Their traits can be incorporated into bread wheat cultivars. Among the A-genome-based SHs studies with RAPD markers, five genotypes show maximum genetic diversity of 1 and least genetic diversity of 0.3 was shown by 45 genotypes. For the SSR markers, 20 genotypes had the maximum genetic diversity of 1 and the least genetic diversity of 0 was shown by 16 genotypes. The remaining genotypes fall in between. Comparing the RAPD and SSR results, the maximum number of diverse genotypes were shown using SSR markers and the results are more reliable.

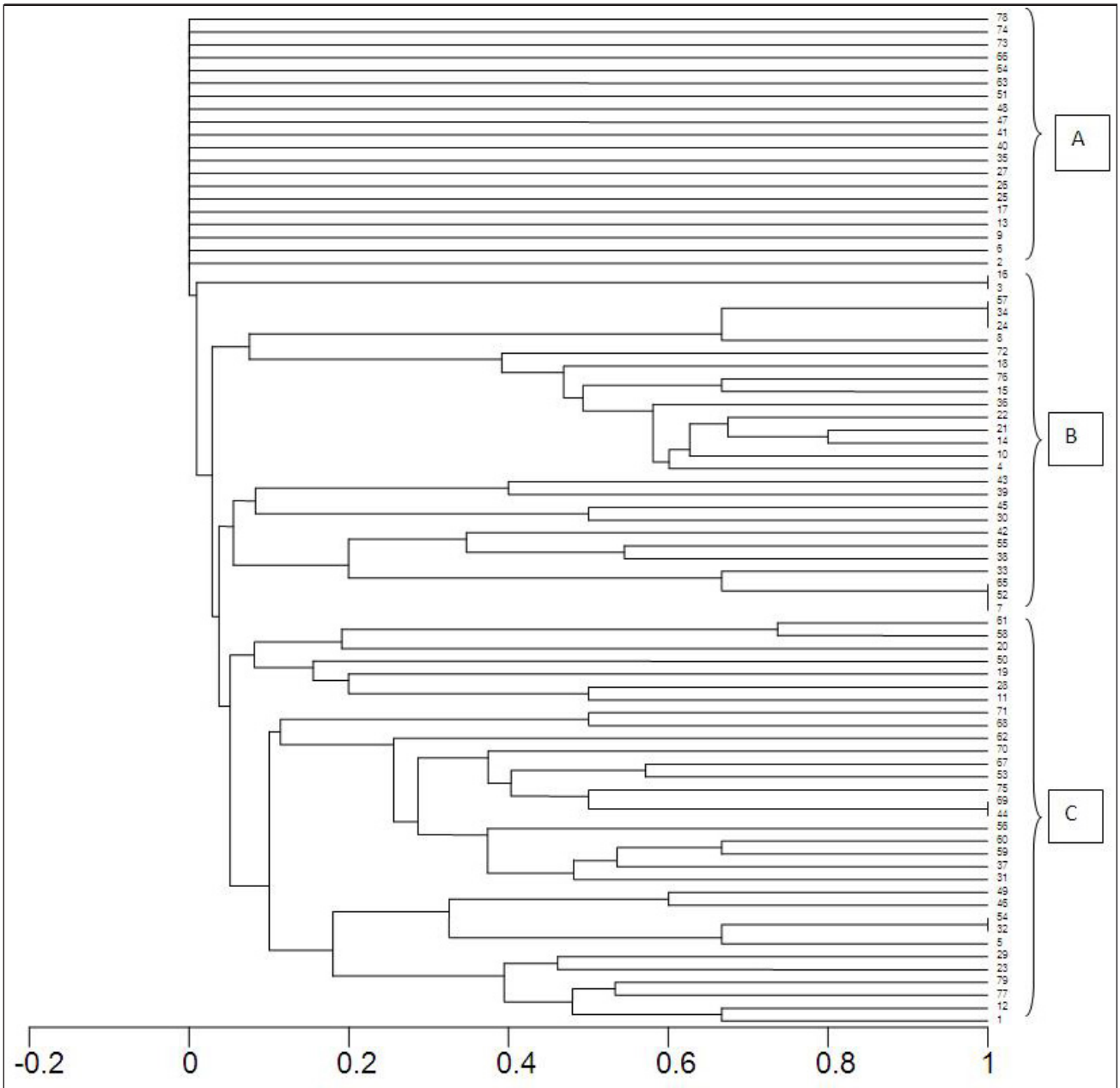

Fig. 9. Grouping of A-genome-based synthetic hexaploids (2n=6x=42; AAAABB) on the basis of genetic distance from SSR analysis.

for drought tolerance.

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Twenty-three drought tolerant, D-genome synthetic hexaploid wheats (Table 30, p. 181) were analyzed using 47 D-genome specific SSR primers to detect genetic diversity (Table 31, p. 181). A total of 136 alleles were detected with an average of 2.89 alleles/locus. The value of the polymorphic information content (PIC) ranged from 0 to 0.81. For phenological evaluation, the genotypes were grown under two environmental conditions; (a) in field conditions (control) with normal seasonal irrigation and (b) in rain shelters (drought stress) with only one presowing irrigation. Five plants/entry for each of the two growing conditions were used for phenolotypic evlaution. Morphological data was recorded for parameters days-to-heading, days-to-physiological maturity, plant height, awns, pubescence, spike length, number of grains/spike, and 1,000-kernel weight.

Table 30. Pedigrees of 23 genotypes of synthetic hexaploid wheats derived from durum wheat x *Ae. tauschii* cross combinations. * is the *Ae. tauschii* accession number in Wide Crosses Program working collection, CIMMYT, Mexico.

Name	Pedigree
S-1	Doy1/Ae. squarrosa (188)*
S-2	Altar 84/Ae. squarrosa (191)
S-3	D67.2/P66.270//Ae. squarrosa (213)
S-4	D67.2/P66.270//Ae. squarrosa (217)
S-5	Dverd-2/Ae. squarrosa (221)
S-6	D67.2/P66.270//Ae. squarrosa (223)
S-7	GAN/Ae. squarrosa (446)
S-8	Doy1/Ae. squarrosa (515)
S-9	68.111/RGB-U//Ward/3/FGO/4/Rabi/5/ Ae. squarrosa (629)
S-10	D67.2/P66.270//Ae. squarrosa (257)
S-11	Scot/Mexi_1//Ae. squarrosa (314)
S-12	Croc_1/Ae. squarrosa (507)
S-13	Croc_1/Ae. squarrosa (444)
S-14	Altar84/Ae. squarrosa (502)
S-15	Doy1/Ae. squarrosa (526)
S-16	Ceta/Ae. squarrosa (1024)
S-17	Dverd_2/Ae. squarrosa (1027)
S-18	Doy1/Ae. squarrosa (1018)
S-19	Ceta/Ae. squarrosa (1026)
S-20	Doy1/Ae. squarrosa (1026)
S-21	Doy1/Ae. squarrosa (1029)
S-22	Ceta/Ae. squarrosa (1031)
S-23	Dverd_2/Ae. squarrosa (1031)

Table 31. Microsatellite primers used for PCR amplification of alleles at 47 loci.

Locus	PIC	Locus	PIC
Xgwm2-3D	0.81	*Xgwm271-5D*	0.50
Xgwm3-3D	0.38	*Xgwm295-7D*	0.54
Xgwm16-5D	0.33	*Xgwm296-2D*	0.22
Xgwm30-2D	0.62	*Xgwm314-3D*	0.67
Xgwm33-1D	0.72	*Xgwm325-6D*	0.09
Xgwm37-7D	0.00	*Xgwm337-1D*	0.42
Xgwm52-3D	0.50	*Xgwm341-3D*	0.75
Xgwm55-6D	0.00	*Xgwm349-2D*	0.00
Xgwm102-2D	0.59	*Xgwm350-7D*	0.66
Xgwm106-1D	0.20	*Xgwm358-5D*	0.73
Xgwm111-7D	0.70	*Xgwm383-3D*	0.50
Xgwm157-2D	0.33	*Xgwm455-2D*	0.67
Xgwm161-3D	0.00	*Xgwm458-1D*	0.00
Xgwm165-4D	0.50	*Xgwm469-6D*	0.35
Xgwm182-5D	0.00	*Xgwm484-2D*	0.42
Xgwm183-3D	0.49	*Xgwm497-3D*	0.00
Xgwm192-5D	0.49	*Xgwm515-2D*	0.46
Xgwm194-4D	0.49	*Xgwm539-2D*	0.60
Xgwm205-5D	0.00	*Xgwm565-5D*	0.73
Xgwm210-2D	0.00	*Xgwm583-5D*	0.66
Xgwm212-5D	0.62	*Xgwm608-2D*	0.40
Xgwm232-1D	0.00	*Xgwm635-7D*	0.00
Xgwm249-2D	0.37	*Xgwm642-4D*	0.25
Xgwm261-2D	0.00		

We used 23 SH genotypes with drought tolerance to characterize and evaluate the genetic diversity by means of morphological and molecular parameters for drought tolerance. We assumed that the variation observed in the morphological parameters was due to water inavailability and not to any other factor, such as high temperature. Awns were present in all genotypes, which is considered a drought-tolerance trait because it increases the net rate of photosynthesis of the spike and causes a considerable increase in yield under conditions of limited water supply (Evans et al. 1972). Pubescence also is considered a trait that enables moisture retention due to its hairy nature. Pubescence on the spike glumes also is known to contribute towards water stress tolerance (Erdei et al. 1990). Breeders select drought-tolerant material based on pubescence. Most of the SH wheats used in this study were pubescent on the outer side of the glume.

Table 32. Basic statistic parameters for morphological traits in genotypes from control (C.) and drought stress (DS) conditions. ** indicated significance at p = 0.01.

Trait	Conditions	Mean	S.E.	CV (%)	t-Value
Days-to-heading	Control	140.87	1.51	5.15	36.46 **
	Drought	83.13	0.29	1.7	
Days-to-phyisological maturity	Control	175.61	1.1	3.01	45.12**
	Drought	112.65	0.6	2.56	
Plant height (cm)	Control	125.4	3.11	11.91	13.6**
	Drought	80.29	1.85	11.05	
Spike length (cm)	Control	12.5	0.35	13.45	14.89**
	Drought	6.46	0.21	15.61	
Grains/spike	Control	32.8	3.05	44.64	5.57**
	Drought	15.45	0.67	20.92	
1,000-kernel weight	Control	43.6	2.1	23.2	3.9**
	Drought	33.15	1.07	15.57	

In Table 32, the t-values indicate that highly significant differ-

ences were present between the means of all parameters of the two treatments. Average spike length under control conditions was significantly different from the mean value in drought-stress conditions. Similarly, the number of grains/spike in the control was double that in drought stress. Ehdaie and Waines (1996) reported that drought stress at grain-filling reduces yield dramatically. Significant differences in means also were observed for 1,000-kernel weight, suggesting that drought also affects this trait. The effect of drought on these characteristics also were reported by Dencic et al. (2000) and Blum (1993). Our results show that despite the low yield of SH wheats in extremely water limited conditions, they were able to survive in conditions that are lethal for most other wheat genotypes. On the other hand, the mean values for plant height, spike length, number of grains/spike, and 1,000-kernel weight were found to be higher than the mean values for these parameters in Nesser, the check cultivar in the experiment. Among the genotypes studied, S-5 and S-21 were found to be the best phenotypically. They performed well in water-stressed conditions, suggesting that these SH wheats have the diversity to perform well under drought conditions. These lines will provide an efficient source to enrich and improve the wheat germ plasm by exploiting the variation present in the D genome.

