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

Islamabad, Pakistan.

Wheat wide crosses and general wheat improvement trends: initiatives and the course ahead.

Mujeeb-Kazi and Alvina Gul Kazi.

Wheat production in 2010 reached near 24 x 10^6 tons at approximately 2.6 t/ha. The yield projection for the approaching harvest in 2011 has been projected between 24.5 to 25 x 10^6 tons, and this is primarily due to congenial environmental conditions across the country. No change in the production constraints that prevail to achieve projection targets was observed. Abiotic stresses of drought and salinity/sodicity remain with heat merging as a major concern due to the cropping systems that are in place. The rusts still rank as number one biotic stress priority, with yellow and leaf rust the biggest. Stem rust, around the local race prevalent in lower Punjab and in the province of Sindh, is carefully monitored because, if confounded by Ug99 when it reaches Pakistan, will pose a serious hazard. National scientists have released varieties that are Ug99 resistant based upon screening in Kenya and advanced breeding materials in conventional national breeding programs also are added sources of new resources. Extensive new genetic diversity is crucial for achieving security against this biotic stress and an on-base research program is a dire need.

A strong prebreeding program firmly in place in Pakistan is paramount and has been initially planned by mid-2010. Unfortunately, both CIMMYT and ICARDA leadership have set in place an operational plan where the CIMMYT Pakistan representative has stated that all prebreeding under the Pak-U.S. bilateral alliance will be done in U.S. This is a set-back to our national efforts, where the forward direction should be to evolve and upgrade the developing country programs promoting scientific advancement integrated with U.S. elite scientific institutions, which was the spirit earlier advocated by the Pak/U.S. partners in 2009 and 2010. Our current program is actively involved in prebreeding and has made impact. International center decisions have not helped our national cause in moving ahead swiftly around volatile young, human, resource strength that is being generated progressively. Despite this temporary constraint, our wide crossing program is moving ahead and has rapidly restructured around new partners hoping that the earlier linkages will fall back in place.

Our new Wheat Wide Crosses Program has identified Ug99 resistant lines through crosses involving D-genome synthetic hexaploid germ plasm. Furthermore, the derivatives also are resistant against the local race of stem rust identified upon screening in Sindh. The nature of the local race has to be elucidated and the Cereal Disease Program of NARC is on the front line for this informational sharing. Resistant, derived lines from Wheat Wide Crosses to Ug99 are shown in Table 1 (p. 82).

Our Wheat Wide Crosses Program, due to prevalent circumstances, has shifted its major focus towards intensive prebreeding areas exploiting novel alien genomic diversity that could be readily adapted to national environmental regimes. Our main breeding effort now is structured around alliances with the private sector and the four agricultural centers of the Pakistan Atomic Energy Commission (PAEC). The PAEC has centers in Sindh and two in Punjab (Upper) and KPK (former NWFP). The Sindh alliance will allow the province of Baluchistan to be covered and then other selected provincial partners shall be tapped to facilitate. Selected locations across the country allow for hotspot sites to be tapped that permit screening for heat, drought, salinity, stem rust local race (SINDH) plus bread-making quality aspects, leaf and stem rust, spot blotch, drought (Lower Punjab), drought (Upper Punjab), yellow rust, aphids, BYDV (KPK), and drought (Baluchistan).

Table 1. Advanced Ug99 stem rust resistant derivatives from bread wheat cultivars recombined with D-genome synthetic hexaploid wheats screened in Kenya under the BGRI initiated facilitation at the Kenyan Agricultural Research Institute. Reactions are R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlap of MR–MS, MSS = moderately susceptible to susceptible, and S = susceptible; control disease 90–100S.

Pedigree	Stripe rust	Stem rust	
	25 February	4 March	17 March
Altar 84/*Ae. tauschii* (224)//2*YACO/3/ Mayoor//TKSN1081/*Ae. tauschii* (222)/4/ Kukun/5/Altar 84/*Ae. tauschii* (221)/YACO	15M	10M	20M
KAUZ/5/68.111/RGB-U//WARD RESEL/3/STIL/4/*Ae. tauschii* (431)	5MR	10M	15M
Opata//DOY 1/*Ae. tauschii* (255)	10M	5MR	10MR
BKH93/Flycatcher	30MSS	20M	30M
Bakhtawar 94/5/68.111/RGB-U//WARD RESEL/3/STIL/4/*Ae. tauschii* (431)	15M	15M	20M
CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (208)/5/OAPTA/5/68.111/RGB-U// WARD RESEL/3/STIL/4/*Ae. tauschii* (783)	30S	5MR	10MR
Mayoor//TK SN1081/*Ae. tauschii* (222)/3/ OPATA/6/68.111/RGB-U//WARD/3/ FGO/4/RABI/5/*Ae. tauschii* (878)	40M	15M	20M
Opata//CETA/*Ae. tauschii* (1027)	0	15M	20M
Worrakatta/Pastor//SHR	50S	10M	15M
PFAU/Weaver*2//Kiritati/3/WAFAQ	5MR	5M	10M
WL6736/5/2*BR12*3/4/IAS55*4/CI14123/3/ WAFAQ	5MR	10M	15M

The pivot location where Wheat Wide Crosses Program is located (Islamabad) concentrates on prebreeding plus basic research to feed material to all locations and maintain wider international ties in addition to those with CIMMYT/ ICARDA and embrace all regional programs that surround Pakistan to the west, east, and northeast. This modified structure allows the wide cross group to concentrate on basic prebreeding and strategic aspects of genetic recombinations with the applied parts exploited by other location partners through their major expertise on breeding.

Our group now focusses on screening for karnal bunt, biochemical quality aspects, all in vitro testings for abiotic stresses, molecular diagnostics, doubled haploidy, micronutrient assays and cytogenetics plus maintenance of genetic stocks and wild species with depositions of appropriate categorized germplasms in the national gene bank also in NARC in PGRI at Islamabad.

Current progress in the Wide Cross Program during mid-2006 to mid-2010, via the support of three major research projects, led to the modest development of an infrastructure including greenhouses, screenhouses, field, and laboratories that undertake activities on stress physiology, cytogenetics, biochemical genetics, molecular diagnostics, doubled haploidy, growth rooms and seed storage capacity around the programs working with the germ plasm collection.

The second major output has been a human resource development from internees, degree holders at the M. Phil and Ph.D. levels, research fellows and assistants, plus trained support staff that hold high school degrees (ayudantes in Spanish or skilled labor). The total output number has been 70 in approximately four years (September 2006 until June 2010).

The third component of the program has been research output, and the current status is the generation of advanced F_7 lines (KAZI 1–9, 11–13) that have seed distributed across the country for testing and increase. These are introduced and adapted lines from international nurseries and also those generated from wheat/synthetic hexaploid crosses made within our program. The SSR-based diversity profiles of these lines will allow us to differentially deploy cultivars in the near future across the country and safeguard our food security through possessed genetic variation across a wide genetic base. The line KAZI 11 is early maturing, possesses multiple resistances, and is a good candidate for the province of Sindh. KAZI 11 is resistant to both the local stem rust race and Ug99. A field plot at a private partner location in Punjab (RCA Seeds) exemplifies our applied effort (Fig. 1).

Fig. 1. A collaborating scientist from the RCA seeds in the KAZI 11 plot at Khanewal, Punjab, during the 2010–11 crop cycle.

In addition, 1,000 elite selections from the F_5 to F_6 generations, derived from our main recombination programs where hexaploids of the A and D genome have been crossed onto elite bread wheat cultivars, are being studied for various attributes. We also have 60,000 derivatives from F_2 to F_4 generations, various mapping populations, cytogenetic stocks, and wild species from all three Triticeae gene pools.

Students who have been involved in the program are major contributors and the articles that follow this introduction show the potential of the germ plasm that has been generated for various production stress constraints. These introductory perceptions address investigations conducted by our young human resource talent on resistance/tolerance to rust, Karnal bunt, powdery mildew, spot blotch, heat, salinity, drought, DNA fingerprint diversity using RAPDs and SSRs, and bread-making quality. The outputs are based upon national testing, collaborative alliances (national/international), and field and controlled environment testing.

The way forward: some perceptions.

National organizational changes have set in and will be implemented by mid-2011. Food security will remain a key concern and, as wheat research progresses, a substantial strategic change is advocated. The immediate major benefit will come from management aspects and these could pay off swift dividends that appear unimaginable if set in place appropriately. The research program alliances of interest will have to be formulated that can address and exploit at least some of the following:

– exploit the global elite durum cultivar diversity for improving bread wheat via pentaploid breeding,
– target tetraploids identified for heat and drought tolerance for bread wheat improvement, e.g., *T. turgidum* subsp. *dicoccum*,
– incorporate bread wheats with large spikes in the breeding program with a selection sieve for optimum grain filling, grain number/spike, and improved tillering capacity,
– investigate early maturity to address climate change and global warming,
– micronutrient enrichment, an interest for wheat breeders, requires greater attention,
– encourage cotton and rice breeders to produce early maturing types as these two crops fall in the wheat cropping system of rice/wheat and cotton/wheat cycles,
– give greater attention to wheat/alien chromosome translocations on the applied dimensions through earlier available stocks or by producing new products mediated by cytogenetic protocols of manipulation,
– have in place a volatile program on doubled haploidy to assist national breeding partners for applying the protocol at least by the F_3 and,
– infuse molecular diagnostics for adding efficiency to breeding to allow marker-assisted trait incorporation to flourish.

There is interest in hybrid wheat and transgenics; two areas that could be contemplated upon and addressed through multidisciplinary integration of expertise. Double haploidy has a role in both programs where for heterotic F_1s to be fixed or transgenics made homozygous on T_0 plants, a massive effort is required. At present, using the maize protocol is the key, but for the future, microspore culture if genotype nonspecific could do wonders for wheat breeding efficiency. Other aspects that can augment yield need to be observed and should be integrated into programs as these

become available. Some are experimental but a C4 wheat or bringing the rice resistance for rust into wheat may be a long shot. However, innovative means of using genomic diversity through gene pyramiding, another look at Triticale or even durum as a new crop addition. should be within our national reach to address when we are projecting to the national vision of 2050 based upon prebreeding, genetic diversity, and the 'Green-to-Gene Revolution'.

Evaluation of Elite-I synthetic hexaploid germ plasm for various phenological, molecular, and disease attributes.

Alvina Gul Kazi, Awais Rasheed, Farrukh Bashir, Hadi Bux, Abdul Aziz Napar, and Abdul Mujeeb-Kazi.

In the primary gene pool of wheat, the species included are hexaploid landraces, cultivated tetraploids (2n=4x=28, AABB), wild *T. turgidum* subsp. *dicoccoides*, and the diploid (2n=2x=14) donors of the A and D genomes to durum/bread wheats. In this gene pool, genetic transfers result through direct hybridization, homologous recombination, and relatively simple breeding strategies. Some combinations requirethe assistance of embryo rescue and are of greater interest for enhancing diversity in bread wheat. The goat grass *Ae. tauschii* (2n=2x=14, DD) currently occupies a very high priority in wheat breeding.

Conventional wheat breeding programs are built around diverse cross combinations of germ plasm residing in the same gene pool that undergo genetic recombination followed by trait segregation, evaluation, and ultimately cultivar release. In order to amplify the genetic diversity of the crop, novel genetic resources become a focus and the close progenitors of wheat are preferred; these are the numerous accessions of the A, B, and D genomes. Within this spectrum, the A and D genomes have greater advantage than B essentially because of their proximity to the A and D sets present in bread wheat and also based upon cytogenetic test analyses that indicate greater closeness of the seven chromosomes of the D-genome wild diploids than the A-genome chromosomes with their respective D and A genomes. Accessions of these two diverse sources reside in the primary gene pool, can be hybridized with ease, allow for swift gene transfer via homologous recombination, and have extensive diversity for global biotic/abiotic stress/constraints that limit wheat production.

Greater genetic proximity tilts the optimum choice towards the exploitation of the D-genome diploid *Ae. tauschii* and also because few accessions were involved in the natural hybridization/amphiploidization event, thus giving rise to a crop with an extremely narrow genetic base. Complementary to this are observations associated with the *Ae. tauschii* role that have enabled current investigators to focus their wheat improvement efforts around this wild diploid via various protocols.

Elite-I subset advance. The 95 primary synthetics were studied for their phenotypic and molecular characterization together with screening against Karnal bunt, stripe rust, and powdery mildew. The phenotypic characters were days-to-flowering, days-to-physiological maturity, height at maturity, presence or absence of pubescence and pigmentation, and 1,000- kernel weight. Molecular characterization for establishing DNA diversity profiles was done via RAPDs and SSR microsatellite markers.

All the 95 Elite-I entries were screened in pot trials in the greenhouse at Murree. Fourty-four accessions of 95 the Elite-1 SH wheats showed a resistant reaction at seedling stage (Table 2, pp. 84-86). Infection type ranged from 0–6 at the seedling stage indicating the presence of major genes for resistance. Some of these resistant accessions exhibited different reaction types against powdery mildew under field conditions. The Elite-I entries also were screened under field conditions at Kaghan and majority showed APR and were found to be resistant to completely resistant (immune).

In the Elite-I, 44 accessions showed resistance against powdery mildew at both seedling and adult-plant stages, including 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, 62, 64, 68, 69, 70, 71, 78, 79, 81, 83, 84, 90, 91, and 93. All these lines with excellent resistance against powdery mildew can be used in direct crosses with elite commercial wheat cultivars for further exploitation as sources of resistance against powdery mildew for wheat improvement.

Another category of the germ plasm included the accessions with APR but susceptible at the seedling stage. This high value APR is needed by breeders and agronomists for introducing durable powdery mildew resistance in elite commercial cultivars and is controlled by different minor genes independently or group of genes working together mak-

Table 2. Phenological and disease characterization of the D-genome synthetic hexaploids in the Elite-I set. FLOW = days-to-flowering; HT = plant height at maturity (cm); AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown); PMA = days-to-physiological maturity; TKW = 1,000-kernel weight (g); G/S = number of grains/spike; SL = spike length (cm); KB = Karnal bunt (– = immune, + = susceptible); Pm (S) = powdery mildew screening at the seedling stage; Pm (A) = powdery mildew screening at the adult-plant stage; Yr (S) = stripe rust screening at the seedling stage; Yr (A) = stripe rust screening at the adult-plant stage where R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlapping of MR–MS, MSS = moderately susceptible to susceptible, S = susceptible, and TS = trace susceptible.

No.	FLOW	HT	AWN	PMA	TKW	G/S	SL	KB	Pm (S)	Pm (A)	Yr (S)	Yr (A)
1	104	100	LB	144	52.3	22	12.0	–	1-1	6	1	R
2	89	110	LB	130	59.6	43	12.0	–	1-1	7	1	0
3	102	95	LB	136	59.7	31	12.0	–	2-1	6	0	5MRR
4	85	115	W	120	43.0	23	8.0	+	1-1	6	78	20MR
5	99	120	LB	140	57.0	38	12.0	+	0-0	5	78	5R
6	99	100	DB	136	54.4	20	12.2	+	0-0	3	1	5R
7	96	105	W	132	46.7	10	10.2	–	1-1	2	0	20S
8	100	110	W	132	59.4	25	7.2	–	1-1	3	0	0
9	96	135	W	134	53.7	14	8.0	–	1-1	4	78	0
10	117	85	DB	152	52.4	9	14.0	–	1-1	4	12	TMS
11	100	110	DB	144	55.6	13	12.0	–	3-1	2	12	10S
12	85	115	DB	127	62.2	14	11.0	+	1-1	4	56	20MS
13	110	105	DB	148	55.5	48	7.0	–	1-1	4	7	10MS
14	93	115	W	130	59.0	42	14.0	–	1-1	3	0	40S
15	106	115	B	144	60.0	10	11.0	–	1-1	4	7	40MS
16	100	95	W	134	55.9	9	9.0	–	1-1	5	4	40S
17	99	100	DB	134	60.0	54	13.7	–	1-1	4	78	50S
18	110	105	B	148	58.8	10	13.2	–	3-2	5	7	20MSS
19	96	105	B	134	57.7	15	12.0	–	1-1	5	1	30MR
20	99	100	W	136	50.8	14	10.0	+	2-1	5	12	20MRMS
21	110	105	W	144	59.6	12	10.5	–	2-1	4	34	MSS
22	110	105	LB	148	53.1	15	9.0	–	2-1	4	89	S
23	106	105	DB	148	55.9	18	12.3	–	1-1	4	67	40MRMS
24	99	90	LB	134	54.8	16	12.0	–	1-1	4	12	40MS
25	100	105	LB	136	52.6	12	13.0	–	1-1	3	0	10MS
26	85	125	DB	127	60.5	49	9.2	–	1-1	6	67	40MSS
27	117	90	B	152	53.2	28	7.3	–	1-1	3	1	10R
28	119	100	LB	152	49.5	11	12.5	–	1-1	7	34	20MSS
29	100	105	W	132	47.5	6	11.0	+	1-1	4	12	20MRMS
30	110	100	B	148	38.0	12	14.0	–	1-1	5	12	40MRMS
31	96	115	LB	134	55.6	23	14.2	–	1-1	5	45	60MRMS
32	99	100	W	134	54.0	16	14.0	+	1-1	3	0	0
33	96	120	W	134	33.4	14	13.0	+	1-1	4	89	50S
34	96	110	LB	130	58.1	10	14.0	–	1-1	2	0	20S
35	106	100	LB	148	32.5	17	14.3	–	1-1	4	0	10R
36	96	110	LB	144	60.1	13	14.5	–	1-1	2	0	20MRR
37	96	125	LB	134	58.9	15	12.0	–	1-1	2	12	40MS
38	99	110	LB	136	52.1	23	14.0	–	1-1	3	56	50MS
39	99	100	LB	134	30.2	38	14.0	–	1-1	5	12	40MS
40	100	115	LB	138	51.0	10	15.5	+	2-1	2	0	TR
41	99	120	LB	136	48.7	16	14.2	–	1-1	4	0	TR
42	96	105	LB	134	46.5	9	14.8	+	1-1	3	12	20RMR
43	104	120	LB	144	50.7	13	11.0	–	1-1	3	12	40MRMS
44	93	125	B	134	58.2	17	12.0	–	1-1	3	12	20MRMS
45	93	125	B	130	55.4	28	9.0	–	1-1	2	23	60MSS

Table 2. Phenological and disease characterization of the D-genome synthetic hexaploids in the Elite-I set. FLOW = days-to-flowering; HT = plant height at maturity (cm); AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown); PMA = days-to-physiological maturity; TKW = 1,000-kernel weight (g); G/S = number of grains/spike; SL = spike length (cm); KB = Karnal bunt (– = immune, + = susceptible); Pm (S) = powdery mildew screening at the seedling stage; Pm (A) = powdery mildew screening at the adult-plant stage; Yr (S) = stripe rust screening at the seedling stage; Yr (A) = stripe rust screening at the adult-plant stage where R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlapping of MR–MS, MSS = moderately susceptible to susceptible, S = susceptible, and TS = trace susceptible.

No.	FLOW	HT	AWN	PMA	TKW	G/S	SL	KB	Pm (S)	Pm (A)	Yr (S)	Yr (A)
46	96	100	LB	144	57.4	18	12.0	–	2-1	6	45	20MS
47	96	125	LB	130	55.9	20	12.0	–	1-1	2	45	40MS
48	100	100	LB	148	54.9	22	12.0	+	1-1	2	1	0
49	76	135	DB	115	44.7	5	10.0	–	1-1	3	1	0
50	85	110	LB	127	57.8	20	10.0	–	1-1	3	0	0
51	85	110	LB	127	51.2	6	12.3	–	1-1	2	1	5MRR
52	99	105	LB	134	54.2	12	14.0	–	1-1	3	0	0
53	96	90	LB	127	53.4	8	14.2	–	1-1	3	4	40MSS
54	110	120	LB	148	42.3	23	14.0	–	0-0	3	0	40MSS
55	99	125	LB	144	54.7	23	14.0	+	0-0	3	8	5R
56	112	115	LB	148	54.8	9	10.0	–	1-1	3	1	30MS
57	112	110	LB	148	50.0	5	12.0	–	2-1	1	45	50S
58	110	115	LB	144	49.0	8	12.0	–	1-1	3	78	10MS
59	99	90	B	134	49.5	11	10.2	+	0-0	4	45	40MS
60	96	120	B	134	52.4	16	14.0	–	1-1	3	1	20MR
61	100	115	LB	144	54.1	61	14.2	–	1-1	3	1	70S
62	96	110	LB	136	52.8	46	13.0	–	2-1	5	67	5MRMS
63	96	115	LB	136	52.7	8	11.0	–	1-1	2	12	10R
64	100	115	LB	152	38.8	58	9.3	+	1-1	4	0	0
65	117	125	LB	152	43.8	22	15.2	–	2-1	3	78	0
66	106	115	LB	152	41.4	15	14.0	+	1-1	5	4	60MS
67	106	110	B	144	67.6	14	12.0	–	1-1	5	8	40MS
68	117	105	LB	150	58.3	4	8.0	–	1-1	5	8	30MS
69	112	120	LB	144	59.5	10	14.0	–	1-1	2	8	0
70	99	115	LB	134	57.9	15	10.2	–	1-1	2	34	60S
71	110	105	LB	144	47.1	30	12.0	–	1-1	3	12	10MS
72	100	105	LB	136	60.8	21	14.3	–	1-1	1	12	80S
73	96	120	LB	130	56.8	9	13.3	–	1-1	5	78	80S
74	96	105	LB	130	57.6	18	11.2	–	1-1	6	34	10R
75	99	95	LB	130	53.7	17	14.3	–	2-1	4	89	50S
76	96	90	LB	134	48.7	18	14.3	–	1-1	5	0	40MS
77	99	95	LB	134	49.3	5	12.0	–	1-1	6	1	40MSS
78	100	95	LB	136	49.1	15	14.0	–	1-1	5	78	0
79	108	90	DB	140	55.7	12	16.0	+	1-1	1	89	0
80	99	140	LB	134	59.0	12	16.0	–	1-1	1	0	0
81	115	110	LB	152	46.8	18	16.0	–	1-1	1	89	70S
82	115	105	LB	152	45.0	20	16.0	–	1-1	5	0	50R
83	96	135	LB	130	60.4	14	15.2	–	1-1	2	0	20MRMS
84	89	95	LB	127	56.9	13	16.0	–	1-1	6	78	5R
85	102	100	LB	138	56.8	19	15.0	–	1-1	3	1	10MR
86	99	105	LB	136	55.7	15	15.0	–	1-1	2	0	10MR
87	96	100	LB	138	54.4	16	15.0	–	1-1	5	0	10MR
88	96	120	LB	134	49.0	18	14.0	–	1-1	5	0	5R
89	108	110	DB	146	58.1	15	14.0	–	1-1	5	0	0
90	84	140	B	137	63.2	16	16.0	–	1-1	4	12	50S
91	96	90	LB	134	57.1	13	15.2	–	1-1	4	0	0

Table 2. Phenological and disease characterization of the D-genome synthetic hexaploids in the Elite-I set. FLOW = days-to-flowering; HT = plant height at maturity (cm); AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown); PMA = days-to-physiological maturity; TKW = 1,000-kernel weight (g); G/S = number of grains/spike; SL = spike length (cm); KB = Karnal bunt (– = immune, + = susceptible); Pm (S) = powdery mildew screening at the seedling stage; Pm (A) = powdery mildew screening at the adult-plant stage; Yr (S) = stripe rust screening at the seedling stage; Yr (A) = stripe rust screening at the adult-plant stage where R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlapping of MR–MS, MSS = moderately susceptible to susceptible, S = susceptible, and TS = trace susceptible.