For molecular characterization, 47 D-genome-specific microsatellite markers for 47 loci were used. A total of 136 loci were detected. Some allelic variation also was observed for some loci . The number of alleles/locus ranged from one for *Xgwm*37-1D, *Xgwm55-6D, Xgwm161-3D, Xgwm182-5D, Xgwm205-5D, Xgwm210-2D, Xgwm232-1D, Xgwm261-2D, Xgwm349-2D, Xgwm458-1D, Xgwm497-3D*, and *Xgwm635-7D* to seven for *Xgwm2-3D*. The average number of alleles/locus was 2.89.

The average PIC value for these primers was 0.38, ranging from zero to 0.81 (Table 31, p. 181). No polymorphism was observed for the primers having a zero PIC value, whereas primer *Xgwm2-3D* showed the maximum polymorphism with a maximum PIC value.

To estimate the genetic diversity and relatedness among the 23 genotypes, SSR amplification data was used to generate a similarity matrix. The similarity coefficient value of these genotypes ranged from 0.114 to 0.795. A minimum similarity of 11.40% was for S-2 with S-18. The genotypes with maximum similarity were S-15 and S-16. Das et al. (2007) also reported that the similarity coefficient among drought-tolerant synthetic and conventional wheat ranged between 0.16 and 0.79.

The genetic distances among 23 SH genotypes were used to construct a dendrogram for determining grouping among these genotypes on the basis of similarities and differences. These genotypes were grouped in two main clusters (Fig. 10). Cluster A had nine genotypes. Genotypes S-21, S-11, S-20, S-19, S-12, and S-10 clustered together in group AI (Fig. 10, p. 184). Genoptyes S-2, S-3, and S-18 formed cluster AII. Except for S-1, which was found to be the most diverse genotype of cluster B, the other remaining 13 genotypes formed two groups. Genotypes S-4, S-5, S-6, S-13, and S-14 were clustered in group BI and S-7, S-8, S-9, S-15, S-16, S-17, S-22, and S-23 formed the BII cluster (Fig. 10). Morphological evaluation showed that S-1 had the highest mean values for the number of grains/spike (40.6) and 1,000-kernel weight (60.60 g) in the control environment compared to the other genotypes. Under drought

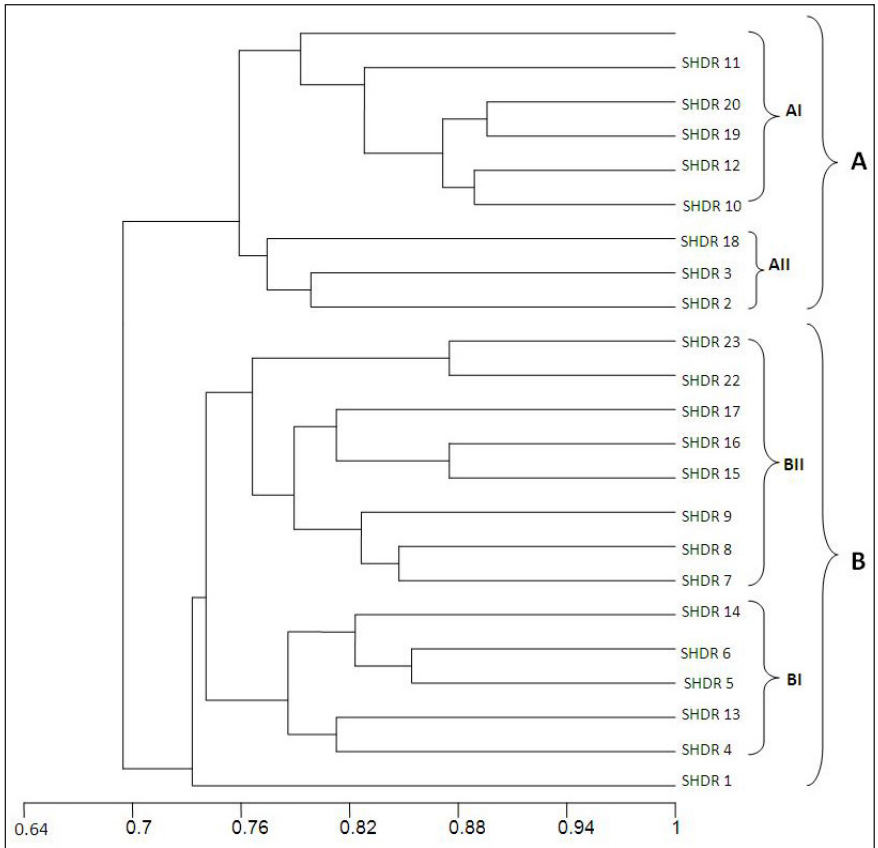

Fig. 10. SSR-based cluster formation of 23 genotypes of synthetic hexaploid wheats.

stress, however. the mean values for grains/spike was 16.7 and 1,000-kernel weight was 24.6 g, which clearly indicates the effect of drought on the yield components. A plant height 136.4 cm was observed in the control conditions, which was reduced to 76 cm under water stress. The same trend was observed for spike length.

These findings demonstrate the substantial amount of genetic diversity of D genome in SH wheats, which possess novel genes for tolerance against abiotic stresses such as drought (Damaina et al. 1992). The unique drought-tolerance genes present in these SH wheats have made them ideal germ plasm for incorporating useful genes into elite Pakistani cultivars.

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Molecular and morphological evaluation of diversity for drought tolerance in a D-genome-based, double-haploid mapping population, its parents, and local drought-tolerant cultivars of wheat using SSRs.

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A morphological and molecular diversity analysis using SSRs, for 20 D-genome-based, double-haploid (DH) plants from mapping population (158 DH plants); the parent lines, Opata M-85 (drought susceptible, 2n=6x=42) and a synthetic hexaploid (SH-257, 2n=6x=42, drought tolerant); and the local drought-tolerant cultivars Nesser, Zarghoon, and Margalla, was conducted in the Wheat Wide Crosses (WWC) Program at the National Agriculture Research Centre (NARC), Islamabad, Pakistan. For SSR statistical analysis, unweighted pair group of arithmetic mean (UPGMA) function (Nei and Li 1979) was used to estimate genetic distance among the genotypes. Fifty-nine SSR (Table 33) primers yielded a total of 177

Table 33. SSR primers used for genetic analysis of 20 D-genome-based, double-haploid (DH) plants from mapping population (158 DH plants); the parent lines, Opata M-85 (drought susceptible, 2n=6x=42) and a synthetic hexaploid (SH-257, 2n=6x=42, drought tolerant); and the local drought-tolerant cultivars Nesser, Zarghoon, and Margalla.

#	Locus/primer	#	Locus/primer	#	Locus/primer
1	*Xgwm 2-3D*	21	*Xgwm194-4D*	41	*Xgwm358-5D*
2	*Xgwm3-3D*	22	*Xgwm205-5D*	42	*Xgwm383-3D*
3	*Xgwm16-5D*	23	*Xgwm210-2D*	43	*Xgwm428-7D*
4	*Xgwm30-2D*	24	*Xgwm212-5D*	44	*Xgwm437-7D*
5	*Xgwm33-1D*	25	*Xgwm232-1D*	45	*Xgwm455-2D*
6	*Xgwm37-7D*	26	*Xgwm249-2D*	46	*Xgwm456-3D*
7	*Xgwm52-3D*	27	*Xgwm261-2D*	47	*Xgwm458-1D*
8	*Xgwm55-6D*	28	*Xgwm269-5D*	48	*Xgwm497-3D*
9	*Xgwm71-3D*	29	*Xgwm271-5D*	49	*Xgwm539-2D*
10	*Xgwm102-2D*	30	*Xgwm272-5D*	50	*Xgwm565-5D*
11	*Xgwm111-7D*	31	*Xgwm292-5D*	51	*Xgwm583-5D*
12	*Xgwm121-7D*	32	*Xgwm295-7D*	52	*Xgwm608-2D*
13	*Xgwm157-2D*	33	*Xgwm296-2D*	53	*Xgwm608-4D*
14	*Xgwm161-3D*	34	*Xgwm314-3D*	54	*Xgwm624-4D*
15	*Xgwm165-4D*	35	*Xgwm320-2D*	55	*Xgwm635-7D*
16	*Xgwm174-5D*	36	*Xgwm325-6D*	56	*Xgwm642-1D*
17	*Xgwm182-5D*	37	*Xgwm337-1D*	57	*Xgwm645-3D*
18	*Xgwm183-3D*	38	*Xgwm341-3D*	58	*Xgwm654-5D*
19	*Xgwm190-5D*	39	*Xgwm349-2D*	59	*Xgwm664-3D*
20	*Xgwm192-5D*	40	*Xgwm350-7D*		

polymorphic bands in sizes ranging from 50–900 bp. The dendrogram demonstrated that the genotypes from the mapping population were genetically distinct from the local drought-tolerant cultivars and the parent lines Opata M-85 and SH-257. The seven best DHs with ample genetic distance were 2 (85.80%), 9 and 15 (74.19%), 18 and 20 (66.67%), 1 (63.00%), and 14 (62.31%). The genetic distance of these DHs was nearly similar or better than that of SH-257 (65.20%). Genetic distance of the local drought-tolerant cultivars were Nesser (42.17%), Margalla-99 (42.17%), and Zarghoon (58.49%), all less than SH-257 (65.20%). Opata M-85, with genetic distance of 83.49%, was distinct from SH-256 and the mapping population.

Under stress, good variability for morphological characters was observed in DHs 2, 6, 9, 14, 18, and 19. Doubled haploids 1, 2, 9, 14, 18, and 20, with good morphological and molecular diversity, are recommended for wheat yield improvement programs in Pakistan (Table 34). Overall, the DH mapping population depicted a good deal of genetic diversity for drought tolerance over the local/elite drought-tolerant cultivars because of the D-genome in the synthetic parent SH-257. These results suggest that using SH wheat is an efficient way to enrich the genetic background of wheat, especially with the genetic variation of the D genome from *Ae. tauschii*. The results also demonstrate the utility of microsatellite markers in detecting DNA polymorphism and estimating genetic diversity.

Evaluating genetic diversity. The 59 SSR primers yielded a total of 177 polymorphic bands ranging from 50–900 bp. The highest number of scorable bands was obtained with primer *Xgwm174-5D* (40) and the lowest with primer *Xgwm2-3D* (2). Maximum genotypes (19) were amplified by both primers *Xgwm261-2D* and *Xgwm271-5D* and minimum (1) by *Xgwm2-3D*. Different primers varied in their ability to detect polymorphism. Primers *Xgwm232-1D* and *Xgwm349-2D* showed highest polymorphism with five polymorphic bands each.

Table 34. Comparative morphological data (mean values) of mapping population entries under control (irrigated) and stress (rain-sheltered) conditions. For growth habit, a + indicates a prostrate habit; for pubescence, a + indicates hariness; for awn color, AW = amber white and LY = light yellow.

DH no.	Plant height (cm)		Days-to-flowering		Days-to-maturity		Awn length (cm)		Spike length (cm)		Grains/spike		1,000-kernel weight (g)		Growth habit		Pubsence		Awn color	
	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
2	125	62.5	112	84	165	122	8.2	6.6	11.7	4.5	42	19	49.0	24.6	–	–	–	–	AW	AW
4	128	72.5	117	75	163	121	8.5	8.8	13.0	7.0	28	10	48.0	19.6	–	–	–	–	AW	AW
14	116	69.0	117	75	169	120	8.8	6.5	12.3	5.5	60	14	37.4	22.5	–	–	–	–	LY	LY
41	132	87.0	118	77	167	118	9.5	7.0	11.5	6.0	68	17	51.0	34.1	–	–	–	–	AW	AW
52	122	57.0	117	75	163	114	7.7	5.5	15.8	6.0	46	12	47.3	28.2	–	–	–	–	LY	LY
61	135	79.0	119	85	163	116	10.2	7.7	15.7	5.5	50	28	45.1	31.6	–	–	–	–	AW	AW
68	116	72.5	119	84	163	123	6.7	6.3	11.0	5.5	32	15	27.3	38.9	–	–	–	–	LY	LY
73	112	67.0	118	77	161	119	8.3	6.5	10.3	5.0	56	11	39.0	30.5	–	–	+	+	LB	LB
74	138	68.0	119	79	161	118	10.2	8.9	11.6	4.5	42	11	49.5	39.3	–	–	–	–	AW	AW
76	127	63.0	113	84	171	114	4.6	4.3	11.1	4.5	24	15	39.5	38.4	–	–	–	–	LY	LY
78	129	79.0	113	77	162	116	8.8	5.6	12.7	5.0	49	12	42.0	32.0	–	–	–	–	AW	AW
80	149	65.0	112	77	166	125	7.6	8.0	11.1	4.0	27	10	38.7	23.6	–	–	–	–	LY	LY
81	110	67.0	114	75	166	113	7.3	7.2	10.3	5.0	45	12	38.1	21.0	–	–	–	–	LY	LY
114	119	77.0	121	86	176	119	8.3	6.4	11.3	7.0	50	19	30.0	29.8	–	–	–	–	AW	AW
117	140	66.0	124	86	176	118	7.2	7.3	10.7	6.0	43	19	44.0	30.3	–	–	–	–	LY	LY
123	120	75.0	125	88	171	120	8.7	6.0	11.1	6.0	39	11	39.0	18.4	–	–	–	–	LY	LY
127	121	67.0	117	104	165	117	8.6	4.5	12.1	5.0	48	11	34.2	21.5	–	–	–	–	AW	AW
144	111	51.0	113	77	173	115	9.5	6.5	11.0	4.0	47	8	38.4	29.6	–	–	–	–	AW	AW
145	125	72.0	114	73	173	114	7.5	4.7	12.8	5.0	46	6	33.0	19.7	–	–	–	–	AW	AW
148	107	71.0	119	77	163	112	8.5	7.1	12.8	6.0	38	12	24.3	23.8	–	–	–	–	AW	AW

Interpreting the similarity matrix. The SSR amplification data was used to obtain a similarity matrix. The similarity coefficient value ranged from 0 to 70%. Genotypes with 0% similarity were 1 with 2, 7, 19, and Opata M-85; 2 with 5, 6, 8, 14, 16, Nesser, Zarghoon, Margalla, and Opata M-85; 3 with 7 and Opata M-85; 4 with 7; 5 with 7; 7 with 9, 11, 16, 17, 20, Opata M-85, and SH-257; 15 with 18 and 19; 19 with 20; and SH-257 and 20 with Zarghoon. Genotypes with 70% similarity were 5 with 6. The similarity of the remaining genotypes was found to be between 0 and 70%.

Dendrogram interpretation. A dendrogram was formulated based on genetic distance was divided into two main clusters A and B (Fig. 11). Cluster B was subdivided into four subclusters, B1, B2, B3, and B4. Cluster A included five genotypes, all of which belonged to the mapping population. DH 2 had the maximum genetic distance of 85.80% from all other four genotypes of this cluster and, hence, was the most diverse. Subcluster B1 had three genotypes, where DHs 9 and 15, with a genetic distance of 74.19%, were genetically similar. Opata M-85, with a maximum genetic distance of 83.49%, was the most diverse line in this subcluster. Subcluster B2 had four genotypes, the most diverse was SH-257 with a maximum genetic distance of 65.20% with rest of the genotypes. In subcluster B3 with nine genotypes, DHs 5 and 6 were genetically similar. The most diverse DH was line 14 with a maximum genetic distance of 62.31%. The conventional, drought-tolerant cultivars Nesser and Margalla were genetically similar, whereas Zarghoon was distinct from both. In subcluster B4 with four genotypes, DH 1, with a maximum genetic distance of 63.00%, was the most diverse of the genotypes. The DHs 10 and 3 were genetically similar.

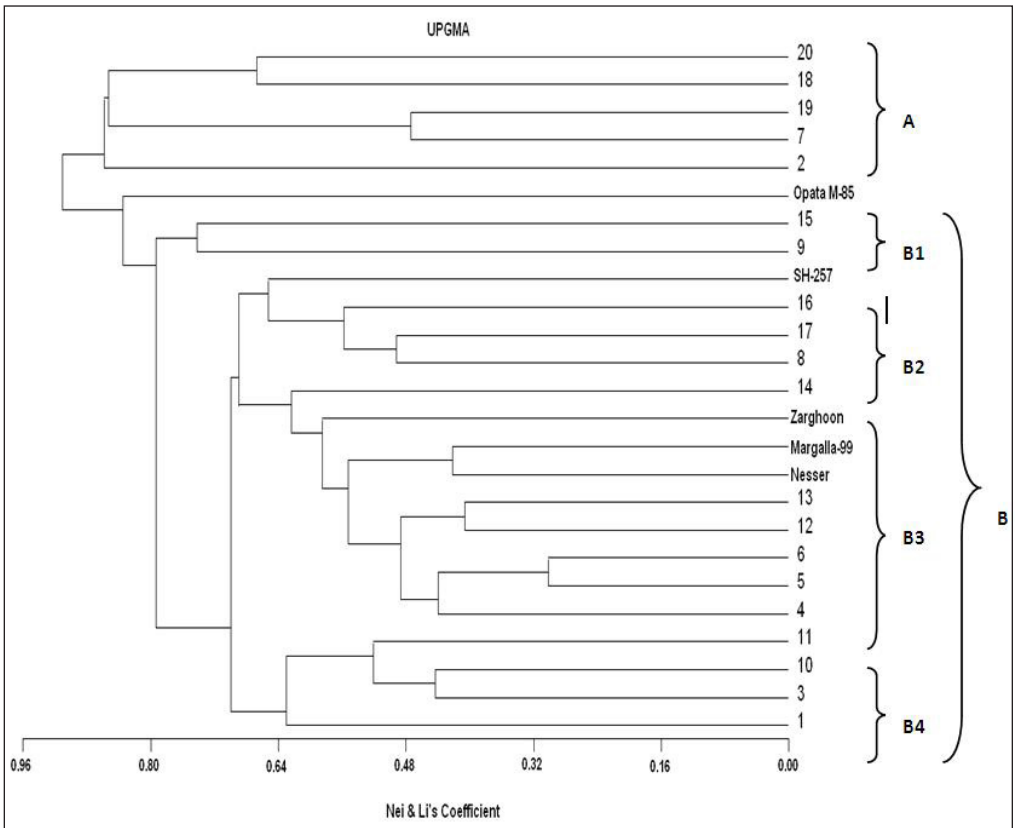

Fig. 11. Dendrogram based on genetic distance for SSR primers analysis of drought tolerance test germ plasm.

Dwarf height is a favorable character to counter lodging. For the mapping population under control conditions, the minimum plant height was 107 cm in DH 20. Under stress conditions, the minimum height was 51 cm in DH 18. The minimum height among the local, drought-tolerant cultivars was 58 cm (Nesser). The heights of Opata M-85 and SH-257 were 94 cm and 99 cm, respectively. The minimum number of days-to-flowering is a desirable character for earliness. Minimum days-to-flowering for the mapping population under control conditions was 112 days in DHs 1 and 12 and under stress condition was 73 days (DH 19). The local, drought-tolerant cultivars had minimum of 75 days (Margalla). Opata M-85 flowered in 110 days and SH-257 in 134 days. The minimum number of days-to-maturity for the mapping population under control conditions was 161 for DHs 8 and 9, whereas under stress conditions was 112 days (DH 20). The minimum number of days in the local, drought-tolerant cultivars was 109 days (Margalla) and for Opata M-85 and SH-257 were 159 and 170, respectively (see Table 35, p. 188).

For the mapping population under control conditions, maximum spike length was 15.8 cm in DH 5 and 7 cm in DHs 2 and 14 under stress conditions. The minimum spike length of the local, drought-tolerant cultivars was 5.5 cm (Nesser). The maximum number of grains/spike was 68 in DH 4 under control conditions and 28 for DH 6 under stress conditions. The maximum grains/spike of the local, drought-tolerant cultivars was 23 (Margalla). For Opata M-85 and

Table 35. Morphological data (mean values) of the parents of a double-haploid mapping population, Opata M-85 (drought susceptible, 2n=6x=42) and a synthetic hexaploid (SH-257, 2n=6x=42, drought tolerant); and the local drought-tolerant cultivars Nesser, Zarghoon, and Margalla. For growth habit, a + indicates a prostrate habit; for awn color, AW = amber white and LY = light yellow.

Trait	Nesser	Zarghoon	Margalla	Opata M-85	SH-257
Height (cm)	58.0	76.5	73.5	94.0	99.0
Days-to-flowering	82	91	75	110	134
Days-to-physiological maturity	112	113	109	159	170
Awn length (cm)	7.0	3.2	4.0	6.4	8.0
Spike length (cm)	5.5	6.0	6.0	19.5	10.0
Grains/spike	12	20	23	28	10
1,000-kernel weight (g)	34.8	37.8	29.3	23.5	49.0
Growth habit	–	–	–	–	–
Pubescence	–	–	–	+	–
Awn color	AW	AW	AW	LY	AW

SH-257, grains/spike were 28 and 10, respectively. The maximum 1,000-kernel weight in the mapping population under control conditions was 49.5 g for DH 9 and under stress conditions was 39.3 g for DH 9. Maximum 1,000-kernel weight for the local, drought-tolerant cultivars was 37.8 g (Zarghoon), 23.5 g for Opata M-85, and 49.0 g for SH-257.

Observing this phenotypic data, we established that the mapping populaiton had better morphological characters than modern wheat cultivars because of D genome of synthetic parent (SH-257). Morphological analysis of the mapping populaiton under control conditions showed that DHs 1, 4, 5, 8, 9, 12 and 20 were the most diverse for the different morphological traits. Under stress conditions, DHs with good variability were 2, 6, 9, 14, 18, and 19. The seven best DHs from the mapping population with high molecular diversity were 2 (85.80%), 9 and 15 (74.19%), 18 and 20 (66.67%), 1 (63.00%), and 14 (62.31%). The genetic distance of these DHs were nearly similar or better than the genetic distance of the SH-257 (65.20%) parent. The genetic distance of the local, drought-tolerant cultivars Nesser (42.17%), Margalla-99 (42.17%), and Zarghoon (58.49%) were less than that for SH-257. Finally, we recommend DHs 2, 9, 14, and 18, with good morphological and molecular diversity, for wheat yield improvement programs in Pakistan.

The dendrogram generated from SSR data revealed that seven DHs of the mapping population have significant molecular diversity; 2 (85.80%), 9 and 15 (74.19%), 18 and 20 (66.67%), 1 (63.00%), and 14 (62.31%). The genetic distance of these DH lines was similar or better than that of SH-257 (65.20%). The genetic distance of the local, drought-tolerant cultivars Nesser (42.17%), Margalla-99 (42.17%), and Zarghoon (58.49%) was less than that of SH-257. This data establishes that the mapping population was better morphological than modern wheat cultivars and the synthetic parent. Morphological analysis under control conditions showed that DHs 1, 4, 5, 8, 9, 12, and 20 were diverse for the different morphological traits. Under stress, DHs 2, 6, 9, 14, 18, and 19 were the best.