No.	FLOW	HT	AWN	PMA	TKW	G/S	SL	KB	Pm (S)	Pm (A)	Yr (S)	Yr (A)
92	96	115	LB	134	57.2	14	15.0	–	1-1	2	78	0
93	77	127	145	119	48.4	16	15.0	–	1-1	3	12	40MS
94	106	105	LB	138	56.1	30	15.0	–	1-1	5	12	20MS
95	99	105	LB	138	62.0	28	15.0	–	1-1	2	0	0

ing it difficult for a new race of pathogen to overcome the plant resistance. The 49 Elite-I accesions were 2, 3, 4, 5, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 28, 29, 30, 31, 33, 35, 39, 41, 46, 52, 61, 63, 65, 66, 67, 72, 73, 74, 75, 76, 77, 80, 82, 85, 86, 87, 88, 89, and 92.

The third category had moderate resistance or was susceptible to powdery mildew at both seedling and adult-plant stages. The Elite-I accessions with an intermediate resistance or susceptible to powdery mildew at the seedling stage are 1, 3, 4, 5, 9, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 26, 29, 30, 31, 33, 35, 39, 41, 46, 58, 61, 63, 65, 66, 67, 72, 73, 74, 75, 76, 77, 80, 82, 85, 86, 87, 88, 89, and 92.

Stripe rust studies. Seedling screening showed that 63 out of 95 (66.3%) in Elite-I exhibited seedling resistance to stripe rust (Table 2, pp. 84-86). These genotypes also were screened for APR under field conditions at NARC; 40 of the 95 (42.1%) were resistant genotypes. Thirty-one (32.6%) genotypes had both seedling and APR (1, 2, 3, 6, 8, 19, 27, 32, 35, 36, 40, 41, 42, 48, 49, 50, 51, 52, 55, 63, 64, 74, 80, 82, 85, 86, 87, 88, 89, 91, and 95). All this germ plasm represents the presence of major genes against stripe rust and can be exploited further in breeding programs.

Adult-plant resistance involving susceptibility at the seedling stage and resistance only at the adult-plant stage indicates the presence of minor genes, which are considered of great importance against rust diseases in acquiring durable resistance. In the Elite-I, nine lines (9%), including 4, 5, 9, 65, 69, 78, 79, 84, and 92, showed APR and are good candidates for providing durable resistance to wheat cultivars.

Karnal bunt studies. Karnal bunt evaluation was done by examining the grains following artificial inoculation. Grains from each entry were examined separately after hand threshing. The rating scale was from 0 to 5 (Fig 2). Only a rating of 0 was considered acceptable and 1–5 as susceptible. In the Elite-I, 79 entries (83.1%) were found to be completely immune to Karnal bunt, including 1, 2, 3, 7, 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24, 25, 26, 27, 28, 30, 31, 34, 35, 36, 37, 38, 39, 41, 43, 44, 45, 46, 47, 49, 50, 51, 52, 53, 54, 56, 57, 58, 60, 61, 62, 63, 65, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 80, 81,82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, and 95 (Table 2, pp. 84-86).

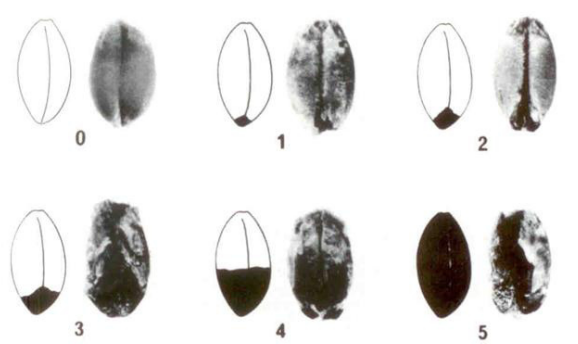

Fig. 2. Infection severity scale caused by Karnal bunt, 0 = zero infection to 5 = a completely bunted grain (Warham et al. 1986).

Molecular studies. ***Genetic diversity evaluation using random amplified polymorphic DNA RAPD primers.*** RAPD primers were used for genetic diversity evaluation of these D-genome synthetic hexaploids. All 520 RAPD primers of the Operon Series were screened, and working primers were identified and applied to detect genetic polymorphism at DNA level. Samples that did not amplify were not included in the analysis.

Genetic analysis was performed only on the scorable bands. Each single band was considered as a single locus/allele. The loci were scored as present/absent. Bivariate data 1–0 were used to estimate genetic distances (GD). Un-

weighted pair group of arithmetic means (UPGMA) function estimated genetic distances between the genotypes as follows: $GD_{xy} = 1 - d_{xy}/d_x + d_y - d_{xy}$, where GD_{xy} = genetic distance between two genotypes, d_{xy} = total number of common loci (bands) in two genotypes, d_x = total number of loci (bands) in genotype 1, and d_y = total number of loci (bands) in genotype 2.

The efficiency of primers to amplify the genotypes ranged from a maximum of 41 genotypes (OPG-12) to a minimum of two genotypes (OPH-4) in Elite-I (Table 3). Scorable bands ranged from five (OPF-3) to 92 (OPG-6) (Table 3). Genetic analysis of the population showed that the total number of loci for Elite-I was 92, of which 82 were polymorphic with a percentage of 89.13% (Table 3). The range of scorable bands was from 250–3,000 bp.

Table 3. Molecular fingerprinting pattern by RAPD analysis in the Elite-I D-genome synthetic hexaploid set.

Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable Bands	Amplification product range (bp)
OPF-1	3	3	100%	24	36	500–1,500
OPF-3	2	2	100%	5	5	500–1,000
OPF-4	2	2	100%	21	37	1,500–2,000
OPF-8	4	4	100%	14	19	500–1,500
OPF-10	4	4	100%	6	11	1,000–2,000
OPF-13	7	7	100%	28	70	500–2,000
OPF-18	5	5	100%	10	14	500–2,000
OPF-20	5	3	60%	8	14	500–2,500
OPG-2	5	5	100%	33	58	750–2,000
OPG-4	1	1	100%	14	14	1,500–2,000
OPG-5	2	0	0%	6	12	250–750
OPG-6	9	9	100%	36	92	250–2,500
OPG-9	4	4	100%	15	30	750–2,000
OPG-11	4	4	100%	7	13	750–2,500
OPG-12	8	8	100%	41	89	250–2,500
OPG-13	3	3	100%	7	17	250–1,000
OPG-17	1	1	100%	5	5	250–500
OPG-18	2	2	100%	8	8	250–1,000
OPG-19	5	5	100%	18	47	750–1,500
OPH-2	2	0	0%	3	6	250–1,000
OPH-3	5	5	100%	5	12	500–2,000
OPH-4	5	3	60%	2	6	500–1,500
OPH-9	4	2	50%	6	16	500–2,000

Similarity matrix.
A bivariate analysis generated a similarity matrix and dendrogram using Nei and Li's coefficient to estimate genetic diversity. The value of the similarity matrix ranged from 63.0% (minimum), between genotypes 49 and 51 and 51 and 53, and 100% (maximum), between genotypes 18 and 68, 18 and 89, 18 and 91, 18 and 93, 68 and 89, 68 and 92, 68 and 94, 80 and 83, 80 and 84, 89 and 92, 89 and 94, and 92 and 94, in the Elite-I.

Dendrogram interpretation. The genetic distance between genotypes were used to construct a dendrogram by UPGMA analysis for determining the grouping of the lines on the basis of similarities and differences. In the Elite-I, the dendrogram represents one main cluster with subclusters A, B, and C (Fig. 3, p.88). Subcluster A has 15 genotypes containing genotype 15 as the most diverse line in the entire Elite-I. Other good lines in subcluster A are 46, 47, and 49. In subcluster B, there are 21 entries of which 73 is the most diverse; lines 81, 83 and 84, and 78 and 80 are 100% similar to each other. Line 73 is the most diverse line in this subcluster and 69, 70, and 77 are other good lines. Subcluster C can be further divided into five groups, from C1 to C5. Group C1 contains four genotypes with entry 3 as the most diverse line. Group C2 has seven genotypes of which 25 and 30 are the best to recommend. Group C3 has seven genotypes, again with 11 and 14 as the best genotypes. Group C4 represents the largest group in subcluster C with 32 genotypes, lines 18, 68, 89, 92, and 94 show 100% similarity. Line 59 stands out as the best line, followed by 23, 37, and 42. Subcluster C5 has nine genotypes of which 1 and 4 represent the best lines.

Molecular studies. ***Evaluation of genetic diversity using simple sequence repeat (SSR) primers.*** SSR primers were used for genetic diversity evaluation of A-, B-, and D-genome synthetic hexaploids. All 275 SSR primers were applied to each set to detect genetic polymorphism at DNA level (Table 4, pp. 89-90). Samples that did not amplify were not included in the analysis.

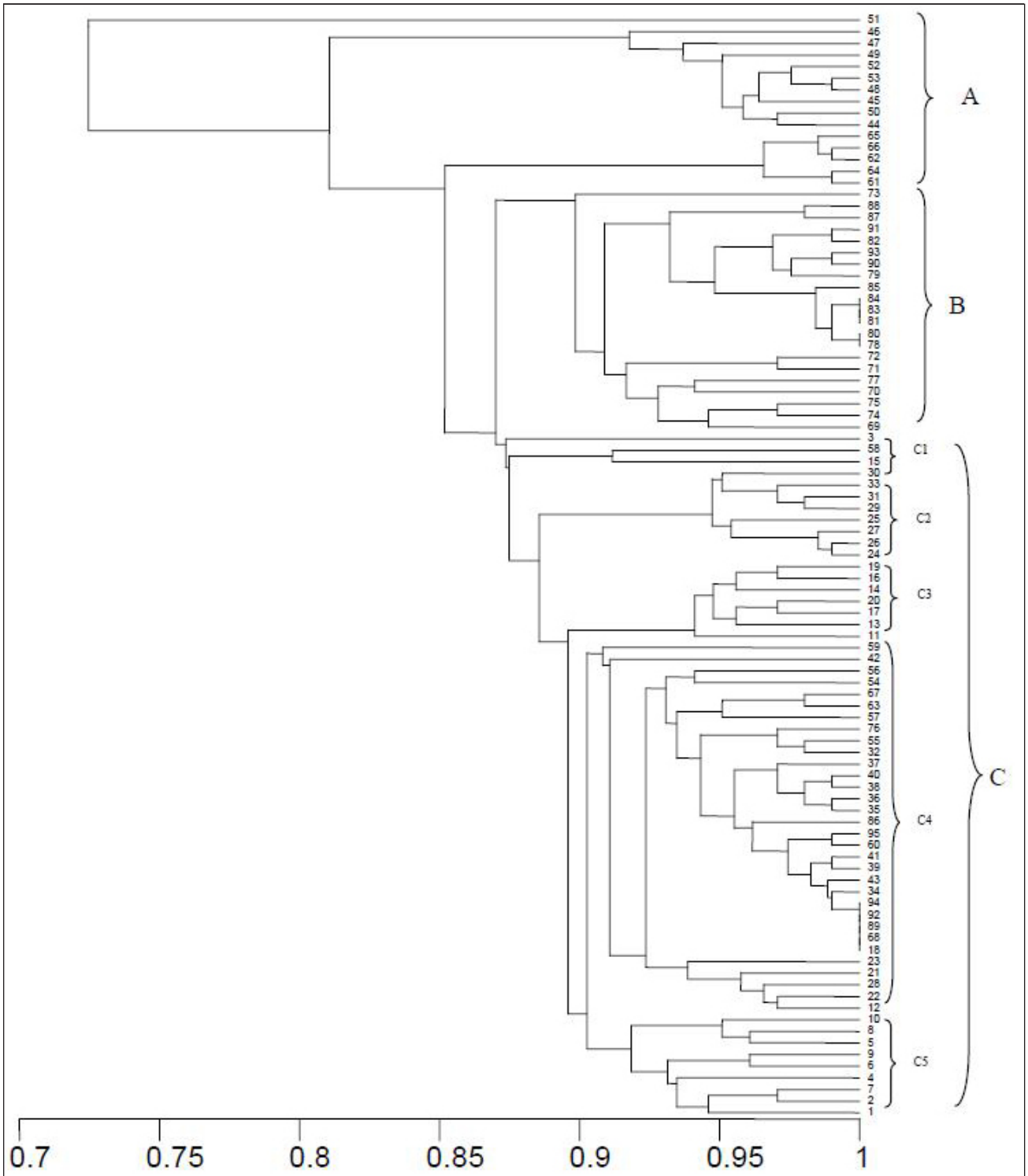

Fig. 3. A dendrogram of the genetic diversity in the Elite-I synthetic hexaploids, evaluated using random amplified polymorphic DNA (RAPD) primers, with one main cluster and three subclusters A, B, and C.

Genetic analysis was performed only on the scorable bands. Each single band was considered as a single locus/allele. The loci were scored as present/absent. Bavariate data 1–0 were used to estimate the GD as for the RAPD markers. The efficiency of the primers to amplify the genotypes ranged from maximum 92 genotypes (*Xgwm645-3D*, *Xgwm149-4B*, *Xgwm550-1B*, *Xgwm264-1B*, *Xgwm169-6A*, and *Xgwm4-4A*) to the minimum of two genotypes (*Xgwm459-6A*) in the Elite-I (Table 4, pp. 89-90). Scorable bands ranged from two (*Xgwm459-6A*) to 430 (*Xgwm219-6B*) (Table 4, pp. 89-90). A genetic analysis of the population showed that the total number of alleles for the Elite-I was 452, of which 431 were polymorphic (95.35%).

Table 4. Molecular fingerprinting pattern using SSR markers in Elite-I set of D-genome synthetic hexaploids.

Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)	PIC
Xgwm33-1A	8	8	100%	90	185	50–250	0.14
Xgwm99-1A	7	7	100%	86	175	50–150	0.54
Xgwm135-1A	4	4	100%	27	44	150	0.53
Xgwm136-1A	6	6	100%	72	86	250–400	0.90
Xgwm164-1A	4	4	100%	80	131	100–150	0.39
Xgwm497-1A	4	4	100%	46	85	100	0.42
Xgwm71.1-2A	5	5	100%	86	257	50–150	0.90
Xgwm359-2A	2	2	100%	8	8	250	0.72
Xgwm497-2A	4	4	100%	77	148	100–150	0.41
Xgwm558-2A	5	5	100%	85	183	50–150	0.55
Xgwm2-3A	7	3	33.33%	82	109	50–100	0.82
Xgwm391-3A	6	6	100%	28	39	150	0.65
Xgwm4-4A	10	10	100%	92	304	50–600	0.75
Xgwm160-4A	6	6	100%	75	115	50–250	0.72
Xgwm610-4A	4	4	100%	81	175	50–150	0.73
Xgwm126-5A	5	5	100%	12	24	800–1,000	0.55
Xgwm617-5A	7	7	100%	89	212	50–150	0.62
Xgwm169-6A	4	4	100%	92	114	200	0.87
Xgwm459-6A	2	2	100%	2	2	50	0.73
Xgwm494-6A	2	2	100%	78	124	100–150	0.67
Xgwm570-6A	4	4	100%	29	122	200	0.71
Xgwm130-7A	4	4	100%	27	44	50–150	0.05
Xgwm332-7A	9	9	100%	90	230	50–500	0.57
Xgwm350-7A	3	3	100%	35	38	50–150	0.51
Xgwm635-7A	4	4	100%	25	38	50–100	0.73
Xgwm140-1B	8	8	100%	86	107	50–400	0.28
Xgwm264-1B	8	8	100%	92	313	50–200	0.69
Xgwm403-1B	2	2	100%	19	20	100	0.57
Xgwm550-1B	12	12	100%	92	359	50–200	0.75
Xgwm47-2B	8	8	100%	81	149	200	0.62
Xgwm210-2B	5	3	100%	90	225	50–200	0.86
Xgwm257-2B	12	12	100%	91	216	200–400	0.72
Xgwm112-3B	5	5	100%	89	159	50–100	0.40
Xgwm264-3B	11	11	100%	74	224	150–1000	0.84
Xgwm284-3B	4	4	100%	37	99	500–1000	0.75
Xgwm493-3B	4	4	100%	85	113	150–200	0.22
Xgwm533.1-3B	3	3	100%	36	36	100–150	0.46
Xgwm6-4B	6	6	100%	36	105	50–300	0.06
Xgwm149-4B	4	4	100%	92	104	150	0.14
Xgwm191-5B	10	10	100%	82	138	50–150	0.54
Xgwm234-5B	16	16	100%	87	346	200–1,000	0.90
Xgwm371-5B	3	3	100%	65	126	50–150	0.39
Xgwm193-6B	6	6	100%	91	137	50–150	0.42
Xgwm219-6B	16	16	100%	88	430	50–1,000	0.90
Xgwm508-6B	5	5	100%	63	120	150–200	0.72
Xgwm613-6B	3	3	100%	7	11	150	0.41
Xgwm626-6B	4	4	100%	86	163	50–150	0.55
Xgwm43-7B	5	5	100%	90	160	50–1,000	0.82

Table 4. Molecular fingerprinting pattern using SSR markers in Elite-I set of D-genome synthetic hexaploids.

Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)	PIC
Xgwm46-7B	5	5	100%	15	23	200	0.65
Xgwm68-7B	5	5	100%	90	286	50–200	0.75
Xgwm146-7B	4	4	100%	76	162	50–200	0.72
Xgwm344-7B	6	6	100%	64	93	100–150	0.73
Xgwm106-1D	4	4	100%	81	124	50–150	0.55
Xgwm232-1D	5	5	100%	84	123	50–150	0.62
Xgwm458-1D	4	4	100%	67	93	50–100	0.53
Xgwm642-1D	11	11	100%	80	301	50–1,000	0.87
Xgwm102-2D	6	6	100%	64	69	150–200	0.73
Xgwm261-2D	6	6	100%	91	184	50–200	0.67
Xgwm515-2D	4	4	100%	30	85	50–150	0.71
Xgwm645-3D	2	2	100%	92	104	50–300	0.05
Xgwm3-3D	3	3	100%	24	28	50–100	0.57
Xgwm183-3D	7	7	100%	75	128	50–150	0.51
Xgwm383-3D	8	5	62.5%	75	153	50–150	0.73
Xgwm608-4D	8	8	100%	85	216	100–1,000	0.28
Xgwm182-5D	5	5	100%	79	106	50–200	0.69
Xgwm190-5D	5	5	100%	88	135	150–200	0.57
Xgwm292-5D	6	6	100%	23	27	50–200	0.75
Xgwm565-5D	10	10	100%	78	332	50–1,000	0.62
Xgwm583-5D	4	4	100%	91	115	150–200	0.86
Xgwm55-6D	4	4	100%	87	152	50–100	0.72
Xgwm325-6D	4	4	100%	69	90	50–150	0.40
Xgwm469-6D	13	11	84.61%	87	253	50–1,000	0.84
Xgwm44-7D	8	8	100%	88	276	50–1,000	0.75
Xgwm428-7D	12	2	16.66%	79	100	50–1,000	0.22
Xgwm437-7D	5	5	100%	86	102	50–100	0.46

Similarity matrix. A bivariate analysis was conducted to generate a similarity matrix and dendrogram using Nei and Li's coefficient (1979) to estimate genetic diversity (Fig 4, p. 91). The value of similarity matrix ranged from 64.9 (minimum) between genotypes 55 and 40, 79 and 28, 80 and 40 and 81 and 20 while 92.6% (maximum) between genotypes 52 and 51 in the Elite-I.