Finally, DHs 2, 9, 14, and 18, with good morphological and molecular diversity, are recommended for wheat yield improvement programs in Pakistan. Overall, the primary and derived synthetic wheats analyzed in this study showed mmore genetic diversity for drought tolerance than the local/elite, drought-tolerant cultivars because of D genome of synthetic parent (SH-257).

Evaluating wheat germ plasm for salt tolerance.

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Various approaches to improve the salt tolerance of wheat, such as the introduction of new salt-tolerant gene/s, screening of large germ plasm/cultivar collections, conventional breeding, and nonconventional crossing with wheat relatives, have been used. The ultimate aim is to exploit the salt-tolerance variation within wheat and its progenitors or close relatives to produce new wheat cultivars with greater tolerance. Success has been achieved in finding tolerant wheat germ plasm by screening the large number of international wheat collections.

Physiological approaches, based on the mechanisms of salt tolerance using physiological traits to select germ plasm with low sodium uptake or with high selectivity for K^+ over Na^+, have successfully contributed in selecting for diversity in salt tolerance. In this study, the genetic diversity of conventional and novel (synthetic hexaploids) wheat germ plasm was assessed on the basis of K^+:Na^+ ratio, chlorophyll content, soluble sugar levels, soluble protein content, and biomass. *In vitro* experiments were conducted in growth chambers in the Plant Physiology Program of the Crop Sciences Institute (CSI) at NARC, Islamabad.

Ten-day-old seedlings were exposed to 75 mM NaCl stress in a hydroponics culture solution. The testing protocols were essentially similar to those established by Gorham et al. (1987) and Shah et al. (1987). Based on physiological parameters, genotypes Calafia, S-24, Shorawaki, Chinese Spring, Galvez S87, Cochimi, SH-13, Oasis, SH-10 from the Elite 1 SH subset (Mujeeb-Kazi 2003), and SH-12 from the SH salinity subset (Mujeeb-Kazi 2002) were found to be salt tolerant. SH-6, SH-9 (from Elite 1), Ciano, and SH-11 were semitolerant. The remaining genotypes were sensitive at the 75 mM NaCl salinity testing level (Fig. 12). The K^+:Na^+ ratio in the tolerant genotypes ranged from 3 to 6.64 and from 1.0 to 2.40 in the semitolerant. Sensitive materials had a range level from 0.23 to 0.77. Susceptibility of the international standard PDW 34 and the tolerance of Chinese Spring prove to be the valid indices for our data. Shoot biomass of the tolerant genotypes was relatively higher than that of the semitolerant and sensitive genotypes. Tolerant genotypes showed higher chlorophyll content, soluble sugar and soluble protein contents compared with semi tolerant and sensitive genotypes (Table 36). The chlorophyll content of the tolerant genotypes ranged from 13–15 mg/g D wt, 10–12.4 mg g D wt in the semi-tolerant lines, and 8.3–9.0 mg/g D wt in the sensitive genotypes. The soluble sugar content in tolerant genotypes ranged from 24 to 30 mg/g F wt, 19–23 mg/g F wt for the semitolerant genotypes, and 13.3–19.0 mg/g F wt for the susceptible genotypes. Soluble protein content in the tolerant genotypes ranged from 1.5 to 1.96 mg g F wt, 1.27–1.4 F wt in the semitolerant, and 0.67–1.19 F wt in the sensitive lines.

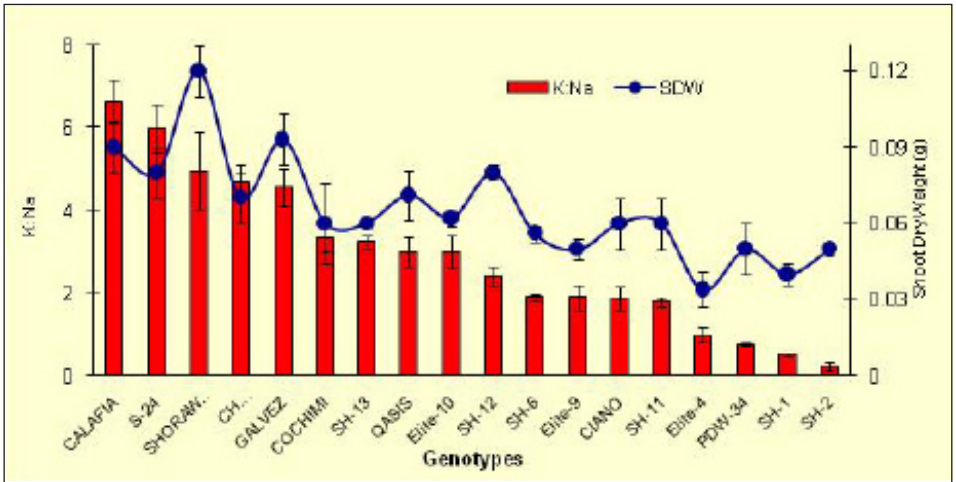

Fig. 12. K^+:Na^+ ratio and shoot dry weight (SDW) of wheat genotypes at 75 mM NaCl stress. Each bar represents the mean value of ten plants with standard error of mean.

Table 36. Chlorophyll, soluble sugar, and soluble protein content of wheat genotypes at 75 mM NaCl stress. The data represents the mean value of ten plants with standard error of mean. Values followed by the same letter (s) are not significantly different at $P < 0.05$ according to the DNMRT test.

Cultivar	Chlorophyll (mg/g D wt)	Soluble sugar (mg/g F wt)	Soluble protein (mg/g F wt)
Calafia	15±0.53 a	30±2.4 a	1.83±0.07 ab
S-24	14±0.7 a	28±3 ab	1.77±0.11 abc
Shorawaki	14±0.3 a	28±1.5 ab	1.96±0.21 a
Chinese Spring	14.5±2.4 a	24±3.4 abc	1.77±0.38 abc
Galvez	14±1.7 a	24±3.2 abc	1.75±0.15 abc
Cochimi	13±0.6 abc	25±1.1abc	1.59±0.17 abcd
SH-13	14±1.3 a	26±3.2 abc	1.7±0.17 abc
Oasis	13.8±0.5 a	23±2.6 abcd	1.60±0.48 abc
Elite-10	13.2±1 ab	24±2 abc	1.5±0.3 abcde
SH-12	13.3±1.7 ab	24±3 abc	1.54±0.10 abcd
SH-6	12±1.5 abc	20±2.9 bcde	1.4±0.18 bcdef
Elite-9	12.4±1.2 abc	22±1.6 abcd	1.27±0.08 cdef
Ciano	11.5±0.28 abc	23±3.2 abcd	1.38±0.10 bcdef
SH-11	10±2 bc	20±2.9 bcde	1.31±0.08 bcdef
Elite-4	9±2.2 c	19±2.5 cde	0.67±0.07 c
PDW-34	8.3±0.4 c	17.8±3.4 cde	1.19±0.06 def
SH-1	8.5±0.5 c	13.7±1.6 e	0.89±0.09 efg
SH-2	9±0.8 bc	13.3±1.2 e	1.04±0.15 e

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New sources of salt tolerance identified in wheat landraces evaluated at different growth stages and environments.

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Introduction. Salt stress is one of the major factors affecting wheat productivity in arid and semiarid regions of the world, including Pakistan. Breeding for salt-tolerant crops has been neglected over the years with few efforts to breed salt-tolerant wheat. The lack of interest by plant breeders mainly is due to complex nature of salt tolerance and its high level of dependence on environmental factors. This project was designed to identify new sources of salt tolerance in wheat by tapping in unexplored landrace collections maintained at Gene Bank of Plant Genetic Resources Program of the NARC. This collection consists of landraces collected from the salt- and drought-prone areas of Pakistan Pakistan and other parts of the world, including Syria, Iran, and Egypt. About 200 landraces were tested at germination, seedling, and maturity under different salt-stress regimes (from 200 to 300 mM salt stess). Some check cultivars with known salt tolerance also were used. The genotypes were tested for two years at germination stage under laboratory conditions at 200, 250, and 300 mM stress, and hydroponically at the vegetative stage in at 200 and 250 mM salt stress. The same set of genotypes also was tested at three locations for two years in salt-affected field conditions with variable stress levels (from moderate to high stress).

2007–08 season. About 200 wheat accessions, including some known salt-tolerant genotypes, were collected and analyzed in the laboratory for their germination at 200 mM salt stress during 2007–08. The same 200 lines also were tested in hydroponic cultures in the vegetative stage at 200 mM salt stress. A salt-tolerance trait index (STTI) for the germination and vegetative stages was calculated by the formula:

$$\text{STTI} = \frac{\text{Value of each trait under stress condition}}{\text{Value under controlled condition}} \times 100$$

Salt-tolerance index (STI) was calculated as the mean of salt-tolerance trait indices (STTIs).

The accessions were tested at reproductive stage at three locations in salt-affected areas at the Soil Salinity Research Institute, Pindi Bhattian District, Hafizabad; the Postgraduate Agriculture Research Station (PARS). and the Biosaline Agriculture Research Station, Pacca Anna, Faisalabad. The genotypes behaved differently at different growth stages. The wheat lines that performed better under different growth-stage testing were 10756 10783, 10790, 10793, 10800, 10806, 10807, 10810, 10812, 10821, 10824, 10828, 10831, 10833, 10841, 10850, 10851, 10859, 11186, 11214, 11287, 11299, 11383, 11385, 11401, 11409, 11417, 11453, 11454, 11460, 11466, 11478, 11526, 11545, 11898, 11907, 11909, 11915, 11917, 11922, 11925, 4098775, 4098805, SARC IV, and SARC VII.

2008–09 season. The selected wheat lines again were tested at germination (250 and 300 mM salt stress), hydroponically (250 mM salt stress), and in two salinity hot spots in the field at the Soil Salinity Research Institute Farm and the Biosaline Research Station during 2008–09. Overall, 21 lines gave good results, including 10756, 10783, 10807, 10833, 10851, 11186, 11299, 11383, 11454, 11460, 11466, 11545, 11898, 11907, 11915, 11917, 4098805, SARC IV, SARC VII, the local white cultivar, and Pasban 90. Pasban 90 is the national salt-tolerance standard and a derivative of *Th.*

distichum (Inia/*Th. distichum*//Inia/3/Genaro). A few accessions also survived the 300 mM stress at germination stage including 11299, 11466, 11907, 11460, 11915, 11454, and 11383. The selected germ plasm will enter our recombination breeding program after determining its diversity status.

Phenotypic characterization and SSR-based diversity estimates of conventional and novel wheat germ plasm with salinity tolerance.

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Genetic diversity of 32 genotypes, consisting of both conventional and novel (synthetic hexaploid) wheat germ plasm, was assessed using SSR primers. The material also was evaluated for its salt-tolerance potential and phenotype. The average K^+/Na^+ value for conventional germ plasm was 3.20 and 1.24 for synthetic hexaploids. Genotypes Calafia, Lu26 S, Chinese Spring, Shorawaki, Galvez S 87, Kharchia 65, and SH-13 were found to be the most tolerant to salinity. Based on phenotypic parameters and yield attributes, Kharchia 65, LU26 S, Mepuchi, Oasis F 86, PBW-343, Calafia, and Pericu showed good performance for days-to-heading, days-to-physiological maturity, plant height, grains/spike, and grain weight. On the other hand, genotypes SH-3, SH-5, SH-7, SH-8, and SH-12 had the highest 1,000-kernel weight and a higher number of grains/spike. Ninety SSR primers specific for the A, B, and D genomes were used to estimate genetic diversity of germ plasm. A total of 347 polymorphic loci was obtained with an average of 3.86 loci/primer. The average similarity coefficient for conventional germ plasm was 0.419 and 0.399 for the synthetics. Conventional genotypes showed a high level of tolerance and the synthetics showed higher genetic diversity. The results suggested that along with salt tolerance, ample genetic diversity is available in both types of germ plasm, especially in synthetic hexaploids, which can be exploited in wheat breeding programs for development of material tolerant to salinity.