Dendrogram interpretation. Genetic distances between the genotypes were used to construct a dendrogram by UPGMA analysis for determining grouping of the lines on the basis of similarities and differences. The dendrogram for Elite-I represents only one main cluster with six subclusters (Fig. 4, p. 91). Subcluster 1 has only six genotypes in which 78 and 88 are the most diverse lines. Subcluster 2 has 18 genotypes in which 20, 21, and 29 are the best lines. Subcluster 3 has 18 genotypes also, with 39, 40, 45, and 53 as the genetically diverse lines. Subcluster 4 has 17 genotypes of which 65 is truly the best line. Subcluster 5 also includes some lines that are 100% similar, such as 55 and 80. A total 22 genotypes in this and 8, 9, 28, 82, 86 and 95 are highly diverse lines in this subcluster 5. Subcluster 6 has 14 genotypes of which 3, 6, 7, and 16 are comparatively better lines.

The Elite-I set of synthetic hexaploids. All SH entries were tall, late maturing, hard to thresh, and showed phenology characteristics that exhibited enormous diversity for various traits analyzed (Table 2, pp. 84-86). Important parameters for breeding generally are days-to-flowering, days-to-physiological maturity, plant height at maturity, spike length, grains/spike, and, most significantly, 1,000-kernel weight. These traits figured in the selection of SHs in crossing with bread wheat. Synthetics are generally tall with a range from over 85 cm to a maximum of 140 cm. The tall height does not negate their utilization in crossing, because height can be rectified via the genetic contribution of the wheat involved. This holds true for the other parameters such as days-to-flowering and physiological maturity, which are, in essence, crucial indices for successful utilization in crossing. If these two traits are later than wheat, having various planting

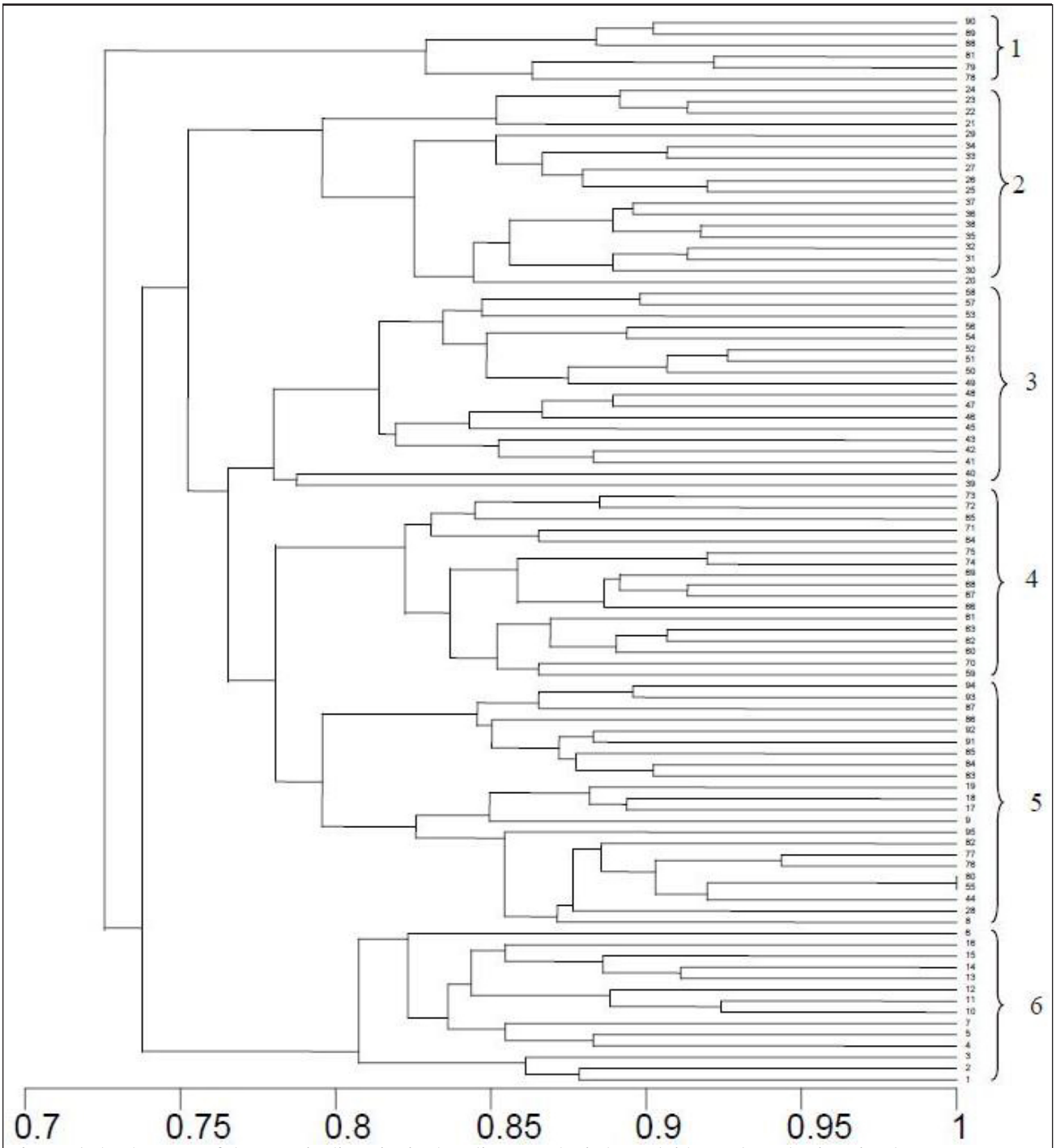

Fig. 4. A dendrogram of the genetic diversity in the Elite-I synthetic hexaploids, evaluated using simple sequence repeat (SSR) primers, with one main cluster and three subclusters A, B, and C.

dates overrides the constraint of lateness. Of greater significance for yield enhancement is the spike detail, where a 1,000-kernel weight greater than 45 g is highly desirable. The variation in 1,000-kernel weight is between 30.2 to 67.6 g (Table 2). The molecular differences within the germ plasm delineated the materials and can be useful for selecting entries useful as breeding resources. Narrowing down the SH parents for a crossing program requires a diagnostic step that permits smart selections to be made; and for this, the molecular diversity is crucial. The diversity exhibited in the Elite-I after RAPD and SSR analyses is shown (Figs. 3 (p. 88) and 4). From this data, desired SHs were identified as parents for a crossing effort. Elite-I lines 1, 3, 4, 11, 13, 15, 23, 25, 30, 37, 42, 46, 47, 49, 59, 69, 70, 73, and 77 were the best SHs according to their diversity status based upon RAPD analysis. Lines 3, 6, 7, 8, 9, 16, 20, 21, 28, 29, 39, 40, 45, 53, 65, 78, 82, 86, 88, and 94 exhibited greater diversity when screened by SSRs. Narrowing these down to a limited number was made possible by the phenology data (Table 2, pp. 84-56) and stringent evaluation of their screening data.

A characteristic spike variation demonstrates the co-dominant genetic expression of the two parents in the SH product (Fig. 5). The tough glumes of *Ae. tauschii* are dominant as are the awns of the durum parent. Spike architecture is modified; awns are inherited and seed has a boldness that is linked with the durum parent. A 1,000-kernel weight much higher (50–65 gm) than that of breadwheat (40–44 g) is common. The Elite-I entries expressed a wide diversity and several emerged as donors for Karnal bunt, powdery mildew, and stripe rust resistance, desirable yield components, and unique plant morphology traits such as leaf waxiness, pubescence, and stay green attributes. The germ plasm tillered well and per plant seed output ranged from 200 to 350 under field increase conditions.

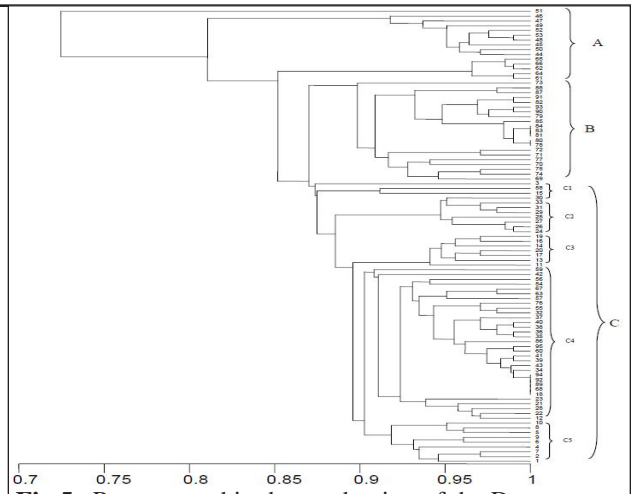

Fig 5. Parents used in the production of the D-genome synthetic hexaploid wheats showing (from left to right): dorsal and ventral spike morphology views of (a) *T. turgidum* subsp. *turgidum* (2n=4x=28; AABB); (b) the synthetic hexaploid (2n=6x=42, AABBDD), and (c) *Ae. tauschii* (2n=2x=14, DD).

The Elite-I SH wheats have been internationally exploited by wheat breeders for the incorporation of biotic and abiotic stress resistances. Often a single trait of interest gives reason to use a SH in a program, but this has been modified as many positive traits are present in a single synthetic, which makes achieving the breeding targets more efficient. Lines with multiple stress resistance are not uncommon, and the screening done in this study has allowed for selecting such elite SHs for breeding utilization. Apart from the stress factors, emphasis also has been given to the molecular diversity within the selected SHs, which serves as a guide for selecting only those SHs in a recombination program that possess the desired traits and also have molecular uniqueness (diversity).

From a holistic perceptive, various traits enable breeders to a select few of the best SHs. A major trait was earliness (days-to-flowering), which is linked with days-to-maturity, height at maturity, and 1,000-kernel weight. A satisfactory number of grains/spike and spike length also were factors. Too many grains/spike was avoided, because a heavier head would promote lodging and the 1,000-kernel weight was heavy in most lines selected. We favored selections that ranged in flowering time, between 76 to 89 days, and had a 1,000-kernel weight between 60.0 and 67.6 g. A maximum spike length of 16 cm was observed in entries 79, 80, 81, 82, 84, and 90. The maximum grains/spike were 64 in entry 58. The maximum 1,000-kernel weight was 67.6 g was in entry number 67 and the minimum days to anthesis was 76 days for entry 49, which reached physiological maturity in 115 days. Seven entries selected for breeding from the Elite-I set of 95 that possessed multiple interesting practical attributes based on phenology alone; 17, 26, 67, 72, 90, 93, and 95. These lines had CIMMYT *Ae. tauschii* accession numbers 220, 309, 629, 877, 502, 1027, and 1030 in their pedigrees. A DNA polymorphic profile was established and stringent utility deduced for application in recombination breeding. Supportive disease data was an additional facet that was considered. For powdery mildew resistance at the seedling and adult-plant stages, entries 90 and 93 indicated the presence of major genes. Adult-plant resistance alone was observed in entries 17, 26, 67, and 72. For stripe rust resistance, entry 95 was identified with both seedling and APR, whereas no line had APR alone. All the seven lines possessed excellent Karnal bunt resistance. These data have enabled the use of these seven lines for location-specific breeding efforts in Pakistan. Powdery mildew resistance is a prerequisite for wheat breeding materials grown in off-season locations where natural prevalence facilitates natural selection on all breeding materials. To this base, by adding in other attributes, one can deploy the best into other sites.

A-genome based diversity status and its practical utilization in wheat.

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One avenue of using the A-genome diversity is via bridge-crossing of AABBAA amphiploids (Fig. 6, p. 93). The 'durum wheat/A-genome accession' crosses are simple and of high frequency. The durum cultivars in these amphiploids are susceptible for the stresses being addressed and a resistant amphiploid implies that a particular A-genome accession contributed the expressed resistance. So far, some diversity has been identified in the AABBAA amphiploids for *Cochliobolus sativus*, *Fusarium graminearum*, and leaf rust resistance but is more extensively observed for *Septoria tritici* resistance.

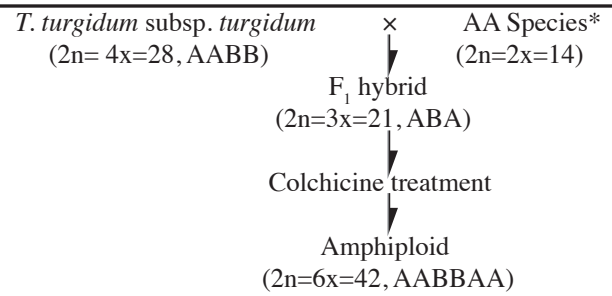


Fig. 6. Schematic showing the production of A-genome 2n = 6x = 42 chromosome stocks as a consequence of hybridizing durum cultivars with A-genome diploid accessions. The A-genome strategy resembles that of the D genome, which demonstrates the utilization of the AABBDD synthetics. Crossing the resistant synthetic hexaploids (SH) with elite but susceptible bread wheat (BW) cultivars yield resistant BW/SH derivatives. *A-genome species include *T. monococcum* subsps. *monococcum* and *aegilopoides* and *T. urartu*.

A set of 194 A-genome synthetics was acquired from the Wheat Wide Crosses Program at CIMMYT, Mexico, and the seed increased in the Wheat Wide Crosses Program based at NARC (Table 6, pp. 94-97). This germ plasm, as a part of the study conducted in Pakistan, was cytologically validated, phenologically characterized, genetically evaluated and screened against Karnal bunt, stripe rust, and powdery mildew during the crop cycles of 2005–06, 2006–07, 2007–08, and 2008–09 (Table 5). The A-genome diploid accessions (2n=2x=14) were *T. monococcum* subsp. *monococcum* and *aegilopoides* and *T. urartu*.

Table 5. The A-genome synthetic hexaploid entries utilized in the study. Synthetic hexaploid entry numbers are the same as those used in the CIMMYT, Mexico, Wide Crosses Program data base. Pedigree details are given in Table 6, pp. 95-97).

Group	A-genome synthetic hexaploid entry	Durum parent	Total entries
1	1, 2	21	2
2	3, 36	28	2
3	4, 6, 10, 16, 21, 23, 24, 26, 29, 30, 31, 106, 113	17	13
4	7, 8, 13, 15	23	4
5	5, 9, 11, 12, 48, 53	34	6
6	14, 27, 49, 63	35	4
7	17, 19, 20, 22, 68, 69, 70, 76, 77, 78, 125, 128, 129	22	13
8	18, 37, 40, 44, 46, 55, 173	45	7
9	25, 32, 33, 34, 41, 42, 45, 47, 51, 52, 54, 56, 57, 58, 59, 64	27	16
10	28, 50, 176, 179, 185, 187, 188, 191, 192	12	9
11	35	37	1
12	38, 39	40	2
13	43, 62, 71, 72, 73, 74, 75	9	7
14	60, 61, 65	25	3
15	66, 67	33	2
16	79, 83	26	2
17	80, 84, 88, 90, 92, 163	5	6
18	81, 82, 85, 89, 91, 93, 94, 101, 102, 103, 104, 105, 111, 112, 119, 120, 123	20	17
19	86, 87, 95, 96, 98, 99, 100, 107, 108, 109, 110, 114, 131, 132, 134, 135, 138, 139, 140, 142, 175, 97, 115, 116, 121, 122, 146, 183, 190, 193, 194	11	31
20	117, 118	14	2
21	124, 170, 156, 160, 161	1	5
22	126	4	1
23	127, 130, 165, 167, 169, 172, 137, 155, 158, 177	13	10
24	133, 136, 141, 143, 157, 159, 174, 178, 144, 145, 147, 148, 149, 150, 151, 152, 153, 154, 162, 180, 181, 182, 184, 186, 164, 166, 168, 171, 189	2	29

The mean crossability data for the production of ABA hybrids across all three categories of diploid progenitors (*aegilopoides*, *monococcum*, and *urartu*) were based on embryos plated from the crosses. The average of all these crosses was 13.0 percent (Table 7, p. 98) for which regeneration and colchicine induced doubling ranged between 90 and 98 percent (Mujeeb-Kazi, unpublished data). Conventional cytological validation protocols provided evidence that the production of F_1 hybrids and their amphiploid production was normal. The F_1 hybrids have 21 chromosomes at mitosis (Fig. 7a, p. 94) and at meiosis show nine univalents + three rod bivalents + three ring bivalents (Fig.

Table 6. Pedigrees of the A-genome synthetic hexaploids.

Number	Parentage / Pedigree
1	YUK/T.BOEOTICUM (1)
2	YUK/T.BOEOTICUM (2)
3	STY-US/CELTA//PALS/3/SRN_5/4/T.BOEOTICUM (3)
4	SCA/T.BOEOTICUM (3)
5	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/T. BOEOTICUM (3)
6	SCA/T.BOEOTICUM (10)
7	GARZA/BOY//T.BOEOTICUM (10)
8	GARZA/BOY//T.BOEOTICUM (12)
9	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/T. BOEOTICUM (13)
10	SCA/T. BOEOTICUM (14)
11	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/T. BOEOTICUM (14)
12	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/T. BOEOTICUM (15)
13	GARZA/BOY//T.BOEOTICUM (16)
14	BOTNO/T.BOEOTICUM (20)
15	GARZA/BOY//T.BOEOTICUM (21)
16	SCA/T.BOEOTICUM (23)
17	DOY1/T.BOEOTICUM (23)
18	SHAG/T.BOEOTICUM (24)
19	DOY1/T.BOEOTICUM (26)
20	DOY1/T.BOEOTICUM (27)
21	SCA/T.BOEOTICUM (28)
22	DOY1/T.BOEOTICUM (28)
23	SCA/T.BOEOTICUM (31)
24	SCA/T.BOEOTICUM (33)
25	SCOOP_1/T.BOEOTICUM (33)
26	SCA/T.BOEOTICUM (34)
27	BOTNO/T.BOEOTICUM (35)
28	D 67. 2/P66. 270//T.BOEOTICUM (35)
29	SCA/T.BOEOTICUM (36)
30	SCA/T.BOEOTICUM (39)
31	SCA/T.BOEOTICUM (40)
32	SCOOP_1/T.BOEOTICUM (40)
33	SCOOP_1/T.BOEOTICUM (46)
34	SCOOP_1/T.BOEOTICUM (50)
35	LCK59. 61/T.BOEOTICUM (52)
36	STY-US/CELTA//PALS/3/SRN_5/4/T.BOEOTICUM (54)
37	SHAG_22/T.BOEOTICUM (55)
38	AJAIA/T.BOEOTICUM (55)
39	AJAIA/T.BOEOTICUM (56)
40	SHAG_22/T.BOEOTICUM (56)
41	SCOOP_1/T.BOEOTICUM (59)
42	SCOOP_1/T.BOEOTICUM (60)
43	68.111/RBG-U//WARD/3/T.BOEOTICUM (61)
44	SHAG_22/T.BOEOTICUM (68)
45	SCOOP_1/T.BOEOTICUM (69)
46	SHAG_22/T.BOEOTICUM (70)
47	SCOOP_1/T.BOEOTICUM (71)
48	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/T. BOEOTICUM (74)

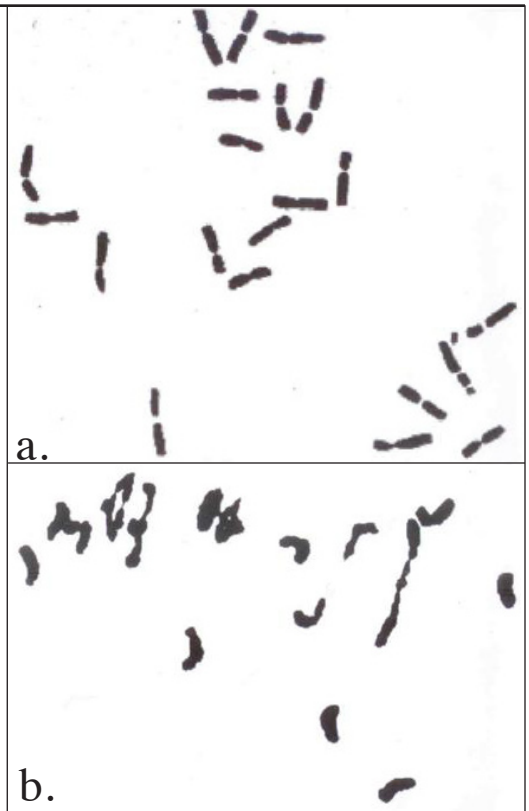


Fig. 7. Progressive stages in the utilization of the A-genome variation showing its cytological validation details as follows: (a) F_1 (2n=3x=21, ABA) hybrid at mitotic metaphase and (b) An F_1 meiocyte at metaphase I with associations of nine univalents + three rod bivalents + three ring bivalents.

7b). The F_1 hybrids after colchicine doubling led to a generation of fertile amphiploids with 2n=6x=42 chromosomes (Fig. 8a, p. 95), AABBAA, that were associated at metaphase I as five rod bivalents + 16 ring bivalents (Fig. 8b, p. 95). Despite the four doses of A-genome chromosomes, bivalency prevailed for most of the AABBAA hexaploids where association of the 42 chromosomes express maximum bivalency; the association is three rod bivalents + 19 ring bivalents (Fig. 9a, p. 96) and two rod bivalents + 19 ring bivalents (Fig. 9b, p. 96). Another line had eight rod bivalents + 13 ring bivalents (Fig. 10a, p. 97), similar to other amphiploids analyzed) at anaphase I with a 21/21 split (Fig. 10b, p. 97).

Mean meiotic associations of several AABBAA amphiploids that were produced involving diverse elite durum wheats and accessions where determined for *T. monococcum* subsp. *aegilopoides* (Table 8, pp. 98-99), *T. monococcum* subsp. *monococcum* (Table 9, p. 100), and *T. urartu* (Table 10, p. 101). The

Table 6. Pedigrees of the A-genome synthetic hexaploids.

Number	Parentage / Pedigree
49	BOTNO/T.BOEOTICUM (75)
50	D 67. 2/P66. 270//T.BOEOTICUM (75)
51	SCOOP_1/T.BOEOTICUM (79)
52	SCOOP_1/T.BOEOTICUM (80)
53	ALG86/4/FGO/PALES//MEXI_1/3/RUFF/FGO/5/ENTE/6/T. BOEOTICUM (83)
54	SCOOP_1/T.BOEOTICUM (87)
55	SHAG_22/T.BOEOTICUM (88)
56	SCOOP_1/T.BOEOTICUM (89)
57	SCOOP_1/T.BOEOTICUM (90)
58	SCOOP_1/T.BOEOTICUM (91)
59	SCOOP_1/T.MONOCOCCUM (98)
60	AOS/T.MONOCOCCUM (98)
61	AOS/T.MONOCOCCUM (111)
62	68.111/RGB-U//WARD/3/T.MONOCOCCUM (112)
63	BOTONO/T.MONOCOCCUM (112)
64	SCOOP _ 1/T.MONOCOCCUM (118)
65	AOS/T.MONOCOCCUM (118)
66	FGO/USA2111//T.MONOCOCCUM (119)
67	FGO/USA2111//T.MONOCOCCUM (122)
68	DOY1/T.URARTU (542)
69	DOY1/T.URARTU (543)
70	DOY1/T.URARTU (550)
71	68.111/RGB-U//WARD/3/T.URARTU (550)
72	68.111/RGB-U//WARD/3/T.URARTU (551)
73	68.111/RGB-U//WARD/3/T.URARTU (553)
74	68.111/RGB-U//WARD/3/FGO/4/RABI/5/T.URARTU (554)
75	68.111/RGB-U//WARD/3/FGO/4/RABI/5/T.URARTU (555)
76	DOY1/T.URARTU (560)
77	DOY1/T.URARTU (563)
78	DOY1/T.URARTU (564)
79	GAN/T.BOEOTICUM (7)
80	DVERD_2/T.BOEOTICUM (18)
81	YAV_2/TEZ//T.BOEOTICUM (18)
82	YAV_2/TEZ//T.BOEOTICUM (25)
83	GAN/T.BOEOTICUM (29)
84	DVERD_2/T.BOEOTICUM (37)
85	YAV_2/TEZ//T. BOEOTICUM (37)
86	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (38)
87	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (41)
88	DVERD_2/T.BOEOTICUM (43)
89	YAV_2/TEZ//T.BOEOTICUM (43)
90	DVERD_2/T.BOEOTICUM (44)
91	YAV_2/TEZ//T.BOEOTICUM (44)
92	DVERD_2/T.BOEOTICUM (45)
93	YAV_2/TEZ//T.BOEOTICUM (45)
94	YAV_2/TEZ//T.BOEOTICUM (47)
95	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (48)
96	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (49)
97	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (53)
98	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (57)
99	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (58)

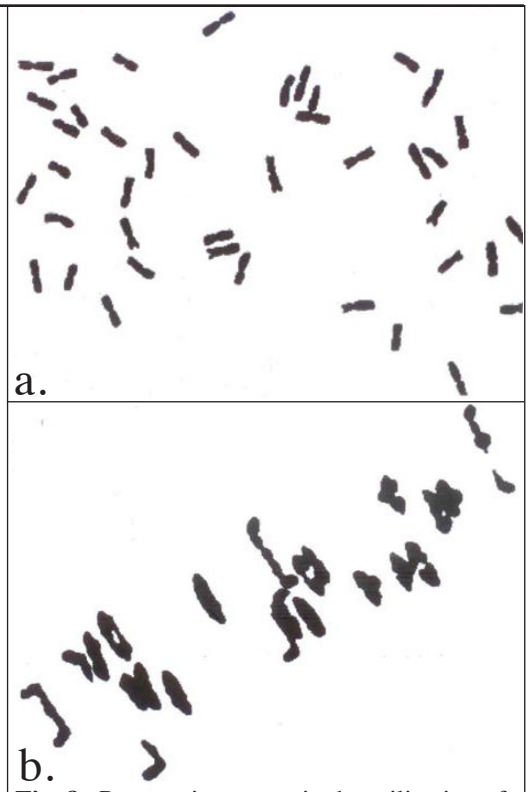


Fig. 8. Progressive stages in the utilization of the A-genome variation showing its cytological validation details as follows: (a) an amphiploid (2n=6x=42; AABBAA) at mitotic metaphase and (b) a 2n=6x=42 meiocyte showing five rod bivalents + 16 ring bivalents.

high frequency of bivalent associations are evident within each group and euploids dominate. These parameters are crucial to fertility and good seed finish, which was the case for all derivatives studied.

Plant morphology characteristics show variation across the amphiploids (Table 11, p. 101). This variation is more pronounced at the spike level (Figs. 11-12, p. 102). The spike characteristics across all accessions of the diploid A-genome resources are subtle (partially listed in Table 11, p. 101). Those for spike length and nodes/spike have priority because these traits determine seed number/spike and the degree of compactness/laxness of the florets.

Powdery mildew studies. All 194 A-genome synthetic hexaploids entries and their 24 durum parents were screened for powdery mildew resistance in pot trials in the greenhouse at Murree; 88 synthetic hexaploids and nine of the durum wheat parents showed resistance reaction at seedling stage. Infection type ranged from 0–6 at the seedling stage indicating the presence of

Table 6. Pedigrees of the A-genome synthetic hexaploids.

Number	Parentage / Pedigree
100	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (58)
101	YAV_2/TEZ//T.BOEOTICUM (62)
102	YAV_2/TEZ//T.BOEOTICUM (64)
103	YAV_2/TEZ//T.BOEOTICUM (65)
104	YAV_2/TEZ//T.BOEOTICUM (67)
105	YAV_2/TEZ//T.BOEOTICUM (73)
106	SCA/T.BOEOTICUM (75)
107	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (76)
108	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (77)
109	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (78)
110	CPI/GEDIZ/3/GOO//JO/ CRA/4/T.BOEOTICUM (81)
111	YAV_2/TEZ//T.BOEOTICUM (82)
112	YAV_2/TEZ//T.BOEOTICUM (83)
113	SCA/T.BOEOTICUM (92)
114	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (93)
115	CPI/GEDIZ/3/GOO//JO/CRA/4/T.MONOCOCCUM (99)
116	CPI/GEDIZ/3/GOO//JO/CRA/4/T.MONOCOCCUM (101)
117	STN/T.MONOCOCCUM (111)
118	STN/T.MONOCOCCUM (112)
119	YAV_2/TEZ//T.MONOCOCCUM (112)
120	YAV_2/TEZ//T.MONOCOCCUM (113)
121	CPI/GEDIZ/3/GOO//JO/CRA/4/T.MONOCOCCUM (114)
122	CPI/GEDIZ/3/GOO//JO/CRA/4/T.MONOCOCCUM (115)
123	YAV_2/TEZ//T.MONOCOCCUM (121)
124	CROC_1/T.URARTU (548)
125	DOY1/T.URARTU (552)
126	ALTAR 84/T.URARTU (558)
127	CETA/T.URARTU (558)
128	DOY1/T.URARTU (559)
129	DOY1/T.URARTU (561)
130	CETA/T.URARTU (562)
131	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (4)
132	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (5)
133	ARLIN_1/T.BOEOTICUM (6)
134	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (9)
135	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (30)
136	ARLIN_1/T.BOEOTICUM (32)
137	CETA/T.BOEOTICUM (42)
138	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (51)
139	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (63)
140	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (72)
141	ARLIN_1/T.BOEOTICUM (84)
142	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (85)
143	ARLIN_1/T.BOEOTICUM (86)
144	ARLIN_1/T.MONOCOCCUM (94)
145	ARLIN_1/ T.MONOCOCCUM (96)
146	CPI/GEDIZ/3/GOO//JO/CRA/4/T.MONOCOCCUM (100)
147	ARLIN_1/T.MONOCOCCUM (102)
148	ARLIN_1/T.MONOCOCCUM (103)
149	ARLIN_1/T.MONOCOCCUM (104)
150	ARLIN_1/T.MONOCOCCUM (105)
151	ARLIN_1/T.MONOCOCCUM (109)

Fig. 9. Meiotic association at metaphase I of some A-genome synthetic amphiploids (hexaploids) with 42 chromosomes and genomically AABBAA: (a) 21 bivalent meiocyte associated as three rod bivalents + 18 rings and (b) 21 bivalent meiocyte associated as two rod bivalents + 19 rings.

major genes for resistance. Some of these resistant accessions exhibited different reaction types against powdery mildew under field conditions.

The A-genome synthetic hexaploids that showed resistance at seedling stage are 1, 4, 6, 18, 19, 24, 25, 26, 27, 28, 37, 39, 40, 42, 43, 45, 47, 48, 50, 53, 54, 55, 56, 57, 58, 60, 62, 68, 72, 75, 76, 78, 79, 81, 85, 87, 88, 93, 95, 96, 97, 98, 101, 102, 103, 105, 106, 107, 108, 109, 110, 113, 115, 116, 117, 118, 119, 124, 126, 127, 129, 131, 133, 134, 135, 136, 138, 144, 145, 150, 155, 156, 157, 162, 167, 168, 169, 177, 179, 182, 183, 185, 186, 188, 192, and 194. The durum parents included 5, 7, 9, 10, 11, 15, 16, 19 and 23.

Stripe rust studies. Seedling screening showed that 29 out of 194 (14.9%) of the synthetics (Table 12, p. 103-107) and 22 out of 23 (95.6%) of the durum wheat parents (Table 13, p. 108) exhibited seedling resistance to strip rust. These genotypes also were screened for APR under field conditions at NARC, which separated 82 out of 194 (42.3%) synthetics and 20 out of 23 (86.9%) of the durum wheat parents as resistant genotypes.

Table 6. Pedigrees of the A-genome synthetic hexaploids.

Number	Parentage / Pedigree
152	ARLIN_1/T.MONOCOCCUM (116)
153	ARLIN_1/T.MONOCOCCUM (117)
154	ARLIN_1/T.MONOCOCCUM (120)
155	CETA/T.BOEOTICUM (72)
156	CROC_1/T.BOEOTICUM (8)
157	ARLIN_1/T.BOEOTICUM (8)
158	CETA/T.BOEOTICUM (8)
159	ARLIN_1/T.BOEOTICUM (11)
160	CROC_1/T.BOEOTICUM (19)
161	CROC_1/T.MONOCOCCUM (97)
162	ARLIN_1/T.MONOCOCCUM (97)
163	DVERD_2/T.MONOCOCCUM (122)
164	ARLIN_1/T.URARTU (544)
165	CETA/T.URARTU (544)
166	ARLIN_1/T.URARTU (546)
167	CETA/T.URARTU (546)
168	ARLIN_1/T.URARTU (547)
169	CETA/T.URARTU (547)
170	CROC_1/T.URARTU (549)
171	ARLIN_1/T.URARTU (549)
172	CETA/T.URARTU (549)
173	SHAG_22/T.URARTU (549)
174	ARLIN_1/T.BOEOTICUM (19)
175	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (19)
176	D67.2/P66.270//T.BOEOTICUM (19)
177	CETA/T.BOEOTICUM (19)
178	ARLIN_1/T.BOEOTICUM (66)
179	D67.2/P66.270//T.BOEOTICUM (66)
180	ARLIN_1/T.MONOCOCCUM (95)
181	ARLIN_1/T.MONOCOCCUM (97)
182	ARLIN_1/T.MONOCOCCUM (107)
183	CPI/GEDIZ/3/GOO//JO/CRA/4/T.BOEOTICUM (107)
184	ARLIN_1/T.MONOCOCCUM (108)
185	D67.2/P66.270//T.MONOCOCCUM (108)
186	ARLIN_1/T.MONOCOCCUM (110)
187	D67.2/P66.270//T.URARTU (542)
188	D67.2/P66.270//T.URARTU (543)
189	ARLIN_1/T.URARTU (548)
190	CPI/GEDIZ/3/GOO//JO/CRA/4/T.URARTU (548)
191	D67.2/P66.270//T.URARTU (550)
192	D67.2/P66.270//T.URARTU (553)
193	CPI/GEDIZ/3/GOO//JO/CRA/4/T.URARTU (553)
194	CPI/GEDIZ/3/GOO//JO/CRA/4/T.URARTU (556)

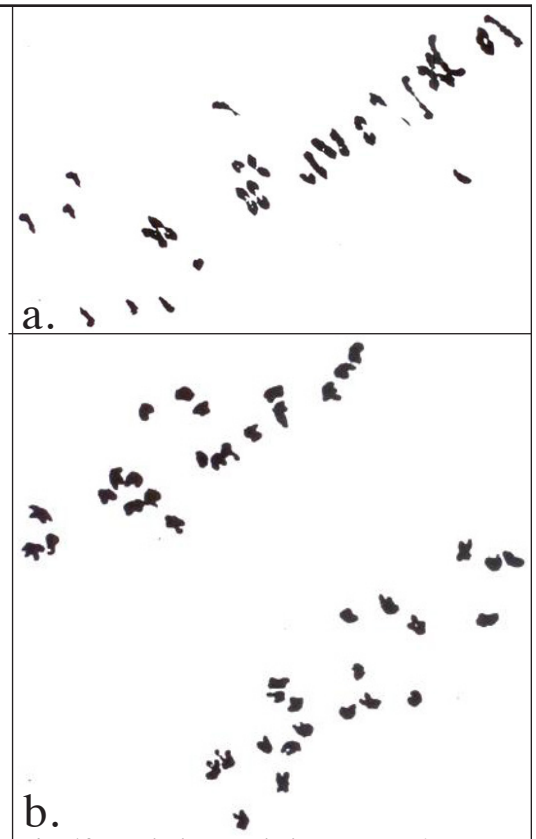


Fig. 10. Meiotic association at metaphase I of some A-genome synthetic hexaploids with 42 chromosomes and genomically AABBAA: (a) 21 bivalents with eight rods + 13 ring associations and (b) anaphase I separation with a 21 + 21 split in a meiocyte.

Seventeen synthetic genotypes (8.7%) were resistant at both seedling and adult-plant stages (5, 15, 19, 20, 24, 30, 32, 35, 58, 59, 60, 61, 95, 108, 109, 119, and 128) and 19 (82.6%) of the durum wheat parents (1, 2, 4, 5, 8, 9, 11, 12, 13, 14, 17, 20, 21, 22, 26, 27, 28, 37, and 40). All this germ plasm represents the presence of major genes against stripe rust and can be exploited further in breeding programs.

Adult-plant resistance involving susceptibility at the seedling stage and resistance only at the adult-plant stage indicates the presence of minor genes, which are considered of great importance against rust diseases in acquiring durable resistance. Sixty-five (33.5%) of the A-genome synthetic hexaploids (1, 8, 10, 11, 14, 17, 21, 25, 29, 31, 33, 34, 38, 40, 41, 45, 49, 50, 51, 55, 56, 62, 64, 67, 68, 69, 88, 89, 96, 103, 104, 105, 114, 115, 116, 117, 118, 120, 121, 122, 123, 125, 126, 127, 135, 136, 137, 139, 141, 142, 143, 148, 150, 151, 159, 161, 168, 169, 178, 179, 182, 187, 189, and 191) and only one (4.35%) durum wheat parent (45) showed APR and are good candidates for providing durable resistance to wheat cultivars.

Molecular studies. ***Genetic diversity evaluation using random amplified polymorphic DNA (RAPD) primers.*** RAPD primers were used to evaluate the genetic diversity of the A-genome synthetic hexaploids. All 520 RAPD primers of

Table 7. The mean crossability data of some *T. turgidum* subsp. *turgidum*/A and B genome diploid species accessions.

Cross combination (AA-genome species)	Florets pollinated	Seed set	Embryos plated
/ *T. monococcum subsp. aegilopoides*	58.5	27.2	25.5
/ *T. monococcum* subsp. *monococcum*	42.0	6.80	6.00
/ *T. urartu*	58.7	12.5	7.50
Average of all three diploid genomes	53.1	15.5	13.0

Table 8. Mean meiotic chromosome associations at metaphase I in AABBAA synthetic hexaploids (amphiploids) involving *Triticum turgidum* subsp. *turgidum* cultivars and A-genome diploid accessions of *T. monococcum* subsp. *aegilopoides*. Abbreviations in table are as follows: I = univalent, rII = rod bivalent, oII = ring bivalent, III = trivalent, cIV = chain quadrivalent, oIV = ring quadrivalent, and Xta = chiasmata.

AABBAA number	Number of cells	I	rII	oII	III	cIV	oIV	Xta	Chromosome number
3	20	1.20	2.05	13.90	0.60	0.80	1.00	37.9	42
5	20	0.20	0.80	13.90	0.00	0.70	2.40	40.3	42
6	20	0.00	2.10	15.50	0.00	0.10	1.60	39.8	42
7	20	1.40	6.20	10.30	0.00	1.00	0.60	33.0	42
8	20	0.00	4.60	13.00	0.00	0.60	1.10	36.8	42
12	20	0.10	3.50	11.30	0.10	1.10	1.90	37.2	42
14	20	0.10	3.80	11.20	0.10	0.90	2.00	37.1	42
16	20	0.00	2.60	13.40	0.00	1.20	1.30	38.2	42
17	20	0.00	1.00	13.40	0.00	1.00	2.30	40.0	42
19	20	0.00	1.80	15.00	0.00	0.20	1.90	40.0	42
20	20	0.10	3.50	11.30	0.10	1.10	1.90	37.2	42
21	20	0.00	2.80	15.80	0.00	0.10	1.10	39.1	42
22	20	0.20	2.90	12.30	0.20	0.80	1.90	37.9	42
23	20	0.20	2.90	13.40	0.00	0.30	2.00	38.6	42
24	20	0.20	2.20	14.00	0.20	0.60	1.60	38.8	42
25	20	0.10	2.10	14.30	0.10	0.50	1.70	39.2	42
26	20	0.30	2.10	12.50	0.90	1.30	1.40	38.4	43
27	20	0.20	3.10	15.80	0.00	0.40	0.60	38.3	42
29	20	0.50	4.10	14.50	0.10	0.40	0.60	36.9	42
30	20	0.30	2.90	15.00	0.10	0.40	1.00	38.3	42
31	20	1.20	3.80	13.40	0.40	0.60	0.70	36.00	42
34	20	0.70	3.60	15.10	0.10	0.40	0.50	37.20	42
36	20	0.60	4.60	12.30	0.00	0.60	1.30	36.20	42
38	20	0.10	3.90	14.70	0.10	0.40	0.70	37.50	42
39	20	1.20	2.60	12.90	0.20	1.10	1.20	36.90	42
40	20	0.40	3.90	13.70	0.00	0.60	1.00	37.10	42
41	20	0.00	2.80	14.40	0.00	0.40	1.50	38.80	42
44	20	0.00	2.80	15.20	0.00	0.50	1.00	38.70	42
45	20	0.00	2.40	15.60	0.00	0.60	0.90	39.00	42
46	20	1.00	5.10	12.60	0.40	0.60	0.50	34.90	42
48	20	0.00	3.10	15.10	0.00	0.50	0.90	38.40	42
49	20	0.50	2.60	15.60	0.10	0.40	0.80	38.40	42
50	20	0.10	3.20	15.20	0.10	0.60	0.60	38.00	42
51	20	0.10	2.80	14.40	0.10	0.50	1.30	38.50	42
52	20	0.20	2.10	16.00	0.00	0.40	1.00	39.30	42
53	20	0.10	2.40	15.60	0.10	0.40	1.00	39.00	42
54	20	0.70	3.30	14.60	0.10	0.50	0.80	37.40	42
55	20	0.20	2.40	15.20	0.20	0.60	0.90	38.60	42

Table 8. Mean meiotic chromosome associations at metaphase I in AABBAA synthetic hexaploids (amphiploids) involving *Triticum turgidum* subsp. *turgidum* cultivars and A-genome diploid accessions of *T. monococcum* subsp. *aegilopoides*. Abbreviations in table are as follows: I = univalent, rII = rod bivalent, oII = ring bivalent, III = trivalent, cIV = chain quadrivalent, oIV = ring quadrivalent, and Xta = chiasmata.