Phenotypic characterization (Table 37, p. 190). All genotypes in the conventional germ plasm had an erect growth habit. Among the SHs, three genotypes had a prostrate growth habit, four were moderately prostrate, and the rest were erect. Pubescence was absent in most of the entries in both conventional genotypes and synthetics. Genotypes Sakha 8, Ciano T 79, Mepuchi, PBW 34, and Cochimi, and SH-1, SH-6, and SH-8, were pubescent. Plant height ranged from 86 to 111 cm in the conventional entries. The synthetics were taller, ranging from 83 to 156 cm. Awn color in both types of material was brown, yellow, or amber white. The majority of entries in conventional set had amber white awns; brown was most common in the synthetics. Only Chinese Spring was awnless.

The conventional entries were early heading, with days-to-heading ranging from 110 (PDW34) to 126 (LU26 S). In the synthetics, days-to-heading ranged from 119 (SH-2 and SH-6) to 144 (SH-4). Days-to-maturity ranged from 174–186 days in conventional entries with a majority of the genotypes maturing in 174–180 days. In the synthetics, maturity was from 173–189 days. Spike length ranged from 10–15 cm in conventional entries; the synthetics had longer spikes 10–17 cm. The number of spikelets/spike ranged from 19 (Kharchia 65) to 26 (Ciano T 79) in thef conventional entries and from 16 (SH-9) to 26 (SH-10 and 13) in the synthetics. The number of grains/spike ranged from 34–79 in conventional entries and 18–66 in synthetics, indicating lesser seed set. Thousand-kernel weight ranged from 21 g to 46 g for the conventional entries. The SHs showed a relatively higher grain weight ,ranging between 37.8–71.8 g. Grain color in both types of germ plasm was brown, dark brown, or light brown (Table 37, p. 190).

K^+:Na^+ Discrimination. The germ plasm was screened at 75 mM NaCl, and a high level of tolerance among the conventional germ plasm was noted (Table 37, p. 190). The average K^+/Na^+ values for the conventional material ranged from 0.96 to 6.5. All genotypes showed a K^+/Na^+ value greater than 1 except PDW 34, which was found to be salt susceptible at 75 mM with a K^+/Na^+ value of 0.96. The genotypes Calafia, LU26 S, Chinese Spring, Shorawaki, Galvez S 87, and Kharchia 65 were the most tolerant to salinity. Genotypes Cochimi, Mepuchi, Sakha 8, KRL 1-4, Oasis F 86, Pericu, and Ciano T 89 were semitolerant. All other genotypes in this group were less tolerant to NaCl salinity with K^+/Na^+ values less than 2.

Among the SHs, the K^+/Na^+ valued ranged from 0.35 to 3.08. Six genotypes had a value greater than 1 and the remaining lines were susceptible at a salinity level of 75 mM NaCl. Among the tolerant genotypes, SH-13 was the most tolerant with a K^+/Na^+ value of 3.08, followed by SH-11 (2.98). The least K^+/Na^+value in this group was 0.35, which was for SH-2.

Table 37. Pedigree, phenotypic data, and mean K^+/Na^+ values of conventional and novel wheat germ plasm. * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. GH = growth habit, prosrtate (–), moderately prostrate (M), or erect (+); PUB = pubescence, absence (–) or prescence (+); FLOW = days–to–flowering; PMA = days–to–physiological maturity; HT = plant height at maturity; SL = spike length (cm); SL/S = number of spikelets/spike; G/S = number of grains/spike; TKW = 1,000–kernel weight; GC = grain color, DB = dark brown, LB = light brown, B = brown, and W = white; AWN = awn color, DB = dark brown, LB = light brown, B = brown, AW = amber white, and Y = yellow; and K/Na = average K^+/Na^+ value.

Line No.	Pedigree	GH	PUB		PMA	HT (cm)	SL (cm)	SL/S	G/S	TKW	GC	AWN	K/Na
1	Shorawaki	–	–	120	179	94.0	13.00	24	34	29.4	B	B	4.96
2	Sakha 8	–	+	112	177	103.0	14.00	20	65	39.0	DB	AW	2.95
3	WH 157	–	–	120	179	111.0	12.00	20	46	33.4	B	AW	1.93
4	Kharchia 65	–	–	114	174	93.0	13.50	19	52	40.0	LB	AW	3.75
5	LU26 S	–	–	126	186	86.0	9.60	18	46	46.0	B	Y	6.00
6	PDW 34 (susceptible durum)	–	–	110	179	102.6	15.50	22	53	34.4	LB	B	0.96
7	SNH.9	–	–	114	177	101.3	13.25	22	66	39.2	LB	AW	1.76
8	KRL 1-4	–	–	116	183	91.3	14.50	26	58	33.2	W	AW	2.79
9	Galvez S 87	–	–	116	173	95.6	13.25	25	70	29.4	LB	AW	4.50
10	Oasis F 86	–	–	125	178	89.3	15.00	24	79	32.0	DB	AW	2.76
11	Chinese Spring	–	–	123	174	120.0	10.25	23	67	24.4	B	Awn-less	5.00
12	Ciano T 79	–	+	122	183	96.0	12.75	26	72	25.2	DB	AW	2.09
13	Yecora F 70	–	–	116	182	100.6	10.50	22	45	38.6	DB	AW	1.20
14	Mepuchi	–	+	118	179	105.0	12.25	20	62	42.0	LB	AW	3.02
15	PBW 343	–	+	116	174	86.0	15.00	22	58	38.6	B	AW	1.70
16	Cochimi	–	+	118	177	90.0	11.25	20	58	21.0	B	AW	3.35
17	Calafia	–	–	123	176	99.0	13.50	23	75	41.8	LB	AW	6.50
18	KRL-19	–	–	122	174	99.6	13.25	21	47	31.6	DB	AW	1.38
19	Pericu	–	–	120	174	90.3	13.50	24	66	39.6	B	AW	2.35
SH-1	68.111/RGB-U//WARD Re-sel/3/STIL/4/Ae.tauschii (781)	–	+	122	181	156.0	12.16	22	37	38.4	LB	B	0.52
SH-2	68.111/RGB-U//WARD/3/ FGO/4/RABI/5/Ae. tauschii (882)	–	–	119	189	115.0	12.40	24	42	45.6	B	AW	0.35
SH-3	68112/WARD//Ae. tauschii (369)	M	–	142	189	132.3	14.00	22	38	67.0	DB	B	0.93
SH-4	Altar 84/Ae. tauschii (224)	M	–	144	189	116.6	10.66	21	23	40.4	LB	AW	0.87
SH-5	Altar 84/Ae. tauschii (502)	M	–	142	186	118.0	9.16	18	19	62.0	DB	B	0.85
SH-6	Altar 84/Ae. tauschii (220)	–	+	119	173	103.0	13.67	24	42	380	B	B	1.50
SH-7	Altar 84/Ae. tauschii (211)	+	–	142	186	121.0	15.67	24	30	57.6	DB	Y	1.30
SH-8	Altar 84/Ae. tauschii (JBAN-GOR)	+	+	142	186	109.6	11.67	20	39	71.8	DB	Y	0.86
SH-9	CETA/Ae. tauschii (1027)	+	–	141	186	83.0	10.67	16	18	37.8	DB	B	0.95
SH-10	CETA/Ae. tauschii (895)	–	–	138	184	137.6	17.16	26	60	52.6	DB	DB	1.17
SH-11	CROC 1/Ae. tauschii (224)	–	–	138	186	144.6	17.16	24	66	48.2	DB	B	2.09
SH-12	D67.2/P66.270//Ae. tauschii (220)	M	–	139	186	115.3	12.33	18	22	55.2	B	B	1.37
SH-13	D67.2/P66.270//Ae. tauschii (213)	–	–	126	173	117.6	11.33	26	46	43.0	LB	AW	3.08

Considering both types of material, genotypes of the conventional set were found to be more tolerant compared to synthetics. Calafia, Chinese Spring, Shorawaki, Kharchia 65, LU26 S, SH-11, and SH-13 were amongs the best of all genotypes.

Molecular evaluation using SSR primers. A total of 90 SSR primer pairs were used for molecular evaluation of both conventional set and synthetic hexaploids. Out of 90, 55 primer pairs were specific for the D genome and used for amplification of both synthetics and conventional set entries, 20 primer pairs were specific for the B genome, and 15 were specific for the A genome. The primers of A and B genome were used for the tester set only.

The SSR primers used for molecular evaluation included *Xgwm3-3D*, *Xgwm469-6D*, *Xgwm16-5D*, *Xgwm484-2D*, *Xgwm30-2D*, *Xgwm497-3D*, *Xgwm33-1D*, *Xgwm515-2D*, *Xgwm37-7D*, *Xgwm539-2D*, *Xgwm52-3D*, *Xgwm565-5D*, *Xgwm55-6D*, *Xgwm583-5D*, *Xgwm71-3D*, *Xgwm608-2D*, *Xgwm106-1D*, *Xgwm645-3D*, *Xgwm111-7D*, *Xgwm654-5D*, *Xgwm121-5D*, *Xgwm30-3A*, *Xgwm121-7D*, *Xgwm33-1A*, *Xgwm157-2D*, *Xgwm33-1B*, *Xgwm161-3D*, *Xgwm47.1-2A*, *Xgwm165-4D*, *Xgwm47.2-2A*, *Xgwm174-5D*, *Xgwm47-2B*, *Xgwm182-5D*, *Xgwm55.1-2B*, *Xgwm183-3D*, *Xgwm55.2-2B*, *Xgwm190-5D*, *Xgwm71.1-2A*, *Xgwm192-5D*, *Xgwm71.2-2A*, *Xgwm194-4D*, *Xgwm77-3B*, *Xgwm205-5D*, *Xgwm95-2A*, *Xgwm210-2D*, *Xgwm99-1A*, *Xgwm212-5D*, *Xgwm107-4B*, *Xgwm232-1D*, *Xgwm108-3B*, *Xgwm249-2D*, *Xgwm120-2B*, *Xgwm261-2D*, *Xgwm122-2A*, *Xgwm271-5D*, *Xgwm124-1B*, *Xgwm272-5D*, *Xgwm131-1B*, *Xgwm292-5D*, *Xgwm136-1A*, *Xgwm295-7D*, *Xgwm148-2B*, *Xgwm296-2D*, *Xgwm153-1B*, *Xgwm314-3D*, *Xgwm191-2B*, *Xgwm320-2D*, *Xgwm210-2B*, *Xgwm325-6D*, *Xgwm257-2B*, *Xgwm337-1D*, *Xgwm264-1B*, *Xgwm341-3D*, *Xgwm374-2B*, *Xgwm349-2D*, *Xgwm388-2B*, *Xgwm350-7D*, *Xgwm413-1B*, *Xgwm358-5D*, *Xgwm448-2A*, *Xgwm383-3D*, *Xgwm512-2A*, *Xgwm428-7D*, *Xgwm515-2A*, *Xgwm437-7D*, *Xgwm614-2A*, *Xgwm455-2D*, *Xgwm630-2B*, *Xgwm456-3D*, and *Xgwm*666-1A.