AABBAA number	Number of cells	I	rII	oII	III	cIV	oIV	Xta	Chromosome number
79	20	3.60	6.20	7.700	0.20	1.10	1.40	30.90	42
82	20	0.20	3.20	14.90	0.00	0.50	0.90	38.10	42
83	20	0.00	3.50	14.50	0.00	0.60	0.90	37.90	42
85	20	0.40	4.30	13.90	0.00	0.50	0.80	36.80	42
86	20	0.00	2.50	15.50	0.00	0.40	1.10	39.10	42
87	20	8.80	6.20	6.000	1.60	0.70	0.30	24.70	42
89	20	0.20	3.50	14.60	0.00	0.50	0.90	37.80	42
91	20	0.20	3.20	14.00	0.20	0.80	0.90	37.60	42
94	20	0.50	3.90	16.70	0.10	0.00	0.00	37.50	42
95	20	0.20	4.70	13.40	0.00	0.70	0.70	36.40	42
96	20	0.10	3.10	15.50	0.10	0.50	0.60	38.20	42
97	20	0.00	1.80	16.80	0.00	0.50	0.70	39.70	42
98	20	1.56	6.33	10.11	0.44	0.78	0.78	32.89	42
99	20	0.30	3.20	14.50	0.10	0.40	1.10	38.00	42
103	20	0.00	2.50	16.50	0.00	0.30	0.70	39.20	42
104	20	0.20	3.00	15.40	0.20	0.40	0.70	38.20	42
106	20	0.00	2.50	15.50	0.00	0.50	1.00	39.00	42
108	20	0.20	2.60	15.60	0.20	0.40	0.80	38.60	42
109	20	0.00	2.80	15.20	0.00	0.40	1.10	38.80	42
133	20	0.10	2.40	17.40	0.10	0.00	0.50	39.40	42
134	20	0.10	1.80	17.20	0.10	0.30	0.60	39.70	42
135	20	0.00	1.70	17.20	0.20	0.30	0.60	39.80	42
136	20	0.30	2.80	16.70	0.10	0.30	0.30	38.50	42
137	20	0.10	1.80	16.20	0.10	0.40	1.00	39.60	42
139	20	0.10	3.40	14.40	0.10	0.40	1.10	38.00	42
142	20	0.70	4.30	13.80	0.10	0.40	0.80	36.50	42
143	20	0.50	3.70	14.40	0.30	0.70	0.40	36.80	42
101	20	0.60	3.10	13.80	0.60	0.60	1.10	38.10	43
102	20	0.60	3.30	13.30	0.20	0.50	1.40	37.40	42
110	20	1.50	3.60	13.20	0.40	0.50	0.80	35.90	42
111	20	0.50	2.80	15.30	0.30	0.20	0.90	38.20	42
112	20	0.40	4.20	14.60	0.00	0.40	0.60	37.00	42
113	20	0.20	1.90	15.80	0.00	0.20	1.40	39.70	42
114	20	0.10	2.50	14.70	0.10	0.60	1.20	38.70	42
131	20	0.40	3.80	13.40	0.00	0.40	1.40	37.40	42
132	20	0.30	1.20	15.00	0.30	0.10	2.00	40.10	42
138	20	2.80	3.50	10.80	1.20	0.60	0.90	32.90	41
156	20	1.00	2.70	13.40	0.40	0.40	1.50	37.50	42
157	20	1.60	3.20	12.90	0.20	0.90	1.00	36.10	42
158	20	0.70	3.40	11.30	0.10	1.40	1.50	36.40	42
159	20	0.20	2.50	13.20	0.00	0.40	2.20	38.90	42

the Operon Series were screened, working primers identified, and applied to detect genetic polymorphism at DNA level. The samples that did not amplify were not included in the analysis. Genetic analysis was performed only on the scorable bands. Every single band was considered as a single locus/allele. The loci were scored as present/absent. Bivariate data 1–0 were used to estimate the genetic diversity (GD). The unweighted pair group of arithmetic means (UPGMA) func-

Table 9. Mean meiotic chromosome associations at metaphase I in AABBAA synthetic hexaploids (amphiploids) involving *Triticum turgidum* subsp. *turgidum* cultivars and A-genome diploid accessions of *T. monococcum* subsp. *monococcum*. Abbreviations in table are as follows: I = univalent, rII = rod bivalent, oII = ring bivalent, III = trivalent, cIV = chain quadrivalent, oIV = ring quadrivalent, and Xta = chiasmata.

AABBAA number	Number of cells	I	rII	oII	III	cIV	oIV	Xta	Chromosome number
18	20	0.00	1.90	16.9	0.00	0.20	0.90	39.9	42
59	20	0.10	3.30	14.3	0.10	0.60	1.00	37.0	42
64	20	0.20	2.80	14.8	0.20	0.50	1.00	38.3	42
65	20	0.30	2.80	14.3	0.10	0.60	1.20	38.2	42
115	20	1.60	2.20	15.4	0.00	0.40	0.90	37.8	42
116	20	1.90	3.30	14.1	0.30	0.40	0.70	36.1	42
117	20	0.70	3.20	14.3	0.10	0.70	0.70	37.2	42 (one VI)
119	20	0.80	2.80	14.5	0.20	0.40	1.10	37.8	42
120	20	0.30	2.20	14.5	0.50	0.40	1.30	38.6	42
121	20	1.70	3.10	12.6	0.30	0.60	0.40	36.3	42
122	20	1.00	3.70	14.1	0.20	0.40	0.80	36.7	42
123	20	1.20	5.30	13.3	0.40	0.40	0.20	34.7	42
144	20	0.30	2.60	15.3	0.20	0.30	1.00	38.7	42
145	20	0.80	3.40	15.3	0.20	0.60	0.20	37.0	42
146	20	0.20	3.20	15.6	0.20	0.20	0.70	38.2	42
147	20	0.40	2.80	16.0	0.00	0.30	0.70	38.5	42
148	20	0.80	4.70	14.3	0.40	0.30	0.20	35.8	42
149	20	1.80	5.40	13.2	0.20	0.40	0.20	34.2	42
150	20	1.80	4.60	13.3	0.40	0.60	0.20	34.6	42
151	20	0.90	3.90	14.3	0.10	0.30	0.80	36.8	42
152	20	0.80	4.20	14.8	0.40	0.10	0.40	36.5	42
153	20	0.70	3.20	14.2	0.10	0.70	0.70	37.2	42
154	20	0.00	2.20	16.8	0.00	0.60	0.40	39.2	42
161	20	0.20	2.20	15.5	0.00	0.30	1.30	39.3	42
162	20	1.90	4.00	12.7	0.50	0.20	1.10	35.4	42
163	20	0.20	2.90	13.2	0.00	0.40	2.00	38.5	42

tion estimated the GDs between the genotypes as follows: $GD_{xy} = 1 - d_{xy}/d_x + d_y - d_{xy}$, where GD_{xy} = GD between two genotypes, d_{xy} = total number of common loci (bands) in two genotypes, d_x = total number of loci (bands) in genotype 1, and d_y = total number of loci (bands) in genotype 2.

The efficiency of primers to amplify the genotypes ranged from 124 (OPE-3 and OPR-8) to one (OPG-10, OPG-17, OPH-11, OPI-2, OPI-4, OPM-14, and OPN-1) in A-genome synthetic hexaploids (Table 14, pp. 109-111) and from nine (OPN-6) to one (OPA-4, OPE-11, OPJ-1, OPW-5, OPX-2, and OPY-8) in B-genome synthetic hexaploids (Table 15, pp. 111-112). Scorable bands ranged from 1 (OPI-4 and OPM-14) to 666 (OPE-3) in A-genome synthetic hexaploids (Table 14, pp. 109-111).

A genetic analysis of the population showed that the total number of loci for A-genome synthetic hexaploids equaled 1,095 of which 1.062 were polymorphic (Table 14, pp. 109-111). The percentage of polymorphism was very high (96.68%). The range of scorable bands was from 250–3,000 bp in A-genome synthetic hexaploids.

Similarity matrix. A bivariate analysis was conducted to generate a similarity matrix and dendrogram to estimate genetic diversity. The A-genome synthetic hexaploids exhibited a minimum value of similarity matrix of 68.9% between 1 and 107 and the maximum value of 98.8% between 121 and 152.

Dendrogram interpretation. The GD between genotypes was used to construct a dendrogram by UPGMA analysis for determining grouping of the lines on the basis of similarities and differences. In A-genome synthetic hexaploids, the

Table 10. Mean meiotic chromosome associations at metaphase I in AABBAA synthetic hexaploids (amphiploids) involving *Triticum turgidum* subsp. *turgidum* cultivars and A-genome diploid accessions of *T. urartu*. Abbreviations in table are as follows: I = univalent, rII = rod bivalent, OII = ring bivalent, III = trivalent, cIV = chain quadrivalent, OIV = ring quadrivalent, and Xta = chiasmata.

AABBAA number	Number of cells	I	rII	oII	III	cIV	oIV	Xta	Chromosome number
52	20	5.20	5.10	9.80	1.40	0.20	0.00	28.1	40
70	20	0.60	2.10	17.6	0.00	0.30	0.20	39.0	42
72	20	0.00	2.90	14.7	0.00	0.80	0.90	38.3	42
74	20	0.00	3.50	14.5	0.00	0.80	0.70	37.7	42
75	20	0.00	4.00	14.4	0.00	0.60	0.70	37.4	42
76	20	2.00	5.00	9.60	0.20	0.90	1.40	32.9	41
77	20	0.40	2.50	15.3	0.00	0.60	0.90	38.5	42
78	20	1.70	6.80	8.60	0.30	1.00	0.90	31.2	41
125	20	1.50	4.00	10.5	0.50	1.20	1.30	34.8	42
126	20	0.70	3.30	14.8	0.10	0.30	0.90	37.6	42
127	20	1.20	2.60	12.6	0.00	0.20	2.40	38.0	42
128	20	1.80	5.60	9.90	0.60	0.20	1.40	32.8	41
129	20	1.10	5.20	10.6	0.30	0.60	1.50	34.8	42
130	20	0.50	3.60	11.6	0.10	1.20	1.50	36.8	42
165	20	1.20	3.40	12.6	0.00	0.60	1.60	36.8	42
166	20	0.70	3.70	10.1	0.30	1.30	1.90	36.0	42
168	20	0.00	2.20	14.0	0.00	0.20	2.20	39.6	42
169	20	0.40	2.60	15.2	0.40	0.40	0.80	38.2	42
170	20	1.30	2.30	12.4	1.50	0.40	0.80	34.5	40
172	20	0.00	2.40	15.4	0.00	0.40	1.20	39.2	42
173	20	0.10	2.70	14.1	0.10	1.00	1.00	38.1	42
154	20	0.00	2.20	16.8	0.00	0.60	0.40	39.2	42
161	20	0.20	2.20	15.5	0.00	0.30	1.30	39.3	42
162	20	1.90	4.00	12.7	0.50	0.20	1.10	35.4	42
163	20	0.20	2.90	13.2	0.00	0.40	2.00	38.5	42

Table 11. Mean phenotypic characteristics of selected AA-genome amphiploids involving *Triticum monococcum* subsps. *aegilopoides* and *monococcum* and *T. urartu*.

	Spike				Spikelet				Glume		Lemma		
No.	Length (cm)	Width (cm)	Nodes /spike	Internode length (cm)	Length (cm)	Width (cm)	/spike	Florets /spikelet	Length (cm)	Awn length (cm)	Body length (cm)	Awn length (cm)	Anther length (cm)
T. monococcum* subsp. *aegilopoides													
28	21.45	1.21	22.00	0.44	11.13	0.65	22.00	4.00	0.91	0.24	1.17	9.17	0.35
86	20.60	1.25	22.00	0.46	11.48	0.81	22.00	4.00	1.12	0.44	1.23	10.58	0.32
133	20.40	1.01	26.00	0.57	10.83	0.81	26.00	4.50	1.22	0.40	1.29	9.15	0.35
T. monococcum subsp. monococcum													
185	14.90	1.70	26.00	0.30	8.33	1.02	26.00	3.75	1.05	0.21	1.16	6.97	0.32
115	20.65	1.10	24.50	0.41	12.18	0.72	24.50	3.25	1.01	0.31	0.93	10.33	0.40
148	17.65	1.21	27.00	0.43	10.73	0.87	27.00	4.25	1.24	0.41	1.06	7.99	0.33
185	14.90	1.70	26.00	0.30	8.33	1.02	26.00	3.75	1.05	0.21	1.16	6.97	0.32
T. urartu													
191	15.40	1.41	19.50	0.61	9.08	0.89	19.50	4.00	0.96	0.35	1.26	8.43	0.31
193	17.85	1.08	24.50	0.46	9.25	0.81	24.50	4.00	0.87	0.28	1.06	6.88	0.21
189	19.60	1.31	26.00	0.40	11.50	0.84	26.00	4.00	1.17	0.80	1.26	9.96	0.40

Fig. 11. Spike morphology of some AABBAA synthetic hexaploids involving the diploid progenitor species *T. monococcum* subsp. *aegilopoides* and *monococcum* and *T. urartu*, (a) *T. turgidum*/accession 086; (b) *T. turgidum*/ accession 115; (c) *T. turgidum*/accession 133; (d) *T. turgidum*/accession 148 and (e) *T. turgidum*/accession 185.

only main cluster has three subclusters, A, B, and C (Fig. 13, p. 113). Subcluster A has five genotypes and also contains the most diverse line of the group, 107. Other good lines in this subcluster include 3, 68, 92, and 111. The highly diverse lines in subcluster B include 20, 39, 82, 95, 118, and 177 in total of 188 genotypes. Subcluster C has only one genotype, 1.

Genetic diversity evaluation using simple sequence repeat (SSR) Primers. SSR primers were used for to evaluate the GD of the A-genome synthetic hexaploids. All 275 SSR primers were applied to detect genetic polymorphism at DNA level. The samples which did not amplify were not included in the analysis. Genetic analysis was similar to that for the RAPD primers.

The efficiency of primers to amplify the genotypes ranged from 68 (*Xgwm312-2A*) to six (*Xgwm473-2A*) in the A-genome synthetic hexaploids (Table 16, p. 112) and scorable bands ranged from six (*Xgwm473-2A*) to 112 (*Xgwm311-2A*) (Table 16, p. 112).

A genetic analysis of the population showed that the total number of alleles for A-genome synthetic hexaploids scored equaled 126, all of were polymorphic; the the percentage of polymorphism was 100% (Table 16, p. 112). The range of scorable bands was from 50–250 bp.

Similarity matrix. Bivariate analysis was conducted to generate a similarity matrix and dendrogram to estimate genetic diversity. The A-genome synthetic hexaploids exhibited minimum value of similarity matrix of 73.3% between 34 and 130 and the maximum value of 100% in 314 different combinations.

Phenotypic characterization and disease screening. From the wide array of AAAABB synthetic wheats produced, field plantings were utilized to establish descriptive parameters and seed increase. The descriptor characteristics demonstrated extensive genetic diversity for plant height, flowering date, and 1,000-kernel weight (Table 12, pp. 103-107). Utilizating this germ plasm for durum wheat improvement will be an advantage selecting synthetics that are trait positive and agronomically superior. The targeted variation also can be tapped for direct crossing with durum wheat cultivars as shown for bread wheat improvement. Because bread wheat in Pakistan is the main cereal crop, the diversity of the A genome can be exploited via bridge crossing of the AAAABB hexaploid with the AABBDD bread wheat as recombination events across the AA genomes will permit the donor variation to be introgressed. Alternatively, after the durum wheats are screened and resistance sources identified, the desired A-genome accession also can enter into direct hybridization with bread wheat.

Fig. 12. Spike morphology of some AABBAA synthetic hexaploids involving the diploid progenitor species *T. monococcum* subsps. *aegilopoides* and *monococcum* and *T. urartu*, (a) *T. turgidum*/accession 189; (b) *T. turgidum*/ accession 191; (c) *T. turgidum*/accession 193; (d) Altar84/accession 015; (e) Decoy/accession 036 and (f) *T. turgidum*/accession 028.

Table 12. Phenotypic and disease characterization of the 194 A–genome synthetic hexaploids (2n=6x=42; AABBAA). FLOW = days–to–flowering, HT = plant height at maturity (cm); AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown), P MA = days to physiological maturity, TKW = 1,000–kernel weight (g), G/S = number of grains/spike, SL = spike length (cm), KB = Karnal bunt reaction (– = immune, + = susceptible), Pm (S) = reaction to powdery mildew at the seedling stage (– indicates missing data), Yr (S) = reaction to stripe rust at the seedling stage, Yr (A) = reaction to stripe rust at the adult-plant stage (R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlapping of MR–MS types, MSS = moderately susceptible to susceptible, S = susceptible, and TS = trace susceptible).

Synthetic number	FLOW	HT	AWN	PMA	TKW	G/S	SL	KB	Pm (S)	Yr (S)	Yr (A)
1	125	104	LB	175	46.0	18	13.5	–	2	89	5R
2	136	99	LB	169	59.0	18	12.0	–	–	89	30S
3	141	97	LB	171	65.0	2	13.0	–	–	89	30S
4	128	95	LB	173	72.0	4	13.0	–	4	89	30S
5	125	98	LB	179	44.0	29	13.8	–	4	23	5R
6	116	105	LB	180	60.0	15	13.2	–	2	23	30S
7	134	117	LB	174	40.0	30	12.0	–	1	89	30S
8	133	129	LB	184	60.0	40	10.3	–	3	56	5R
9	117	127	LB	179	58.0	39	14.0	–	4	7	30S
10	125	125	LB	185	38.0	5	14.1	–	4	7	10R
11	135	124	LB	181	38.0	7	11.1	–	3	89	10R
12	135	127	LB	184	48.0	33	14.1	–	3	89	90S
13	116	112	LB	181	40.0	1	13.1	–	4	89	90S
14	154	104	LB	186	32.0	7	12.1	–	3	78	10R
15	143	104	LB	186	22.6	24	12.3	–	4	1	5R
16	136	116	LB	184	39.5	3	12.1	–	–	23	30MRMS
17	131	128	LB	187	35.2	2	12.3	–	–	89	10R
18	133	139	DB	180	41.4	7	12.0	–	3	89	30S
19	133	125	LB	186	40.6	13	11.5	–	–	0	10R
20	135	135	LB	186	38.4	4	12.1	–	–	1	10R
21	143	125	LB	185	43.8	18	12.6	–	5	89	10R
22	129	145	LB	181	20.0	2	13.0	–	–	89	30S
23	142	92	LB	181	50.0	11	11.0	–	–	0	30S
24	140	121	LB	187	50.0	5	12.3	–	4	4	10R
25	135	133	LB	187	44.0	9	12.8	–	4	78	10R
26	125	124	LB	181	55.2	8	11.1	–	4	78	10S
27	145	151	LB	176	69.0	5	8.0	–	–	78	30S
28	124	130	LB	175	13.7	7	18.0	–	3	89	30S
29	128	125	LB	181	49.0	21	13.0	–	5	78	10MR
30	108	119	LB	176	43.5	9	12.1	–	4	0	10R
31	133	100	LB	181	44.0	2	11.1	–	–	78	10R
32	135	104	LB	186	42.6	12	10.1	–	3	0	10R
33	133	101	DB	186	48.2	22	10.1	–	2	78	10R
34	147	129	LB	190	27.2	29	11.5	–	2	89	10R
35	135	118	LB	187	48.0	6	10.1	–	–	12	5R
36	127	133	LB	176	43.8	6	8.0	–	3	89	30S
37	128	129	LB	175	52.0	8	9.0	–	–	89	30S
38	135	125	LB	181	42.6	9	11.1	–	1	89	10R
39	127	133	LB	179	56.0	18	15.0	–	1	89	30S
40	145	121	LB	190	60.0	4	13.5	–	1	89	10R
41	149	104	LB	190	40.6	24	11.0	–	2	78	10R
42	118	102	LB	176	54.2	16	10.0	–	1	0	30S

Table 12. Phenotypic and disease characterization of the 194 A–genome synthetic hexaploids (2n=6x=42; AABBAA). FLOW = days–to–flowering, HT = plant height at maturity (cm); AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown), P MA = days to physiological maturity, TKW = 1,000–kernel weight (g), G/S = number of grains/spike, SL = spike length (cm), KB = Karnal bunt reaction (– = immune, + = susceptible), Pm (S) = reaction to powdery mildew at the seedling stage (– indicates missing data), Yr (S) = reaction to stripe rust at the seedling stage, Yr (A) = reaction to stripe rust at the adult-plant stage (R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlapping of MR–MS types, MSS = moderately susceptible to susceptible, S = susceptible, and TS = trace susceptible).