The 90 primers yielded a total of 347 polymorphic loci in the size range of 50 to 1,000 bp out of which 264 were found for the conventional genotypes and 135 for the SHs. The average number of polymorphic loci/primer was 3.86, with a range of minimum of 2 *(Xgwm210-2D)* and a maximum of 15 (*Xgwm33-1B*). The highest number of scorable bands was obtained with primer *Xgwm565-5D* (95) and the lowest with primer *Xgwm210-2D* (1). The maximum number of genotypes (25) were amplified by primer *Xgwm565-5D* and the minimum (1) by *Xgwm210-2D*. All genotypes showed amplification with different primers. Genotype Shorawaki was amplified by the maximum number of primers, 58, and genotype SH-9 was amplified by only one primer, *Xgwm261-2D*. Shorawaki produced the maximum number of bands (85) with all 90 primers, and genotype SH-9 produced the minimum number of bands (2) with 55 D-genome primers.

Similarity coefficient. The similarity coefficient values (Nei and Li's similarity coefficient) for the conventional set ranged from 0 to 0.58. The average similarity coefficient value was 0.419, showing that the genotypes in this group were 58.1% diverse. The values for the SHs ranged from 0 to 0.727. The average similarity coefficient value for the synthetics was 0.399, indicating that these genotypes were 60.1% diverse. The average similarity coefficient value obtained from similarity matrix of all 32 genotypes was 0.418, which showed that there was 41.8% similarity among the genotypes of both groups.

Dendrograms. Dendrograms representing the clustering pattern of the 19 conventional wheat genotypes (Fig. 13) and 13 synthetic hexaploids (Fig. 14, p. 192) were obtained by cluster analysis based on genetic distances. the most distinct genotype of the conventional group was Ciano T 79, which showed an average of 92.1% difference with all other genotypes. A cluster of genotypes, LU26 S, PDW 34, SNH 9, KRL

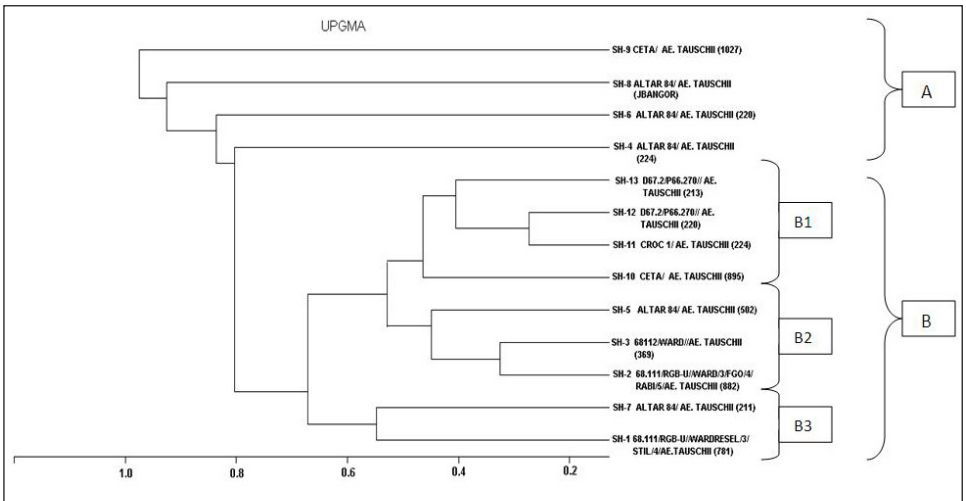

Fig. 13. Dendrogram showing clustering pattern of 19 conventional wheat genotypes based on genetic distance.

1-4, Cochimi, Yecora F 70, Mepuchi, and PBW 343, showed distinctness to another cluster consisting of KRL-19, Galvez S87, Shorawaki, Sakha 8, WH 157, Kharchia 65, Oasis F 86, Chine Spring, Calafia, and Pericu. The average distance between these two clusters was 86%.

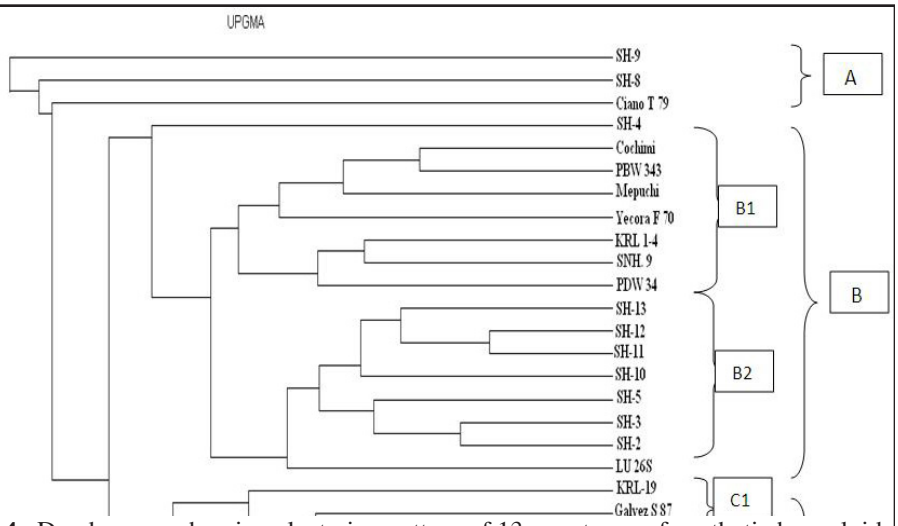

Fig. 14. Dendrogram showing clustering pattern of 13 genotypes of synthetic hexaploid wheats based on genetic distance.

Genotypes SH-9, SH-8, SH-6, and SH-4 were the most distinct among the SHs. In this group, the most diverse genotype was SH-9 with an average genetic distance of 97.5%. SH-8 showed 92.5% genetic distance with 11 other genotypes. SH-6 showed 83.6% genetic distance with ten other genotypes. SH-4 showed 80.4% genetic distance with nine other genotypes. Other genotypes of this group were comparatively less diverse, with a genetic distance ranging from 25% to 65%.

Genotypes of both the conventional and synthetic germ plasm were compared in the dendrogram involving all 32 entries (Fig. 15,). This grouping revealed that a high level of diversity exists between genotypes of conventional and synthetic material. Genotypes of both sets grouped distinctly, showing a relatively low level of diversity within the group anda high level of diversity between the groups. The most distinct genotype in this grouping was SH-9, which was 99% diverse from rest of the genotypes. Ciano T 79 also showed 92.7% diversity with a cluster of 29 other genotypes.

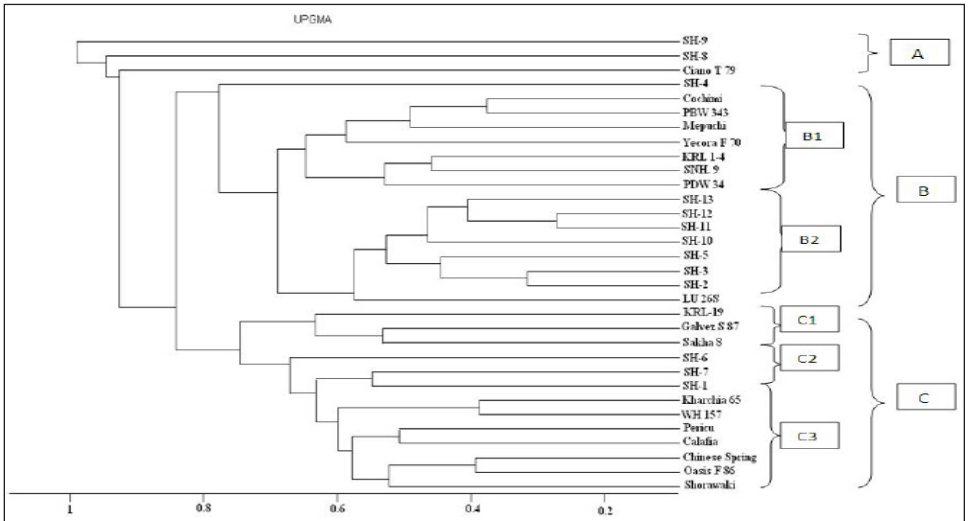

Fig. 15. Dendrogram showing clustering pattern of all 32 genotypes of conventional and synthetic germ plasm based on genetic distance.

Based on the phenotypic evaluation, Kharchia 65, LU26 S, Mepuchi, Oasis F 86, PBW 343, Calafia, and Pericu showed better performance for days-to-heading, days-to-maturity, plant height, grains/spike and grain weight. On the other hand, genotypes SH-3, SH-5, SH-7, SH-8, and SH-12 had the highest grain weight, more grains/spike, and were relatively tall.

Thousand-kernel weight is an important parameter determining yield. Based on grain weight, a genotype can be scored as high or low yielding. Synthetic hexaploid entries had a relatively high grain weight, ranging from 37.8 g to 71.8 g. The highest grain weight was in genotype SH-8. The average 1,000-kernel weight for the conventional material was 34.6 g and 50.5 g for the SH lines.

High K^+/Na^+ values indicate a high level of salt tolerance and a greater ability to exclude Na^+ and accumulate K^+ at high NaCl concentrations. Accumulating more K^+ compared to Na^+ under saline conditions is a character that deter-

mines salinity tolerance of wheat at seedling stage. K^+:Na^+ discrimination of several SHs was determined at different salinity levels. The average K^+/Na^+ value for the conventional set was 3.20 and 1.24 for the synthetics. The synthetic germ plasm deviated from previous results, where the average K^+/Na^+ value among the synthetics was 2.04, whereas that of the conventional germ plasm was 2.26. Data for PDW 34, a susceptible durum wheat, however, was within expected levels.

Analysis of the similarity matrices of both genotype groups revealed that the genetic diversity among the synthetics is greater than that of the conventional genotypes. Based on the dendrogram, Ciano T 79 and SH-9, SH-8, and SH-6 were highly diverse. The average genetic diversity was greater for the synthetics compared to the conventional genotypes. Thus, the SHs will provide a valuable genetic resource for future exploitation.

We concluded that the conventional material is a good source of tolerance to salinity compared to synthetics, however, synthetics are agronomically better with a high level of genetic diversity, which is a prerequisite for any crop-improvement program. Based on overall performance, genotypes LU 26S, Calafia, Galvez S 87, and SH-13 are recommended for their use in wheat breeding programs for the development of salinity-tolerant germ plasm.

Phenotypic evaluation and D-genome-based genetic diversity assessment of winter synthetic wheat germ plasm using SSR primers.

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Nonconventional plant resources for wheat improvement are present in primary, secondary and tertiary *Triticeae* gene pools and, of these, species within the primary gene pool are best for quick and practical output. One such resource is the diploid D-genome donor to bread wheat, *Ae. tauschii*. Of the three modes for utilizing this resource for wheat improvement, the one most utilized is bridge crossing. We focused on capturing the variation within the 58 new synthetic combinations that exploited high-yielding, winter durum wheats and *Ae. tauschii* accessions. The parameters analyzed were related to traits and molecular composition for diversity was differentiated by chromosome-specific SSR markers. According to the phenological data WS-11, WS-24, WS-46, WS-47, and WS-48 performed well in the field, especially for yield-enhancing characters. A cluster analysis using SSR primers revealed that WS-1, WS-24, WS-43, and WS-46 were the best lines.

Breeding and selection has resulted in the loss of a great number of alleles, causing difficulties in wheat improvement that have emerged for the modern agriculture system (Allard 1996; Hoisington et al. 1999). The narrow genetic base weakens the resistance of current wheat cultivars against biotic and abiotic stresses and threatens further advancement of wheat. Studies of synthetic hexaploid wheat developed from *T. turgidum* and *Ae. tauschii* has provided significant information on potentially useful characters in *Ae. tauschii* and/or *T. turgidum* for genetic improvement of hexaploid wheat (Mujeeb-Kazi et al. 2008).

Modern tetraploid durum wheat generally has been used to produce these new primary synthetic wheats. The primary synthetics are agronomically poor, difficult to thresh, generally tall, low yielding, and frequently have poor quality. However, they do carry useful and, often times, new variation for a range of economically important characteristics. Potentially new genetic variation among primary synthetics also has been found for tolerance to drought (Villareal et al. 1998), salinity (Gorham 1990), frost (Maes et al. 2001), heat (Yang et al. 2002), and nutrient stress (Cakmak et al. 1999).

This study is focused on capturing the variation within the 58 new synthetic combinations developed by crossing high-yielding, winter durum wheat with *Ae. tauschii* accessions. Winter SHs were developed for enhancing the diversity and to improve wheat quality and yield. These winter synthetics were grown in field after their production for their phenotypic evaluation and were then molecularly analyzed.

Phenotypic evaluation. Morphological characters of selected new winter SH wheats were assessed phenotypically (Table 38, pp. 194-196). Lines WS-11, WS-24, WS-46, WS-47, and WS-48 exhibited good morphological characters, early maturity, optimum plant height, large spike length, and higher 1,000-kernel weight.

Molecular evaluation. ***Evaluation of SSR primers for diversity estimates in winter SH lines.*** Microsatellite or inter simple sequence repeat (ISSR) markers and RAPD markers are the most polymorphic markers in wheat and are highly useful (Röder et al. 1998; Nagaoka and Ogihara 1997). In this study, SSRs were used to find genetic diversity among 58

winter synthetic wheat lines. These synthetics possess a high degree of polymorphism. The 65 D-genome-specific SSR primers were used to detect genetic diversity at DNA level in new D-genome-derived winter SHs included the following: *Xgwm2-3D, Xgwm295-7D, Xgwm3-3D, Xgwm296-2D, Xgwm16-5D, Xgwm314-3D, Xgwm30-2D, Xgwm320-2D, Xgwm33-1D, Xgwm325-6D, Xgwm37-7D, Xgwm337-1D, Xgwm52-3D, Xgwm341-3D, Xgwm55-6D, Xgwm349-2D, Xgwm71-3D, Xgwm350-7D, Xgwm102-2D, Xgwm358-5D, Xgwm106-1D, Xgwm383-3D, Xgwm111-7D, Xgwm428-7D, Xgwm121-5D, Xgwm437-7D, Xgwm121-7D, Xgwm455-2D, Xgwm157-2D, Xgwm456-3D, Xgwm161-3D, Xgwm458-1D, Xgwm165-4D, Xgwm469-6D, Xgwm174-5D, Xgwm484-2D, Xgwm182-5D, Xgwm497-3D, Xgwm183-3D, Xgwm515-2D, Xgwm190-5D, Xgwm539-2D, Xgwm192-5D, Xgwm565-5D, Xgwm194-4D, Xgwm583-5D, Xgwm205-5D, Xgwm608-2D, Xgwm210-2D, Xgwm608-4D, Xgwm212-5D, Xgwm609-4D, Xgwm232-1D, Xgwm624-4D, Xgwm249-2D, Xgwm635-7D, Xgwm261-2D, Xgwm642-4D, Xgwm269-5D, Xgwm645-3D, Xgwm271-5D, Xgwm654-5D, Xgwm272-5D, Xgwm664-3D,* and *Xgwm292-5D*.

The 65 primers yielded a total of 1,114 bands in the range of 50–1,000 bp out of which 250 were found to be polymorphic. The highest number of scorable bands (74) was obtained with primer *Xgwm*337-1D and the lowest number (1) with primer *Xgwm*437-7D. The maximum number of genotypes (36) were amplified by primer *Xgwm*608-2D and the minimum (1) by *Xgwm*437-7D, *Xgwm358-D*, and *Xgwm583-5D*. Different primers showed variation in their ability to detect polymorphism. Primer *Xgwm529-2D* and *Xgwm325-6D* showed the highest polymorphism and primers *Xgwm15-5D*, *Xgwm106-1D*, *Xgwm261-2D*, and *Xgwm320-2D* had the lowest.

WS-5 was amplified by maximum number of primers (30) whereas WS-56 was amplified by only one primer. Genotypes WS-1, WS-24, and WS-43 showed the maximum diversity. The efficiency of these primers to amplify the

Table 38. Pedigree and morphological data (mean values) of winter synthetic hexaploid wheats (2n=6x=42; AABBDD) derived from 'winter durum/*Aegilops tauschii*' cross combinations. * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. GH = growth habit, prosrtate (–), moderately prostrate (M), or erect (+); PUB = pubescence, absence (–) or prescence (+); FLOW = days–to–flowering; PMA = days–to–physiological maturity; HT = plant height at maturity; #S = total number of spikes/plant; SL = spike length (cm); SL/S = number of spikelets/spike; G/S = number of grains/spike; TKW = 1,000–kernel weight; GC = grain color, DB = dark brown, LB = light brown, B = brown, and W = white; and AWN = awn color, DB = dark brown, LB = light brown, B = brown, AW = amber white, and Y = yellow.

Line No.	Pedigree	GH	PUB		PMA	HT (cm)	#S	SL (cm)	SL/S	G/S	TKW	GC	AWN
1	Aisberg/*Ae.tauschii* (221)*	+	–	159	185	77	15	12.3	19	17	28.4	LB	AW
2	Aisberg/*Ae.tauschii* (310)	+	–	149	185	87	18	13.3	19	14	39.6	B	LB
3	Aisberg/*Ae.tauschii* (369)	+	+	157	186	96	13	11.3	19	5	20.0	B	AW
4	Aisberg/*Ae.tauschii* (446)	+	–	153	186	94	20	11.6	21	3	34.0	LB	DB
5	Aisberg/*Ae.tauschii* (511)	+	–	153	185	98	20	12.0	20	14	42.2	B	AW
6	LEUC762.93/*Ae.tauschii* (409)	+	–	151	185	94	10	14.0	25	3	33.0	LB	DB
7	LEUC762.93/*Ae.tauschii* (424)	+	–	153	181	95	17	11.0	18	13	25.0	LD	AW
8	LEUC762.93/ *Ae.tauschii* (1027)	+	+	156	184	98	14	13.0	18	5	31.0	DB	LB
9	LEUC784693/*Ae.tauschii* (310)	+	–	152	178	93	7	11.0	18	2	20.0	B	LB
10	LEUC84693/*Ae.tauschii* (409)	+	+	150	189	81	10	11.6	16	13	32.0	B	B
11	LEUC84693/*Ae.tauschii* (1024)	+	–	151	183	93	11	13.6	17	3	48.0	D.B	B
12	LEUC84693/*Ae.tauschii* (1026)	+	–	156	189	83	11	14.0	19	5	33.0	LB	DB
13	UKR-OD 1169.91/*Ae.tauschii* (625)	+	–	164	191	54	1	12.0	17	4	27.0	B	LB
14	UKR-OD 1169.91/*Ae.tauschii* (1024)	+	–	154	190	67	3	11.3	15	8	29.0	B	B
15	UKR-OD 761.93/*Ae.tauschii* (191)	+	–	154	191	91	8	11.6	12	6	35.5	B	B
16	UKR-OD 761.93/*Ae.tauschii* (192)	+	+	155	188	98	15	11.6	15	3	35.0	LB	AW
17	UKR-OD 761.93/*Ae.tauschii* (219)	+	+	149	194	105	11	10.3	17	16	34.8	B	AW

Table 38 (continued). Pedigree and morphological data (mean values) of winter synthetic hexaploid wheats (2n=6x=42; AABBDD) derived from 'winter durum/*Aegilops tauschii*' cross combinations. * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. GH = growth habit, prosrtate (–), moderately prostrate (M), or erect (+); PUB = pubescence, absence (–) or prescence (+); FLOW = days–to–flowering; PMA = days–to–physiological maturity; HT = plant height at maturity; #S = total number of spikes/plant; SL = spike length (cm); SL/S = number of spikelets/spike; G/S = number of grains/spike; TKW = 1,000–kernel weight; GC = grain color, DB = dark brown, LB = light brown, B = brown, and W = white; and AWN = awn color, DB = dark brown, LB = light brown, B = brown, AW = amber white, and Y = yellow.

Line No.	Pedigree	GH	PUB	FLOW	PMA	HT (cm)	#S	SL (cm)	SL/S	G/S	TKW	GC	AWN
18	UKR-OD 761.93/*Ae.tauschii* (392)	+	–	150	182	100	15	13.0	21	7	24.5	LB	AW
19	UK- OD 1704.94/*Ae.tauschii* (1031)	+	–	169	193	60	6	11.0	13	M	—	—	DB
20	UKR-OD 1704.94/*Ae.tauschii* (213)	+	+	154	185	100	18	11.0	17	9	33.6	B	AW
21	UKR-OD 1704.94/*Ae.tauschii* (217)	+	+	153	192	93	10	12.7	21	4	31.3	LB	AW
22	UKR-OD 1704.94/*Ae.tauschii* (369)	+	–	154	194	98	12	11.6	19	7	24.5	B	LB
23	UKR-OD 1704.94/*Ae.tauschii* (511)	+	–	161	189	94	11	11.0	15	1	27.7	B	AW
24	UKR-OD 1871.94/*Ae.tauschii* (213)	+	–	152	183	84	10	12.3	19	8	35.0	B	LB
25	UKR-OD 1871.94/*Ae.tauschii* (221)	+	–	153	183	92	17	14.0	19	8	41.0	LB	AW
26	UKR-OD 1871.94/*Ae.tauschii* (323)	+	–	154	183	84	12	12.6	21	3	28.0	B	LB
27	UKR-OD 1871.94/*Ae.tauschii* (1024)	+	–	165	192	52	6	10.3	17	2	29.0	LB	AW
28	UKR-OD 1871.94/*Ae.tauschii* (1027)	+	–	175	192	54	2	16	16	5	27.3	LB	LB
29	UKR-OD 952.92/*Ae.tauschii* (188)	+	–	186	194	45	3	10.0	17	7	29.5	B	AW
30	UKR-OD 952.92/*Ae.tauschii* (304)	+	+	172	190	50	4	11.6	15	8	30.0	LB	B
31	UKR-OD 952.92/*Ae.tauschii* (309)	+	–	172	192	56	9	8.6	16	5	28.0	LB	LB
32	UKR-OD 952.92/*Ae.tauschii* (311)	+	–	166	192	55	5	7.1	15	6	31.0	B	B
33	UKR-OD 952.92/*Ae.tauschii* (326)	+	–	156	186	89	14	12.3	19	5	36.0	LB	LB
34	UKR-OD 952.92/*Ae.tauschii* (358)	+	–	150	188	74	9	14.0	22	8	32.0	LB	LB
35	UKR-OD 952.92/*Ae.tauschii* (372)	+	–	170	187	52	5	10.6	16	7	27.0	B	AW
36	UKR-OD 952.92/*Ae.tauschii* (409)	+	–	166	192	60	3	10.0	13	8	36.0	LB	B
37	UKR-OD 952.92/*Ae.tauschii* (428)	+	+	166	186	49	1	10.0	16	5	29.0	LB	LB
38	UKR-OD 952.92/A*e.tauschii* (511)	+	–	158	185	71	3	11.3	21	7	26.6	B	LB
39	UKR-OD 952.92/*Ae.tauschii* (633)	+	–	153	185	81	9	13.3	20	4	37.3	LB	LB

Table 38 (continued). Pedigree and morphological data (mean values) of winter synthetic hexaploid wheats (2n=6x=42; AABBDD) derived from 'winter durum/*Aegilops tauschii*' cross combinations. * Number in parenthesis is the Wide Crosses working collection accession number maintained at CIMMYT, Mexico. GH = growth habit, prosrtate (–), moderately prostrate (M), or erect (+); PUB = pubescence, absence (–) or prescence (+); FLOW = days–to–flowering; PMA = days–to–physiological maturity; HT = plant height at maturity; #S = total number of spikes/plant; SL = spike length (cm); SL/S = number of spikelets/spike; G/S = number of grains/spike; TKW = 1,000–kernel weight; GC = grain color, DB = dark brown, LB = light brown, B = brown, and W = white; and AWN = awn color, DB = dark brown, LB = light brown, B = brown, AW = amber white, and Y = yellow.