Synthetic number	FLOW	HT	AWN	PMA	TKW	G/S	SL	KB	Pm (S)	Yr (S)	Yr (A)
43	134	118	LB	179	42.8	18	11.0	–	–	56	30S
44	116	106	LB	178	47.5	4	10.0	–	2	34	30S
45	125	94	LB	175	45.2	24	9.5	–	2	89	10R
46	127	112	LB	176	30.0	32	10.5	–	4	89	30S
47	127	113	LB	180	45.0	4	11.0	–	–	89	30S
48	135	115	LB	193	37.8	13	11.1	–	3	89	40S
49	133	102	Y	189	23.5	4	13.3	–	–	89	5R
50	145	80	LB	191	29.0	32	12.1	–	5	89	10R
51	126	110	Y	182	40.0	33	13.5	–	2	89	10R
52	125	126	LB	186	68.0	35	12.0	–	–	89	30MS
53	140	124	LB	180	40.0	33	9.0	–	2	89	30S
54	125	123	LB	181	44.4	21	10.0	–	0	89	30S
55	131	119	DB	187	32.2	21	11.0	–	1	89	30MR
56	146	130	DB	193	37.0	16	11.1	–	1	89	10R
57	126	110	LB	179	56.4	3	8.0	–	4	89	30S
58	136	116	LB	189	56.2	27	11.1	–	4	1	10R
59	131	117	LB	171	56.4	37	12.3	–	–	1	10R
60	127	105	LB	150	55.6	21	11.6	–	3	0	10R
61	127	103	LB	151	41.6	16	11.1	–	1	0	5R
62	121	108	LB	158	24.0	6	12.8	–	3	89	10R
63	127	110	LB	156	30.0	5	8.0	–	4	89	30S
64	139	113	LB	154	50.0	26	11.3	–	5	89	10MR
65	137	127	Y	156	48.0	6	14.0	–	–	89	30S
66	124	141	LB	150	50.0	1	11.5	–	5	89	30MS
67	125	108	LB	146	50.0	1	12.3	–	–	89	5 MR
68	122	114	LB	140	52.2	7	11.6	–	–	89	10MR
69	125	120	LB	150	46.0	43	13.1	–	2	89	5R
70	126	144	LB	179	43.4	31	12.3	–	–	12	10S
71	127	146	LB	171	53.2	22	14.6	–	–	9	90S
72	129	146	LB	173	32.6	6	13.5	–	–	1	10S
73	133	127	LB	172	37.6	16	10.0	–	4	9	30S
74	133	108	LB	185	46.0	26	14.6	–	3	12	20S
75	120	112	LB	172	17.4	21	7.0	–	–	9	30S
76	127	145	LB	175	63.0	11	15.0	–	3	89	40S
77	119	75	LB	171	49.6	20	14.0	–	1	89	30S
78	141	75	LB	183	42.8	33	8.0	–	6	45	30S
79	126	126	DB	176	32.8	33	11.0	–	6	56	90S
80	129	122	LB	180	33.2	18	9.0	–	4	89	30S
81	131	124	LB	179	32.0	17	14.0	–	–	9	30S
82	133	123	LB	176	31.0	4	13.0	–	1	12	30S
83	120	125	LB	175	37.0	35	10.0	–	0	89	30S
84	122	125	LB	173	50.0	11	12.0	–	0	89	30S

Table 12. Phenotypic and disease characterization of the 194 A–genome synthetic hexaploids (2n=6x=42; AABBAA). FLOW = days–to–flowering, HT = plant height at maturity (cm); AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown), P MA = days to physiological maturity, TKW = 1,000–kernel weight (g), G/S = number of grains/spike, SL = spike length (cm), KB = Karnal bunt reaction (– = immune, + = susceptible), Pm (S) = reaction to powdery mildew at the seedling stage (– indicates missing data), Yr (S) = reaction to stripe rust at the seedling stage, Yr (A) = reaction to stripe rust at the adult-plant stage (R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlapping of MR–MS types, MSS = moderately susceptible to susceptible, S = susceptible, and TS = trace susceptible).

Synthetic number	FLOW	HT	AWN	PMA	TKW	G/S	SL	KB	Pm (S)	Yr (S)	Yr (A)
85	125	127	LB	178	41.2	15	13.0	–	3	89	20MS
86	136	112	LB	178	40.0	7	8.0	–	0	9	30S
87	141	110	LB	175	46.0	17	11.0	–	1	9	30S
88	128	115	LB	178	38.0	15	11.0	–	2	9	10MR
89	128	104	LB	183	31.0	6	12.0	–	2	9	10R
90	131	109	LB	172	31.6	5	9.0	–	1	9	30S
91	131	114	LB	180	57.4	19	11.0	–	7	9	30S
92	134	119	LB	175	51.8	25	10.0	–	1	9	30S
93	134	118	LB	176	40.0	17	11.0	–	2	9	30S
94	139	120	LB	180	40.0	13	13.0	–	3	9	30S
95	134	123	LB	184	71.0	6	10.0	–	3	1	10MRMS
96	148	114	LB	191	59.0	6	12.5	–	1	56	10R
97	125	108	LB	177	49.6	7	7.0	–	–	89	30S
98	126	109	LB	178	38.0	16	9.0	–	–	67	30S
99	142	106	LB	184	35.0	14	10.0	–	6	89	30S
100	131	131	LB	183	35.4	23	14.0	–	0	89	30S
101	124	118	LB	179	43.4	6	13.0	–	1	89	30S
102	133	108	LB	180	65.6	13	7.0	–	0	9	30S
103	134	116	LB	183	56.6	38	12.0	–	0	9	5R
104	133	89	LB	181	61.6	14	9.8	–	5	89	5R
105	133	85	LB	179	50.2	51	10.0	–	4	89	5R
106	135	82	LB	176	31.0	1	12.0	–	0	89	30S
107	145	81	LB	179	30.8	21	9.0	–	–	89	30S
108	150	79	LB	188	72.0	36	9.0	–	2	1	10R
109	145	128	LB	186	44.6	21	11.0	–	1	0	10R
110	120	123	DB	178	46.2	14	10.0	–	4	9	80S
111	120	110	LB	182	47.0	1	15.0	–	0	9	40S
112	125	105	LB	185	48.0	15	13.0	–	5	9	30S
113	127	109	LB	182	48.0	21	10.0	–	5	9	30S
114	135	100	LB	187	44.0	20	13.0	–	7	89	10R
115	135	102	LB	187	26.6	9	11.0	–	5	89	10R
116	137	120	LB	186	45.4	10	12.0	–	4	89	10R
117	145	114	LB	188	38.2	26	8.0	–	4	89	5R
118	143	107	LB	188	32.6	7	14.0	–	4	9	5R
119	141	117	LB	189	53.8	34	12.0	–	6	12	10R
120	126	117	LB	189	48.0	27	12.6	–	0	89	10R
121	139	87	LB	187	44.0	7	13.0	–	4	89	10R
122	143	137	LB	191	45.6	10	12.0	–	7	89	10R
123	143	124	LB	191	32.4	8	11.0	–	1	89	5R
124	134	95	LB	186	59.8	32	14.0	–	2	89	30S
125	127	100	DB	189	45.3	6	8.0	–	3	89	5R
126	132	100	LB	183	46.2	19	10.0	–	3	9	5R

Table 12. Phenotypic and disease characterization of the 194 A–genome synthetic hexaploids (2n=6x=42; AABBAA). FLOW = days–to–flowering, HT = plant height at maturity (cm); AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown), P MA = days to physiological maturity, TKW = 1,000–kernel weight (g), G/S = number of grains/spike, SL = spike length (cm), KB = Karnal bunt reaction (– = immune, + = susceptible), Pm (S) = reaction to powdery mildew at the seedling stage (– indicates missing data), Yr (S) = reaction to stripe rust at the seedling stage, Yr (A) = reaction to stripe rust at the adult-plant stage (R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlapping of MR–MS types, MSS = moderately susceptible to susceptible, S = susceptible, and TS = trace susceptible).

Synthetic number	FLOW	HT	AWN	PMA	TKW	G/S	SL	KB	Pm (S)	Yr (S)	Yr (A)
127	127	120	LB	176	36.6	9	7.0	–	2	78	5R
128	131	131	LB	173	35.8	20	6.0	–	2	1	5R
129	138	95	LB	184	48.0	40	14.0	–	4	89	30S
130	122	119	LB	183	41.8	24	8.0	–	1	9	70S
131	147	113	DB	186	45.2	57	14.0	–	3	89	30S
132	133	111	LB	179	46.8	16	11.0	–	5	12	30S
133	128	111	LB	172	34.4	9	12.0	–	4	89	30S
134	143	110	LB	176	35.6	5	10.0	–	2	7	30S
135	140	115	LB	175	36.0	9	12.6	–	7	89	10R
136	140	137	LB	185	31.6	5	13.0	–	3	89	10R
137	140	138	LB	185	35.6	9	10.0	–	3	89	10R
138	125	133	LB	186	42.0	41	12.0	–	2	78	10R
139	140	123	LB	185	40.0	3	17.0	–	3	89	10R
140	140	120	LB	179	58.0	16	11.0	–	1	89	30S
141	140	122	LB	181	27.0	18	12.0	–	1	89	5R
142	140	143	LB	187	41.6	20	13.0	–	1	89	5R
143	139	135	LB	185	37.6	30	10.1	–	1	7	5R
144	139	125	LB	180	36.0	35	12.0	–	–	89	30S
145	137	138	LB	181	42.6	23	10.0	–	9	7	10S
146	140	135	LB	180	24.4	6	11.0	–	8	7	30S
147	127	132	LB	172	24.0	33	14.0	–	–	89	30S
148	133	135	LB	187	30.8	14	10.0	–	2	67	5R
149	142	155	LB	186	33.6	31	9.0	–	6	78	10MS
150	141	155	Y	188	24.0	13	11.0	–	2	78	30MR
151	135	111	LB	186	38.2	11	8.0	–	5	89	10R
152	125	136	LB	185	41.0	30	14.0	–	–	78	30MS
153	128	137	LB	185	40.8	29	14.0	–	3	89	30MS
154	127	137	LB	187	35.0	24	12.0	–	3	89	90S
155	136	126	DB	180	28.2	30	10.0	–	8	89	30S
156	126	115	LB	179	30.0	15	11.0	–	–	89	30S
157	128	119	LB	176	54.7	17	12.0	–	7	8	30S
158	127	122	LB	187	30.6	13	11.0	–	4	89	5S
159	151	130	LB	187	30.2	28	12.0	–	9	89	10R
160	122	125	DB	180	36.0	16	17.0	–	–	89	30S
161	141	131	LB	186	20.4	19	12.0	–	7	9	10R
162	131	104	LB	186	25.6	41	10.0	–	5	9	30S
163	139	108	LB	187	31.8	5	13.0	–	7	89	5MS
164	130	111	LB	180	36.4	18	12.0	–	5	9	30S
165	125	101	Y	187	26.2	23	8.0	–	5	9	MSS
166	144	95	LB	179	39.6	15	13.0	–	4	9	30S
167	140	95	LB	185	22.4	30	7.0	–	4	9	30S
168	141	117	LB	187	22.0	29	7.0	–	3	9	10R

Table 12. Phenotypic and disease characterization of the 194 A–genome synthetic hexaploids (2n=6x=42; AABBAA). FLOW = days–to–flowering, HT = plant height at maturity (cm); AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown), P MA = days to physiological maturity, TKW = 1,000–kernel weight (g), G/S = number of grains/spike, SL = spike length (cm), KB = Karnal bunt reaction (– = immune, + = susceptible), Pm (S) = reaction to powdery mildew at the seedling stage (– indicates missing data), Yr (S) = reaction to stripe rust at the seedling stage, Yr (A) = reaction to stripe rust at the adult-plant stage (R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlapping of MR–MS types, MSS = moderately susceptible to susceptible, S = susceptible, and TS = trace susceptible).

Synthetic number	FLOW	HT	AWN	PMA	TKW	G/S	SL	KB	Pm (S)	Yr (S)	Yr (A)
169	133	119	LB	181	40.4	33	12.0	–	7	9	10R
170	141	121	LB	182	30.0	10	9.0	–	7	9	10S
171	140	120	LB	185	29.0	18	10.0	–	–	9	30S
172	146	102	LB	183	39.8	8	10.0	–	6	9	30S
173	135	112	LB	176	29.3	15	8.0	–	7	9	30S
174	122	116	LB	172	36.4	19	16.0	–	4	89	30S
175	139	112	LB	176	32.4	31	11.0	–	5	89	30S
176	132	116	LB	171	10.0	3	11.6	–	–	9	30S
177	132	108	LB	173	38.4	9	11.0	–	4	89	30S
178	133	100	LB	182	32.6	9	14.0	–	7	9	5R
179	128	108	LB	182	24.0	16	13.3	–	4	89	5R
180	141	118	LB	183	28.5	4	12.6	–	2	89	20S
181	140	131	LB	180	25.2	34	8.0	–	3	89	30S
182	129	144	LB	187	20.7	25	10.0	–	1	78	30MR
183	137	107	LB	182	33.6	12	11.0	–	0	0	30S
184	136	95	LB	180	48.9	12	14.0	–	0	9	30S
185	127	85	LB	182	24.4	18	10.0	–	0	78	30S
186	133	116	LB	186	21.4	52	12.8	–	2	8	80S
187	128	112	LB	176	42.0	32	12.8	–	7	8	5R
188	124	113	LB	175	43.0	25	10.0	–	7	89	30S
189	129	115	LB	177	44.8	22	8.0	–	0	89	5R
190	120	86	LB	182	35.6	27	13.0	–	2	9	40MS
191	122	120	LB	173	45.2	7	13.0	–	0	9	10R
192	135	98	LB	177	25.0	4	7.0	–	1	9	30MS
193	125	150	LB	177	35.8	11	17.0	–	8	9	30S
194	128	145	LB	176	32.0	18	15.0	–	–	89	30S

This study has focussed on having in stock a genetic resource in adequate seed amounts that is phenotypically characterized and molecularly typed for its diversity. Earlier investigations indicated that in the AAB F_1 hybrids (2n=3x=21), mean pairing frequency ranged between 5.5 to 6.0 bivalents across the 194 combinations produced with 25 to 50 meiocytes analyzed per F_1 sample. Because A genome recombination events were possible, the variation of the diploid progenitors could be harnessed in breeding. Producing AAAABB amphiploids provided a resource that has the merit of being globally distributed and utilized by providing a more reliable evaluation of the genetic value of the alien genes in the derived background through a permanent germ plasm base. Although amphiploid instability is a frequent occurrence, all the synthetics analyzed in our field studies in Pakistan showed exceptional cytological stability. The predominance of bivalents (Figs. 8–10, pp. 95-97) with a high seed set with well-filled grains enabled adequate production of seed for national testing against key biotic/abiotic stress production constraints.

The Karnal bunt screening of all the AAAABB synthetic hexaploids reinforced the earlier observations that have expressed durums to be generally field resistant. Bringing in additional diversity from new diploid progenitor accessions did not alter this field resistance trend. The A-genome synthetic hexaploids when tested under field conditions for yellow rust exhibited a parallel trend to the D-genome-based amphiploids. Conclusions drawn in the above study were that the germ plasm tested fell into two categories:

Table 13. Phenotypic and disease characterization of the 194 A–genome synthetic hexaploids (2n=6x=42; AABBAA). FLOW = days–to–flowering, HT = plant height at maturity (cm); AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown), P MA = days to physiological maturity, TKW = 1,000–kernel weight (g), G/S = number of grains/spike, SL = spike length (cm), KB = Karnal bunt reaction (– = immune, + = susceptible), Pm (S) = reaction to powdery mildew at the seedling stage (– indicates missing data), Yr (S) = reaction to stripe rust at the seedling stage, Yr (A) = reaction to stripe rust at the adult–plant stage (R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlapping of MR–MS types, MSS = moderately susceptible to susceptible, S = susceptible, and TS = trace susceptible).

Entry	FLOW	HT	AWN	PMA	TKW	G/S	SL	KB	Pm (S)	Yr (S)	Yr (A)
1	87	86	LB	101	45	45	9	–	7	0	10R
2	86	86	LB	105	18.5	16	9	–	5	0	5RMR
4	89	78	LB	108	33.0	26	6	–	7	0	TR
5	87	76	LB	112	37.6	18	6	–	3	0	10R
8	95	103	LB	108	32.2	30	9	–	3	0	0
9	88	103	LB	103	41.6	38	8	–	3	0	5R
11	85	102	LB	99	46.0	28	6	–	2	0	0
12	98	96	LB	110	37.0	41	10	–	4	0	5R
13	88	102	LB	100	41.1	28	7	–	5	0	10R
14	87	85	LB	106	46.5	31	7	–	6	0	10MR
17	92	83	LB	112	32.0	42	11	–	6	0	40R
20	89	85	LB	114	38.4	47	10	–	–	0	10R
21	87	90	LB	102	35.1	34	9	–	5	0	5R
22	89	103	LB	115	34.8	48	9	–	7	0	0
23	100	68	LB	115	12.5	9	8	–	2	0	20MRMS
25	89	75	LB	100	42.5	36	8	–	–	0	40MS
26	82	104	LB	98	35.5	41	9	–	–	0	5R
27	82	90	LB	95	41.2	45	8	–	–	0	20RMR
28	88	92	LB	93	44.2	28	8	–	–	0	TR
33	92	93	LB	105	32.1	30	5	–	–	0	20MRMS
34	102	104	LB	115	34.3	34	5	–	–	0	20MRMS
35	102	97	LB	116	32.4	23	7	–	–	0	20MRMS
37	100	96	LB	112	29.7	9	9	–	–	0	TR
40	100	78	LB	118	34.2	30	9	–	–	0	0
45	89	87	LB	103	42.7	15	7	–	–	78	TR

1. Dual resistance, where the durum wheat parent and the corresponding synthetic hexaploid entry were resistant. Because the diploid donor was not tested, the resistance of the synthetic could be due to the durum parent alone or the durum parent complimented by the resistance in the diploid donor accession.
2. Resistance in the synthetic and susceptibility in the durum wheat parent. This category provided unequivocal support that the resistance was a contribution of diversity that was expressed from the diploid donor accession.

By analogy, the stripe rust resistance present in the AAAABB synthetics is inferred to emanate either from the durum wheat parent if that was resistant or the A-genome diploid if the durum wheat was susceptible, or as a consequence of both the durum wheat parent and the A-genome parent where both parents were resistant.

Systematic discussion is now built upon addressing the phenology, disease, and genetic diversity of the 194 A-genome synthetics that have been used. The synthetics have been grouped into various categories based upon the durum wheat parent used (Table 5, p. 93) where group 1 has two synthetics with the same durum wheat cultivar entry 21; YARMUK. From the various groups, number 19 contributed to 31 AABBAA synthetics and the durum wheat parent is entry number 11 (CPI/GEDIZ/3/GOO//JO/CRA). In this group, the synthetics have been separated according to the diploid parent used, thus the categories discussed are *T. monococcum* subsp. *aegilopoides* with 23 synthetics, *T. monococcum* subsp. *monococcum* with five, and *T. urartu* with three (Table 11, p. 101).

Table 14. Molecular fingerprinting pattern by random amplified polymorphic DNA (RAPD) primers in the A-genome synthetic hexaploids (2n=6x=42; AABBAA).

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)
1	OPD-20	14	14	100%	84	448	250–3,000
2	OPE-1	12	12	100%	73	321	500–2,500
3	OPE-2	9	9	100%	34	119	500–2,500
4	OPE-3	12	12	100%	124	666	250–2,500
5	OPE-4	12	12	100%	113	476	250–3,000
6	OPE-5	5	5	100%	35	37	750–3,000
7	OPE-6	12	12	100%	63	187	500–2,500
8	OPE-7	13	13	100%	85	422	500–3,000
9	OPE-12	12	12	100%	49	150	500–2,500
10	OPE-14	11	11	100%	79	289	500–2,500
11	OPE-15	14	14	100%	80	382	250–2,500
12	OPE-16	13	13	100%	46	115	250–2,500
13	OPE-19	11	11	100%	35	112	250–2,500
14	OPF-10	10	10	100%	47	152	500–2,500
15	OPF-12	11	11	100%	40	133	500–2,500
16	OPF-13	12	12	100%	65	275	250–2,500
17	OPF-14	13	13	100%	60	292	250–2,500
18	OPF-15	11	11	100%	42	184	500–2,500
19	OPF-16	3	3	100%	3	4	1,000–3,000
20	OPF-20	11	11	100%	41	190	500–2,500
21	OPG-2	10	10	100%	9	27	500–2,500
22	OPG-3	11	11	100%	13	49	500–2,500
23	OPG-8	9	9	100%	74	233	750–2,500
24	OPG-10	8	0	0%	1	8	750–2,500
25	OPG-17	6	0	0%	1	6	1,000–2,000
26	OPG-18	13	13	100%	18	81	250–2,500
27	OPG-19	11	11	100%	52	270	500–2,500
28	OPH-1	8	8	100%	2	10	500–2,500
29	OPH-2	14	14	100%	12	46	250–3,000
30	OPH-4	10	10	100%	51	159	500–2,500
31	OPH-5	12	12	100%	12	57	500–2,500
32	OPH-11	2	0	0%	1	2	500–1,000
33	OPH-12	8	8	100%	10	30	250–1,000
34	OPH-13	11	11	100%	15	48	500–2,500
35	OPH-15	10	10	100%	11	28	250–2,000
36	OPH-17	5	5	100%	6	10	500–2,500
37	OPH-19	10	10	100%	15	37	500–2,500
38	OPI-2	6	0	0%	1	6	500–1,500
39	OPI-4	1	1	100%	1	1	1,500
40	OPI-6	12	12	100%	29	159	250–2,500
41	OPI-7	9	9	100%	8	23	250–2,000
42	OPI-9	14	14	100%	69	200	250–3,000
43	OPI-10	11	11	100%	69	218	250–2,000
44	OPI-12	15	15	100%	121	643	250–3,000
45	OPI-14	11	11	100%	41	138	250–2,500
46	OPI-16	10	10	100%	27	69	250–2,000
47	OPI-17	12	12	100%	53	245	1,000–1,500

Table 14. Molecular fingerprinting pattern by random amplified polymorphic DNA (RAPD) primers in the A-genome synthetic hexaploids (2n=6x=42; AABBAA).