Line No.	Pedigree	GH	PUB	FLOW	PMA	HT (cm)	#S	SL (cm)	SL/S	G/S	TKW	GC	AWN
40	UKR-OD 952.92/*Ae. tauschii* (1018)	+	–	156	180	64	4	8.0	23	12	25.0	AW	AW
41	UKR-OD 952.92/*Ae. tauschii* (1024)	+	–	156	180	77	7	8.7	23	28	23.2	AW	B
42	UKR-OD 952.92/*Ae. tauschii* (1028)	+	+	154	186	75	4	12.0	20	7	24.0	LB	B
43	UKR-OD 952.92/*Ae. tauschii* (1031)	+	–	152	180	76	16	10.0	19	5	23.0	AW	LB
44	UKR-OD 1530/*Ae. tauschii* (217)	+	–	149	180	92	12	11.6	17	12	37.5	LB	LB
45	UKR-OD 1530/*Ae. tauschii* (306)	+	–	152	185	80	9	12.3	19	5	39.5	AW	DB
46	UKR-OD 1530.94/*Ae. tauschii* (311))	+	–	153	187	95	11	11.0	17	7	48.0	AW	B
47	UKR-OD 1530.94/*Ae. tauschii* (310)	+	–	151	185	96	15	10.6	17	13	44.4	LB	B
48	UKR-OD 1530.94/*Ae. tauschii* (312)	+	–	152	185	91	12	13.0	17	7	43.3	LB	DB
49	UKR-OD 1530.94/*Ae. tauschii* (392)	+	+	152	184	85	8	11.3	17	12	35.3	LB	DB
50	UKR-OD 1530.94/*Ae. tauschii* (446)	+	–	157	186	92	9	12.3	18	9	32.5	Br	DB
51	UKR-OD 1530.94/*Ae. tauschii* (458)	+	+	155	181	89	17	14.0	20	10	27.0	AW	LB
52	UKR-OD 1530.94/*Ae. tauschii* (511)	+	–	158	184	94	7	10.2	19	5	32.0	AW	B
53	UKR-OD 1530.94/*Ae. tauschii* (629)	+	+	157	185	77	8	12.3	15	9	16.8	LB	LB
54	UKR-OD 1530.94/*Ae. tauschii* (1024)	+	+	152	182	89	18	13.3	10	2	15.0	AW	AW
55	UKR-OD 1530.94/*Ae. tauschii* (1027)	+	–	153	178	88	16	13.8	21	4	43.0	LB	B
56	PANDUR/*Ae. tauschii* (223)	+	–	150	182	67	4	11.0	18	4	30.0	LB	B
57	PANDUR/*Ae. tauschii* (409)	+	–	152	183	80	14	10.5	17	11	35.0	LB	DB
58	PANDUR/*Ae. tauschii* (515)	+	–	158	185	80	7	10.0	17	2	34.0	LB	B

genotypes ranged from 36 by primer *Xgwm608-2D*, 32 by primer *Xgwm337-1D*, 30 by primer *Xgwm456-2D*, and 28 by primer *Xgwm456-3D*.

SSR amplification data was used to generate a similarity matrix to estimate genetic diversity and relatedness among the 58 newly synthesized winter synthetics. The value of the similarity coefficient of winter synthetic wheat lines ranged from 0 to 75%. The average similarity coefficient value was 32% and, hence, 68% genetic distance.

This set of newly synthesized winter wheat SHs showed minimum similarity. Nearly all genotypes gave a 0% similarity coefficient value with one or more genotypes. Genotypes with 70% or more similarity were WS-49 with WS-

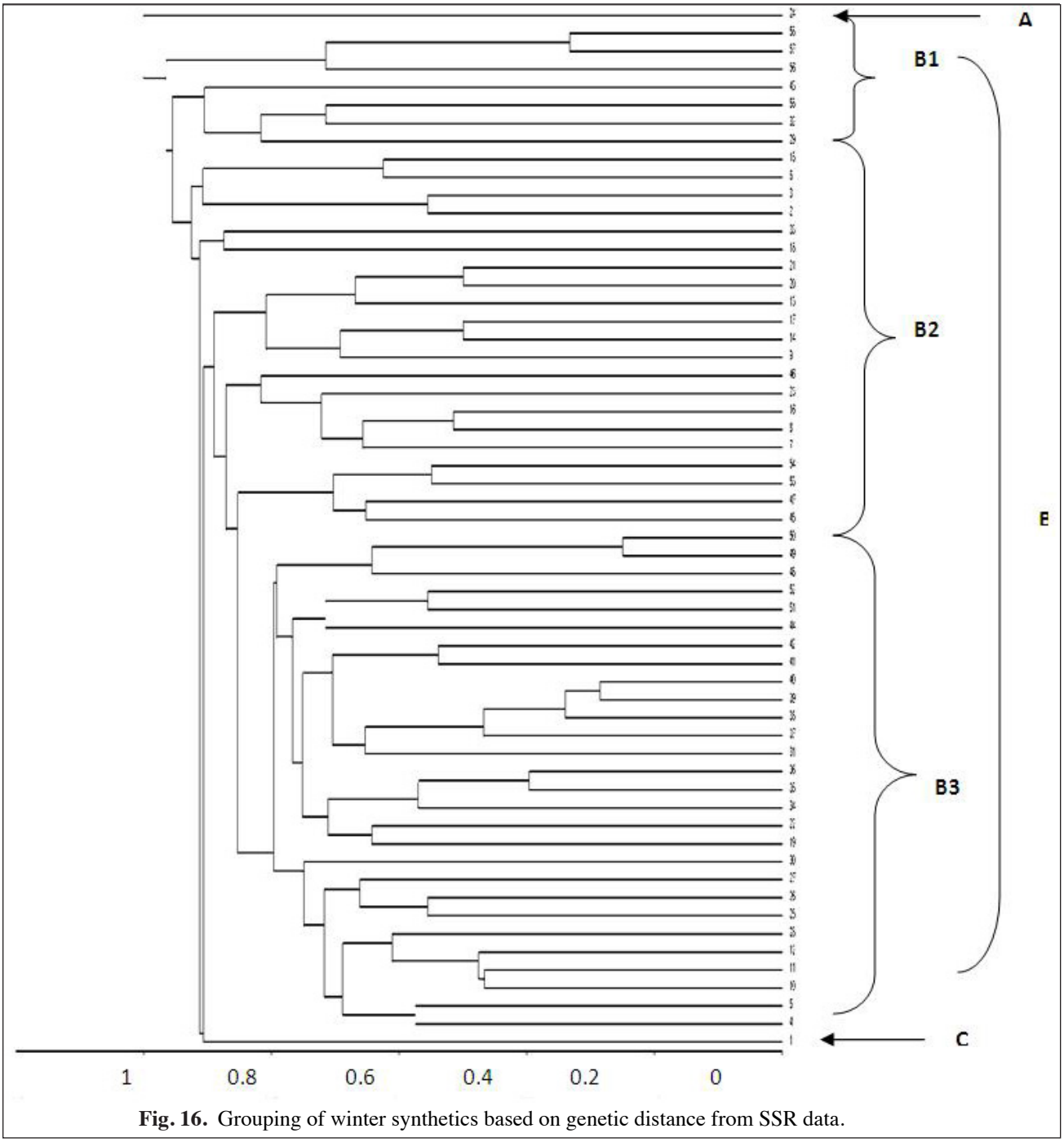

Fig. 16. Grouping of winter synthetics based on genetic distance from SSR data.

50 (75.0%), WS-39 withWS-40 (71.4%). The similarity of the remaining genotypes was between 0 to 70%.

The dendrogram of SSR based genetic diversity evaluation clearly indicates three main clusters, A, B, and C (Fig. 16). Genotypes in cluster A and C exhibited the maximum genetic diversity compared to all other clusters. Genotype WS-24 of cluster A is the most diverse line among the 58 winter synthetics with a maximum genetic distance of 100%. WS-1 also is considered to be a diverse line.

These winter synthetics are genetically diverse. They have traits that can be incorporated into modern cultivars by crosses with *T. aestivum*. Among these winter synthetics, genotypes WS-1, WS-24, WS-43, and WS-46 are recommended. Based on these data, winter wheat breeders can use these suggested winter synthetics expediently in their bread wheat improvement programs for the transfer of desirable genes.

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ITEMS FROM POLAND

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Instability of some endosperm traits in Trtiticum/Aegilops amphiploids.

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We studied endosperm instability in the following amphiploids: *T. turgidum* subsp. *dicoccum*/*Ae. tauschii*, *T. turdidum* subps. *carthlicum*/*Ae. tauschii*, *T. turgidum* subsp. *turgidum*/*Ae. tauschii*, *T. timopheevii* subsp. *timopheevii*/*Ae. longissima*, and *T. timopheevii* subsp. *timopheevii*/*Ae. umbellulata*. All hybrids were obtained from the Plant Germ-plasm Institute in Kyoto, Japan. Anatomy and cytology of this material were observed under light microscopes (polarized Amplival Carl Zeiss Jena and epifluorescence Olympus BX60) and documented with the use of Fuji 400 negatives.

The normal development of endosperm in grasses ends with the creation of interior starch tissue, the sub-aleurone, high-protein layer, and the outer-most aleurone. Normally, the aleurone is composed of one-cell layer with a special protein encapsulated in the aleurone grains. The aleurone layer can disappear or multiply under some genetic or environmental stimuli. An interesting example of multiplication of the aleurone layer in the vicinity of the caryopsis crease is presented in Fig. 1A (p. 199) for the *T. timopheevii* subsp. *timopheevii*/*Ae. umbellulata* amphiploid. A multi-celled layer is created as a result of several periclinal divisions. A two-celled aleurone is showed on the left (red arrow). This type of cell penetrates deeper into the starch tissue on the right (a green arrow). In these two adjacent, anticlinal rows of cells, the expression of aleurone phenotype differs distinctly. A starch phenotype can penetrate outside the starch tissue and appears, often in the form of a large, undivided anticlinally, cell between smaller aleurone cells (Fig. 1B for *T. turdigum* subsp. *carthlicum*/*Ae. tauschii*, p. 199). This tissue also is visible in the cross-section of caryopsis (Fig. 1C in *T. timopheevii* subsp. *timopheevii*/*Ae. umbellulata*, p. 199) where more cells of the starch phenotype develop. The most surprising development is in *T. timopheevii* subsp. *turdigum*/*Ae. tauschii* (Fig. 1D, p. 199), where a very long starch cell, isolated by a hemicellulosic wall expressing blue autofluorescence. grows for a long time and finally is located between