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)
48	OPI-18	1	1	100%	3	3	1,000–1,500
49	OPI-20	11	11	100%	25	87	250–2,500
50	OPJ-1	12	12	100%	14	50	250–2,000
51	OPJ-6	11	11	100%	21	63	750–2,500
52	OPJ-9	12	11	91.67%	48	124	500–3,000
53	OPJ-17	11	11	100%	13	47	500–3,000
54	OPJ-19	11	11	100%	54	235	250–2,000
55	OPJ-20	12	12	100%	116	536	250–2,500
56	OPK-15	5	5	100%	18	34	750–2,000
57	OPK-16	13	13	100%	101	331	250–3,000
58	OPK-19	11	11	100%	70	290	250–2,000
59	OPK-20	7	7	100%	3	14	250–1,500
60	OPL-7	8	8	100%	17	25	250–3,000
61	OPL-8	8	0	0%	2	16	250–2,000
62	OPL-11	12	12	100%	62	300	250–2,500
63	OPL-12	10	10	100%	86	308	250–2,000
64	OPL-14	12	12	100%	52	135	250–3,000
65	OPL-16	11	11	100%	72	236	500–2,500
66	OPL-20	14	14	100%	71	242	250–2,500
67	OPM-1	8	8	100%	27	65	500–1,500
68	OPM-3	12	12	100%	46	184	250–2,500
69	OPM-4	10	10	100%	18	70	500–2,500
70	OPM-5	11	11	100%	11	37	250–2,500
71	OPM-7	11	11	100%	15	64	500–2,500
72	OPM-8	3	3	100%	6	7	1,000–1,500
73	OPM-10	10	10	100%	54	234	250–2,000
74	OPM-12	7	7	100%	11	32	500–2,500
75	OPM-13	12	12	100%	26	92	250–2,500
76	OPM-14	1	1	100%	1	1	500
77	OPM-15	8	8	100%	24	57	750–2,500
78	OPM-16	10	10	100%	27	89	500–2,500
79	OPM-20	14	14	1000%	17	102	500–2,500
80	OPN-1	2	0	0%	1	2	250–500
81	OPN-2	14	14	100%	40	180	250–2500
82	OPN-3	10	10	100%	7	34	250–2500
83	OPN-4	12	12	100%	69	329	250–2500
84	OPN-5	11	11	100%	33	89	250–2,500
85	OPN-7	6	6	100%	9	19	500–2,000
86	OPN-9	13	13	100%	14	53	250–2,500
87	OPN-11	9	9	100%	13	40	250–2,000
88	OPN-12	11	11	100%	34	124	250–3,000
89	OPN-13	12	12	100%	8	39	500–2000
90	OPN-14	13	13	100%	16	73	250–2,500
91	OPN-16	9	9	100%	93	381	500–2,000
92	OPN-18	13	13	100%	71	319	250–2,500
93	OPN-19	13	13	100%	22	60	250–3,000
94	OPN-20	11	11	100%	26	119	250–2,500

Table 14. Molecular fingerprinting pattern by random amplified polymorphic DNA (RAPD) primers in the A-genome synthetic hexaploids (2n=6x=42; AABBAA).

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)
95	OPO-12	8	8	100%	7	16	500–2,000
96	OPO-13	6	6	100%	4	12	1,000–2,500
97	OPQ-6	12	12	100%	13	63	250–2,500
98	OPQ-9	8	8	100%	9	27	250–1,500
99	OPQ-14	15	15	100%	52	277	250–3,000
100	OPQ-16	13	13	100%	72	344	250–2,500
101	OPR-1	13	13	100%	63	163	250–2,500
102	OPR-4	6	6	100%	17	24	1,000–2,000
103	OPR-8	12	12	100%	124	400	250–1,500
104	OPR-9	12	12	100%	39	149	250–3,000
105	OPR-20	13	13	100%	49	160	250–3,000
106	OPT-8	9	9	100%	28	70	250–1,500
107	OPV-3	11	11	100%	18	70	500–3,000
108	OPV-18	09	09	100%	38	110	500–2,500

Table 15. Molecular fingerprinting pattern by random amplified polymorphic DNA (RAPD) primers in the B-genome synthetic hexaploids (2n=6x=42; AABBBB(SS)).

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)
1	OPA-3	8	8	100%	4	15	750–2,500
2	OPA-4	1	1	100%	1	1	1,000
3	OPB-1	8	5	62.5%	5	10	750–3,000
4	OPC-2	5	5	100%	5	7	750–2,000
5	OPE-9	2	2	100%	2	2	1,500
6	OPE-11	1	1	100%	1	1	1,000
7	OPE-14	4	2	50%	2	5	750–1,500
8	OPE-15	9	8	88.88%	5	21	500–2,500
9	OPE-16	7	6	85.71%	4	15	250–2,000
10	OPG-2	3	2	66.66%	2	4	750–1,500
11	OPG-5	8	8	100%	8	26	250–1,500
12	OPG-13	1	1	100%	2	2	1,500
13	OPI-7	9	7	77.77%	4	22	250–2,000
14	OPI-19	9	4	44.44%	4	17	250–2,000
15	OPJ-1	5	1	20%	1	5	500–1,500
16	OPJ-9	3	3	100%	4	7	750–1,500
17	OPJ-20	4	2	50%	6	21	250–1,000
18	OPK-9	2	2	100%	2	2	1500–2,000
19	OPL-1	7	7	100%	4	13	500–1,500
20	OPL-2	9	9	100%	7	20	250–1,500
21	OPL-12	4	3	75%	6	15	750–1,000
22	OPL-20	8	5	62.5%	8	17	250–2,500
23	OPM17	8	8	100%	4	18	250–1,500
24	OPN-1	3	3	100%	2	3	1,000–2,000
25	OPN-2	7	7	100%	6	16	250–2,000
26	OPN-3	2	2	100%	3	4	1,500
27	OPN-4	6	4	66.66%	8	29	500–1,500
28	OPN-5	6	6	100%	7	17	250–1,500

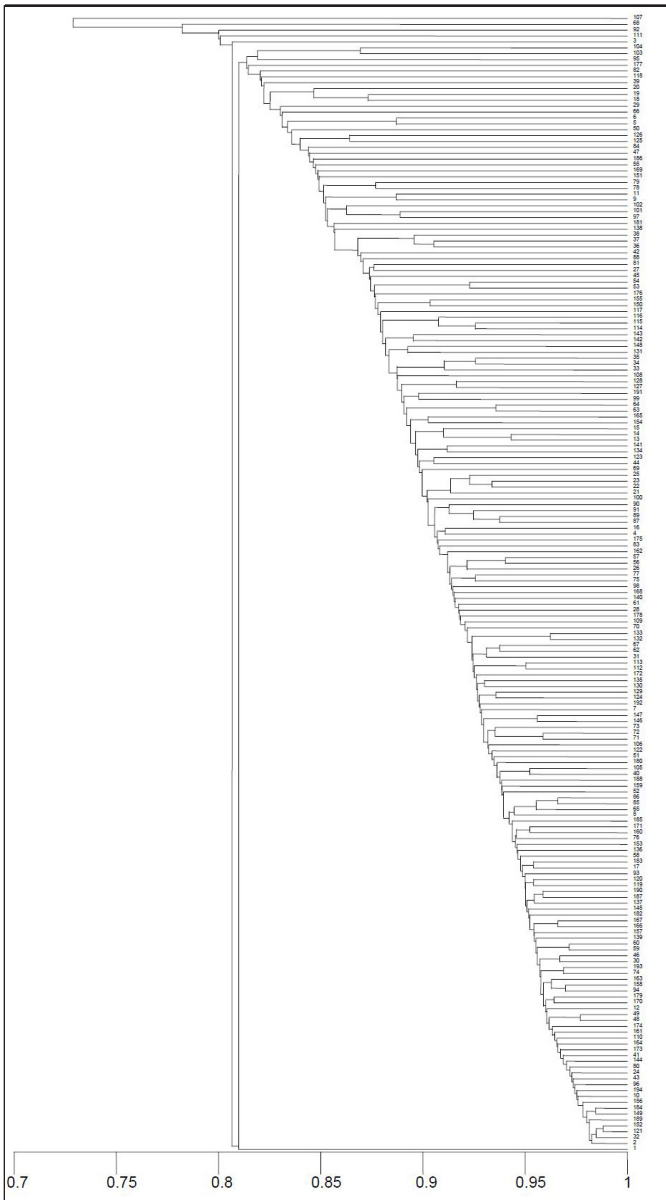
Table 15. Molecular fingerprinting pattern by random amplified polymorphic DNA (RAPD) primers in the B-genome synthetic hexaploids (2n=6x=42; AABBBB(SS)).

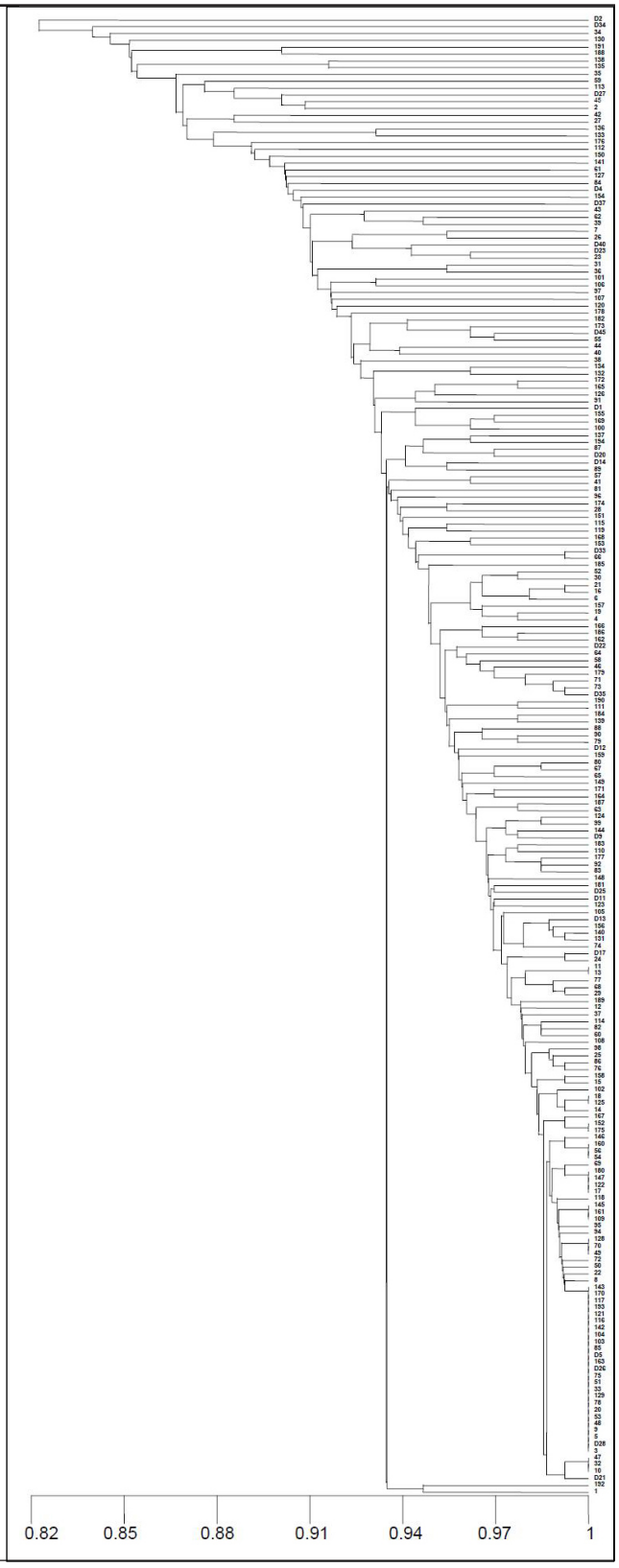
Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)
29	OPN-6	7	7	100%	9	25	250–1,500
30	OPN-9	2	2	100%	2	3	250–1,500
31	OPN-12	4	1	25%	2	7	500–2,000
32	OPN-17	4	4	100%	3	9	500–1,000
33	OPP-16	3	3	100%	5	7	750–1,500
34	OPQ-5	4	4	100%	5	12	250–1,000
35	OPW-4	3	3	100%	3	6	750–2,000
36	OPW-5	1	1	100%	1	1	1,000
37	OPX-2	4	0	0%	1	4	750–1,500
38	OPX-12	4	4	100%	3	9	500–1,000
39	OPY-7	2	0	0%	2	4	1,000
40	OPY-8	3	0	0%	1	3	750-1,500

Table 16. Molecular fingerprinting pattern by simple sequence repeat (SSR) primers in A-genome synthetic hexaploids (2n=6x=42; AABBAA).

Primer number	Primer	Total loci	Polymor-phic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)	PIC
1	*Xgwm10-2A*	7	7	100%	58	73	50–150	0.36
2	*Xgwm47.1-2A*	5	5	100%	31	36	50–200	0.57
3	*Xgwm47.2-2A*	6	6	100%	18	28	50–200	0.51
4	*Xgwm71.1-2A*	6	6	100%	34	80	50–150	0.73
5	*Xgwm71.2-2A*	6	6	100%	27	61	50–150	0.76
6	*Xgwm95-2A*	3	3	100%	56	62	50–150	0.37
7	*Xgwm122-2A*	5	5	100%	17	20	50–150	0.43
8	*Xgwm249-2A*	9	9	100%	50	92	50–200	0.78
9	*Xgwm265-2A*	4	4	100%	16	16	50–200	0.36
10	*Xgwm296-2A*	5	5	100%	15	18	50–100	0.65
11	*Xgwm311-2A*	9	9	100%	46	112	50–250	0.77
12	*Xgwm312-2A*	6	6	100%	68	85	50–150	0.57
13	*Xgwm372-2A*	3	3	100%	15	16	50–200	0.28
14	*Xgwm382-2A*	8	8	100%	25	45	50–200	0.85
15	*Xgwm473-2A*	1	1	100%	6	6	50–100	0.00
16	*Xgwm515-2A*	5	5	100%	44	65	50–150	0.75
17	*Xgwm558-2A*	6	6	100%	22	32	50–100	0.76
18	*Xgwm5-3A*	8	8	100%	31	50	50–200	0.70
19	*Xgwm30-3A*	4	4	100%	12	14	50	0.38
20	*Xgwm162-3A*	4	4	100%	34	38	50–200	0.47
21	*Xgwm391-3A*	4	4	100%	9	12	50–300	0.70
22	*Xgwm666.2-3A*	4	4	100%	25	31	50–150	0.58
23	*Xgwm397-4A*	2	2	100%	22	24	50	0.12
24	*Xgwm601-4A*	5	5	100%	42	49	50–100	0.59
25	*Xgwm637-4A*	1	1	100%	17	17	50	0.00

The *T. monococcum* subsp. *aegilopoides*-based SHs are entries 86, 87, 95, 96, 97, 98, 99, 100, 107, 108, 109, 110, 114, 131, 132, 134, 135, 138, 139, 140, 142, 175, and 183. The range for days-to-flowering in this set is 120–150 days. Among those that are considered early flowering (120–135 d) are 110 (120 d), 97 and 138 (125 d), 98 (126 d), 100





The RAPD- (**Fig. 13 (above)**) and SSR-based (**Fig. 14, right**)) analyses on the 194 AABBAA synthetics indicate a wide diversity. Maximum diversity was highest for entry 107 by RAPD analysis (Fig. 13). Other diverse entries are 95, 97, 138, and 142 (Fig. 13). Two diverse lines based upon SSR analysis were 135 and 138 (Fig. 14); with entry 138 being common.

(131 d), 132 (133 d), 95 (134 d), and 114 (135 d). The range for days to physiological maturity was 175–191 days, of which 11 matured by 180 days (86, 87, 97, 98, 107, 110, 132, 134, 135, 140, and 175). Thousand-kernel weight ranged from 30.8 to 72 g. A majority of lines were up to 50 g, and those higher than 50 g were 95 (71 g), 96 (59 g), and 190 (58 g). Grains/spike ranged from 3 to 57, with a majority between 10 to 20. A maximum of 57 grains/spike was found

in entry 131; 138 had 41 grains/spike. Spike length ranged from 7 to 17 cm, where the greatest was 17 cm in entry 139. Entrys 100 and 131 had 14-cm spikes. The detailed data is in Table 12 (pp. 103-107).

All AABBAA synthetics possessed Karnal bunt resistance (Table 12, pp. 103-107). For powdery mildew seedling resistance, entries showing a susceptible reaction were 99, 114, 132, 135, and 175. All the others were classified as resistant. Seedling resistance for stripe rust was limited to five entries; 95, 108, 109, 132, and 183. The first three entries also had APR and are favored candidates for wheat breeding. Other entries possessing seedling susceptibility but having APR were 96, 114,135, 138, 139, and 142. These lines have minor genes and are highly desirable for durable resistance breeding.

The RAPD- and SSR-based analyses on the 194 AABBAA synthetics indicated a wide diversity. Maximum diversity was highest for entry 107 by RAPD analysis. Other diverse entries are 95, 97, 138, and 142 (Fig. 13, p. 113). Two diverse lines based upon SSR analysis were 135 and 138 (Fig. 14, p. 113); with entry 138 being common. This line also possessed APR for stripe rust, had KB resistance, and some positive phenotypic traits rendering it a potent source for breeding efforts. Line 95 is resistant to Karnal bunt, powdery mildew, and strip rust and has a high 1,000-kernel weight. Such comprehensive checks identify various synthetics for exploitation.

The synthetic lines mentioned above are from five durum wheat entries (115, 116, 121, 122, and 146), and two lines (116 and 121) have multiple traits for resistance and good phenology. Three durum/*T. urartu* synthetics (190, 193, and 194) are suitable for breeding possessing multiple positive attributes (Table 12, pp. 103-107).

A stringent analysis of the phenotypic and biotic stress data across all the 194 AABBAA synthetics studied indicate that the 14 entries are elite in performance and top priority candidates for wheat improvement; 9, 12, 52, 59, 71, 95, 103 108, 111, 116, 121,124, 138, and 190. They possess a maximum of positive traits and also are molecularly diverse. Within each group, this number can be enhanced based upon fewer of positive traits (Table 12, pp. 103-107).

Of the 14 elite entries some are described in detail for their practical attributes:
Entry 9: resistant to KB and powdery mildew, early flowering (117 d), 1,000-kernel weight (58 g), grains/spike (39), spike length (14 cm),
Entry 12: resistant to KB and powdery mildew, grains/spike (33), spike length (14.1 cm),
Entry 52: resistant to KB and powdery mildew, 1,000-kernel weight (68 g), grains/spike (35),
Entry 59: resistant to KB and stripe rust (seedling and APR), 1,000-kernel weight (56.4 g), grains/spike (37),
Entry 71: resistant to KB and powdery mildew, 1,000-kernel weight (53.2 g), spike length (14.6 cm),
Entry 103: resistant to KB and stripe rust (APR), 1,000-kernel weight (56.6 g), grains/spike (38),
Entry 108: resistant to KB and stripe rust (seedling and APR), 1,000-kernel weight (72 g), grains/spike (36),
Entry 111: resistant to KB and powdery mildew, early flowering (120 d), spike length (15 cm), and
Entry 124: resistant to KB, grains/spike (32), spike length (14 cm).

Genomic diversity present in the A-genome diploids is plentiful and a boon for broadening the wheat genetic base. Often, single valuable traits of significance are exploited, but the our studies have shown that multiple traits can be harnessed simultaneously to assist efficiency of breeding. The more traits are present in the starting genetic stock, the easier to isolate recombinants where the wheat parent contributions are additive. These stocks are excellent tools for screening for other stress traits and, with the molecular diversity foundation established here, the meaningful decisions can aid the way forward in breeding.

Cytological, phenoligical, and molecular characterization of B-genome synthetic hexaploids.

Alvina Gul Kazi, Farrukh Bashir, Hadi Bux, Awais Rasheed, Rabia Sultan, Abdul Aziz Napar, and Abdul Mujeeb-Kazi.

The polyploid *Aegilops* and *Triticum* species sharing one genome with wheat are included in the secondary gene pool. Also included are the diploid species of the *Sitopsis* section. Genetic transfers are routine within homologous genomes but require manipulative protocols between nonhomologous types. Embryo rescue is a complementary aid for obtaining hybrids. Although limited use exists for wheat improvement, priority has been suggested for exploiting the *Sitopsis* species *Ae. speltoides* (2n = 2x =14, SS) for durum and bread wheat improvement. Breeding protocols are more complex

because manipulation strategies associated with alien gene transfer often incorporate undesirable traits together with the target gene of interest.

We currently are exploiting *Ae. speltoides* accessions via a hexaploid–amphiploid bridge-cross (2n=6x=42, AABBSS) (Fig. 15). These newly produced amphiploids have shown initial promise for resistance to *C. sativus, F. graminearum, S. tritici*, barley yellow dwarf virus, and leaf and stripe rust. More testing together with exploiting the potential of other *Sitopsis* species diploids appears logical, recognizing that the positive outputs from *Ae. speltoides* accessions genetic is just one example.

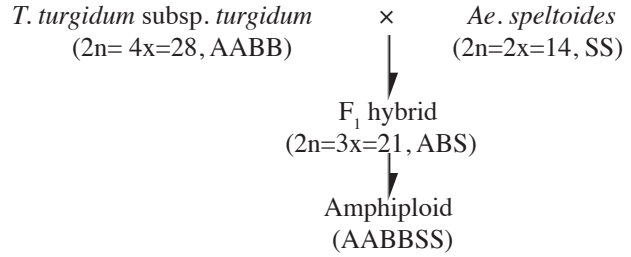


Fig. 15. Schematic for AABBSS production involving *T. turgidum* subsp. *turgidum* and *Ae. speltoides* (2n=2x=14, SS).

Table 17. The B-genome synthetic hexaploid entries utilized in the study, synthetic hexaploid entry numbers are similar to those maintained in CIMMYT, Mexico, Wide Crosses Program. Pedigrees details are given in Table 18.

Group No.	B-genome synthetic hexaploid entry Numbers	Total entries
1	7, 9, 11, 48	4
2	6, 12, 13	3
3	10, 22, 24, 25, 26, 32, 34, 36, 47, 49	10
4	18	1
5	19	1
6	23	1

Table 18. Pedigrees of the B-genome synthetic hexaploids.

Synthetic number	Parentage/pedigree
6	CETA/*Ae. speltoides* (127)
7	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. speltoides* (129)
9	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. speltoides* (133)
10	ARLIN_1/*Ae. speltoides* (134)
11	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. speltoides* (135)
12	CETA/*Ae. speltoides* (135)
13	CETA/*Ae. speltoides* (139)
18	ALTAR 84/*Ae. speltoides* (141)
19	CROC_1/*Ae. speltoides* (149)
22	ARLIN_1/*Ae. speltoides* (126)
23	D67.2/P66.270//*Ae. speltoides* (126)
24	ARLIN_1/*Ae. speltoides* (128)
25	ARLIN_1/*Ae. speltoides* (130)
26	ARLIN_1/*Ae. speltoides* (131)
32	ARLIN_1/*Ae. speltoides* (143)
34	ARLIN_1/*Ae. speltoides* (144)
36	ARLIN_1/*Ae. speltoides* (145)
47	ARLIN_1/*Ae. speltoides* (157)
48	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. speltoides* (157)
49	ARLIN_1/*Ae. speltoides* (158)

A set of 20 B-genome synthetics were cytologically and phenologically characterized and screened against powdery mildew, due to limited seed availability (Table 17).

Since the initial production of the B-genome hexaploids, the number available has decreased to 34. Several of the original 54 total were poorly adapted to the Pakistani conditions at Islamabad. In contrast to the A-genome synthetics, the B-genome synthetics are weaker plants and showed aneuploid meiotic associations (Fig. 16a and b, p. 116) and all expressed a co-dominant spike phenotype (Fig. 17a, p. 116). At meiosis, open bivalents increased in number with increased appearance of multiple chromosomal associations, trivalents, quadrivalents, and to a lesser degree pentavalents and aneuploidy (Table 19, p. 117). Like the A-genome hexaploids, those of the B genome were similar in having a tall plant habit (100–130 cm) and late maturity (145–155 days) under Islamabad conditions. Seed fertility was satisfactory in those that were adapted, but the seed was shriveled. The crossability across all combinations obtained at CIMMYT was of a high frequency (Table 19, p. 117) and regeneration of embryos was generally over 90% with colchicine-induced doubling to yield the AABB(SS) amphiploids also of a similar or higher level (Table 20, p. 118). C-banding was used to validate the presence of four B genomes in the amphiploid.

All the entries were screened in pot trials in the greenhouse at Murree. Nine of 20 B-genome synthetic hexaploids showed a resistant reaction to powdery mildew at the seedling stage (Table 21, p. 119). Infection type ranged from 0–6 in all lines at the seedling stage indicating the presence of major genes for resistance. Some of these resistant accessions exhibited different reaction types against powdery mildew under field conditions. The entries also were screened under field conditions at Kaghan and a majority of the germ plasm showed APR and were found to be resistant to completely resistant (immune). In B-genome synthetic hexaploids, the accessions were found to be completely resistant (immune) to susceptible at seedling stage. The accessions that showed resistance at seedling stage are 1, 3, 4, 5, 6, 7, 8, 13, and 18. Resistant lines at the seedling stage provide a source of major resistance genes but further evaluation at the adult-plant stage is needed for the identification of lines with novel resistance source and, thus, for further exploitation in future breeding programs.

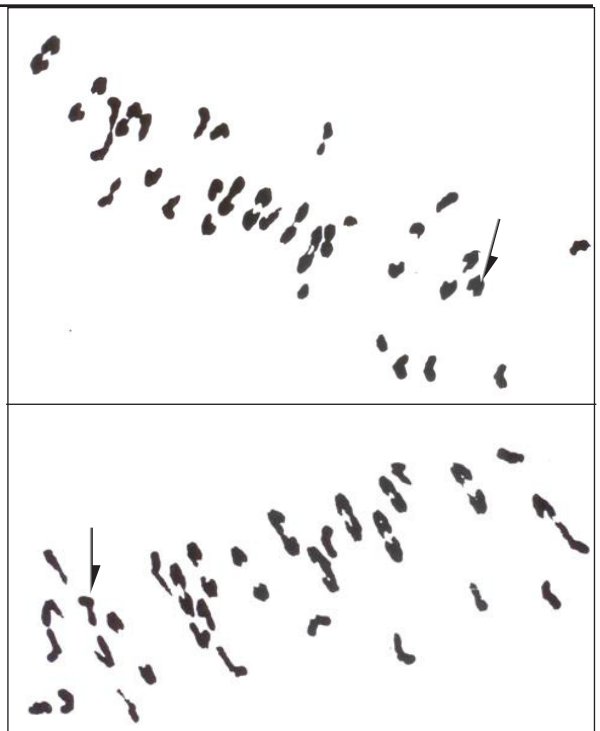


Fig. 16. Meiotic associations at metaphase I of some B-genome synthetic hexaploids, genomically AABBSS, showing (a) an aneuploid synthetic wth 41 chromosomes with one trivalent (arrow) and a mixture of ring and rod bivalents and (b) an aneuploid synthetic derivative with 41 chromosomes and univalents, ring and rod bivalents, and one quadrivalent association (arrow).

Evaluation of genetic diversity using random amplified polymorphic DNA (RAPD) primers. RAPD primers were used for genetic diversity evaluation of A-, B-, and D-genome synthetic hexaploids. All 520 RAPD primers of the Operon Series were screened and working primers were identified and applied to detect genetic polymorphism at DNA level. Samples that did not amplify were not included in the analysis.

Genetic analysis was performed only on the scorable bands. Every single band was considered as a single locus/allele. The loci were scored as present/absent. Bivariate data 1–0 were used to estimate genetic distances (GD). The unweighted pair group of arithmetic means (UPGMA) function estimated GD between the genotypes as follows: $GD_{xy} = 1 - d_{xy}/d_x + d_y - d_{xy}$, where GD_{xy} = genetic distance between two genotypes, d_{xy} = total number of common loci (bands) in two genotypes, d_x = total number of loci (bands) in genotype 1, and d_y = total number of loci (bands) in genotype 2.

Fig. 17. Spike morphology of B-genome (S) genome hexaploids derived from *T. turgidum* subsp. *turgidum* (2n=4x=28, AABB)/*A. speltoides* crosses (2n=2x=14; SS) from left to right (a) *T. turgidum* subsp. *turgidum*, (b) *T. turgidum* cv. Cerceta/*Ae. speltoides* (B-13), and (c) *T. turgidum* cv Cerceta/*Ae. speltoides* (B-02).

The efficiency of primers to amplify the genotypes ranged from nine (OPN-6) to one (OPA-4, OPE-11, OPJ-1, OPW-5, OPX-2, and OPY-8) (Table 22, p. 120). Scorable bands ranged from one (OPA-4, OPE-11, and OPW-5) to 29 (OPN-4).

Genetic analysis of the population showed that the B-genome synthetic hexaploids scored total 190 loci with 151 as polymorphic (79.47%) (Table 22, p. 120). The range of scorable bands was from 500–3,000 bp.

Similarity matrix. Bivariate analysis was conducted to generate a similarity matrix and dendrogram using Nei and Li's coefficient to estimate genetic diversity. The similarity coefficient in B-genome synthetic hexaploids ranged from 54.7% (6 and 7) to 100% (between 6 and 47, 6 and 9, and 9 and 47).

Table 19. Mean meiotic chromosomal associations at metaphase I of AABBBB(SS) synthetic hexaploids (amphiploids) involving *T. turgidum* subsp. *turgidum* cultivars and B-genome diploid accessions of *Ae. speltoides* (I = univalent, rII = rod bivalent, oII = ring bivalent, III = trivalent, cIV = chain quadrivalent, oIV = ring quadrivalent, XTA = chiasmata).

Synthetic No.	No. cells	I	rII	oII	III	cIV	oIV	V	VI	XTA	Chromosome number
1	20	1.40	3.70	15.3	0.20	0.50	0.00	0.00	0.00	36.2	42
2	20	2.90	6.90	7.20	1.00	1.30	0.30	0.10	0.00	28.8	41
6	20	5.30	5.30	7.40	1.10	1.00	0.10	0.20	0.10	26.5	40
7	20	5.70	4.40	8.60	0.60	1.30	0.20	0.10	0.00	27.5	40
8	20	5.30	5.50	7.80	1.00	0.90	0.00	0.10	0.00	25.8	39
9	20	4.80	5.40	7.20	1.80	1.40	0.00	0.20	0.00	27.6	42
10	20	5.20	7.40	7.60	1.80	0.60	0.00	0.00	0.00	28.0	43
11	20	0.00	3.40	16.4	0.00	0.60	0.00	0.00	0.00	38.0	42
12	20	3.20	4.40	11.8	1.00	0.60	0.00	0.00	0.00	38.8	41
13	20	3.00	3.80	14.5	0.40	0.30	0.00	0.00	0.00	34.5	42
16	20	1.90	2.60	12.9	1.00	0.80	0.10	0.10	0.00	33.6	40
17	20	2.40	4.10	12.2	1.40	0.50	0.20	0.00	0.00	33.6	42
19	20	6.60	6.00	5.80	1.80	1.20	0.40	0.00	0.00	26.4	42
22	20	4.50	3.20	9.60	1.10	0.40	0.00	0.00	0.00	25.8	35
23	20	5.70	6.20	3.80	2.20	0.80	0.20	0.10	0.20	22.2	38
27	20	2.30	7.90	7.40	1.10	0.90	0.30	0.00	0.00	28.8	41
30	20	3.50	5.50	10.8	0.90	0.70	0.10	0.00	0.00	31.4	42
32	20	5.00	6.00	7.20	1.80	0.30	0.10	0.00	0.10	25.7	39
34	20	1.90	5.50	12.1	0.60	0.20	0.20	0.10	0.00	32.3	41
37	20	3.00	6.80	11.0	0.60	0.30	0.10	0.00	–	31.3	42
38	20	22.5	7.70	1.40	0.10	0.00	0.00	0.00	0.00	10.7	41
39	20	2.00	4.50	14.2	0.40	0.10	0.00	0.00	–	34.0	41
40	20	9.50	8.90	5.80	0.50	0.20	0.20	0.00	0.00	22.9	42
41	20	6.20	9.30	6.90	0.40	0.20	0.10	0.00	–	24.9	41
42	20	4.40	7.80	7.90	0.80	0.30	0.40	0.00	–	27.7	41
43	20	1.70	5.00	13.7	0.70	0.10	0.10	0.00	–	34.5	42
45	20	7.40	7.60	6.50	1.40	0.20	0.10	0.00	–	24.4	41
47	20	3.90	6.10	9.20	1.10	0.10	0.20	0.00	–	27.8	39
48	20	1.20	4.60	13.6	0.60	0.10	0.30	0.00	–	34.5	41
49	20	2.20	5.30	13.0	0.40	0.20	0.30	0.00	–	33.9	42
52	20	5.20	5.10	9.80	1.40	0.20	0.00	0.00	0.00	28.1	40

Dendrogram interpretation. The GD between genotypes was used to construct a dendrogram by UPGMA analysis for determining grouping of the lines on the basis of similarities and differences. The dendrogram of B-genome (Fig. 18, p. 118) has only one cluster with 7 as the most diverse line with 11, 18, 24, 36, and 48 as other good lines of this group. The genotypes 6, 9, and 47 are 100% similar.

Evaluation of genetic diversity using simple sequence repeat (SSR) primers. SSR primers were used for genetic diversity evaluation of B-genome synthetic hexaploids. All 275 SSR primers were applied on each set to detect genetic polymorphism at DNA level. The samples which did not amplify were not included in the analysis.

Efficiency of primers to amplify the genotypes ranged from 19 (*Xgwm257-2B*, *Xgwm319-2B*, and *Xgwm554-5B*) to one (*Xgwm11-1B*, *Xgwm148-2B*, *Xgwm540-5B*, and *Xgwm569-7B*) in B-genome synthetic hexaploids. Scorable bands ranged from one (*Xgwm11-1B*, *Xgwm148-2B*, and *Xgwm569-7B*) to 61 (*Xgwm213-5B*) (Table 23, pp. 121-122).

Population genetic analysis showed that the B-genome synthetic hexaploids scored total 327 alleles with 299 as polymorphic or 91.43% (Table 23, pp. 121-122). The range of scorable bands was 50–800 bp.

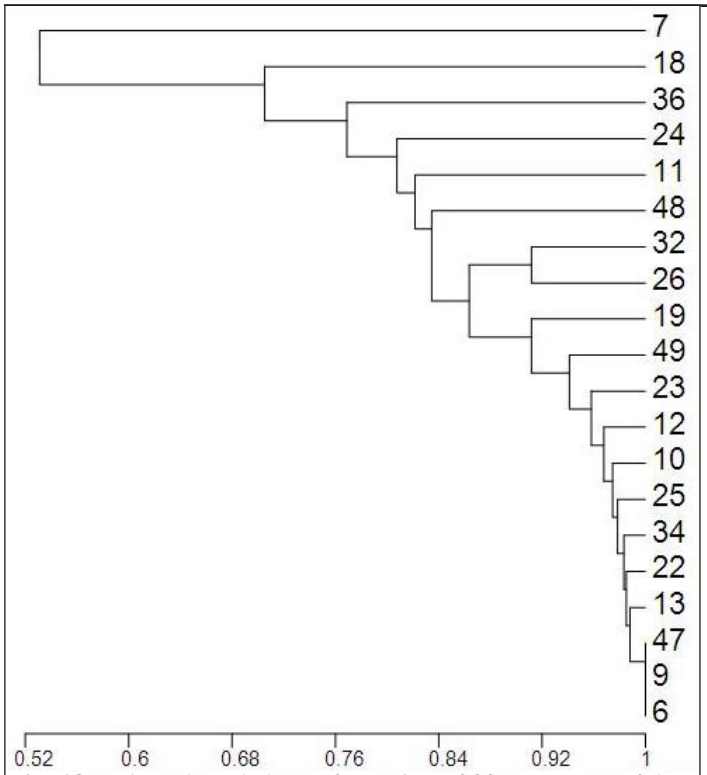


Fig. 18. RAPD-based cluster formation of 20 genotypes of the B-genome synthetic hexaploids (2n=6x=42; AABBSS).

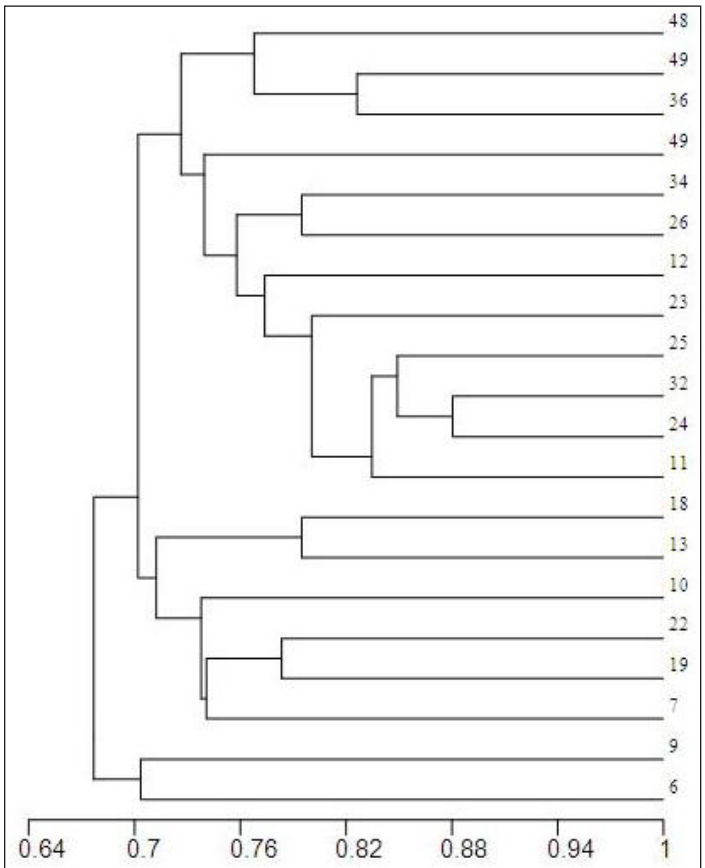


Fig. 19. SSR-based cluster formation of 20 genotypes of the B-genome synthetic hexaploids (2n=6x=42; AABBSS).

Table 20. The mean crossability data of some B-genome synthetic hexaploids (*T. turgidum* subsp. *turgidum*/*Ae. speltoides*).

Synthetic number	Florets pollinated	Seed set	Embryos rescued
22	48	14	14
23	24	15	15
24	48	14	14
25	48	7	7
26	37	20	20
27	48	20	19
28	48	17	15
29	24	16	15
30	96	33	20
31	76	14	13
32	48	20	20
33	48	10	9
34	48	20	20
35	52	2	2
36	48	5	4
37	48	20	18
38	24	21	19
39	48	27	20
40	48	20	18
41	72	21	20
42	48	20	20
43	48	20	18
44	24	20	15
45	48	23	20
46	96	16	10
47	48	26	20
48	48	12	12
49	24	11	11
50	72	3	2
51	24	21	20
52	120	47	20
53	24	11	5
54	48	20	17
55	48	11	10
56	48	4	3

Similarity matrix. A bivariate analysis was conducted to generate a similarity matrix and dendrogram using Nei and Li's coefficient to estimate genetic diversity. The similarity coefficient in B-genome synthetic hexaploids ranged from 58.2% (13 and 49) to 88.1% present between 24 and 32.

The B-genome diploids offer another reservoir of genetic diversity that can provide wheat breeders a unique source of variation for traits that limit productivity. These lines have been limited in practical use essentially because of chromosomal

behavior. The classic example has been of the *Ph* suppression activity, and the alien transfer that has been a standard in cytogenetic manipulation studies. For applied efforts focusing on *Ae. speltoides*, researchers in CIMMYT initiated an ambitious program to make AABBSS synthetics and generated over 50 lines. Of these, 20 were used to study phenology and powdery mildew screening resistance. Four lines appeared to be useful sources for further exploitation in breeding; entries 6, 9, 10, and 11 (Table 21). All possessed seedling resistance for powdery mildew. Some phenotypic attributes of thes four lines are

Table 21. Morphological and disease characterization of 20 B-genome synthetic hexaploids (2n=6x=42; AABBSS). FLOW = days-to-flowering, HT = plant height at maturity (cm), AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown), PMA = days to physiological maturity, TKW = 1,000-kernel weight (g), G/S = grains/spike, SL = spike length (cm), and Pm (S) = powdery mildew reaction at the seedling stage.

Synthetic number	FLOW	HT	AWN	PMA	TKW	G/S	SL	Pm (S)
6	113	72	Y	161	52.0	22	10.0	0
7	140	80	Y	160	40.0	40	12.5	8
9	113	75	Y	160	60.3	56	14.0	0
10	133	90	AW	164	62.7	28	15.0	0
11	112	85	AW	163	44.8	60	12.0	1
12	129	98	Y	164	18.0	5	13.0	0
13	133	93	Y	162	44.6	24	14.5	2
18	133	66	LB	166	60.0	30	13.0	1
19	139	78	AW	163	40.0	36	11.3	1
22	131	85	AW	165	40.0	34	12.6	6
23	130	89	Y	162	40.0	36	12.8	7
24	130	92	AW	164	50.0	6	9.3	6
25	145	74	AW	166	13.2	14	11.0	3
26	113	65	AW	161	44.8	42	8.5	8
32	146	90	Y	165	18.0	12	16.0	8
34	134	87	AW	160	22.4	1	13.0	8
36	141	68	Y	166	15.0	9	16.0	8
47	118	63	AW	161	28.6	46	8.0	0
48	134	77	LB	162	11.0	8	14.0	7
49	133	79	AW	164	19.5	27	18.5	7

Entry 6: days-to-flowering (113), 1,000-kernel weight (52.0 g),

Entry 9: days-to-flowering (113), 1,000-kernel weight (60.3 g), grains/spike (56), spike length (14 cm) (This was the best line in the set),

Entry 10: 1,000-kernel weight (62.7 g), spike length (15 cm), and

Entry 11: days-to-flowering (112), grains/spike (60).

The 20 synthetics (AABBSS) were tested for diversity using RAPDs (Fig. 18, p. 118) and SSRs (Fig. 19, p. 118). The maximum diversity via RAPD markers was for entry 7. Using SSR markers, entries 6 and 9 showed maximum diversity, were linked, and also have been selected for use in breeding based upon resistance plus phenotypic attributes. Entry 10 also showed good diversity and has other positive traits of interest.

The Mantel Test (Z) between RAPD and SSR similarity matrices for the population of B-genome synthetics indicates a RAPD-SSR matrix correlation (r) of –0.113 with a P value of 0.247. The Z value was used to compare the direction of diversity generated by both marker systems (RAPDs and SSRs). For this purpose, the similarity matrix for RAPDs and SSRs of each population was used. A negative diversity spectrum was shown by both markers in the population.

From the limited number of 20 B-genome synthetics studied, that four were superior for use in breeding is a fairly high frequency and encouraging. The meiotic behavior during maintenance of these stocks is a notch below that of the A-genome synthetics; more rod bivalents/aneuploidy is observed, but seed set is adequate. The trait value coupled with the molecular diversity status and a unique genetic resource sparsely used in breeding make this germ plasm important for further exploitation and additional stock production. Target practices can give precision to the efforts whereby screening of all accessions and actively pursuing the direct crossing protocols in use for the A- and D-genome diploids can be applied here also.

Table 22. Molecular fingerprinting by random amplified polymorphic DNA (RAPD) primers in B-genome synthetic hexaploids (2n=6x=42; AABBSS).

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)
1	OPA-3	8	8	100%	4	15	750–2,500
2	OPA-4	1	1	100%	1	1	1,000
3	OPB-1	8	5	62.5%	5	10	750–3,000
4	OPC-2	5	5	100%	5	7	750–2,000
5	OPE-9	2	2	100%	2	2	1,500
6	OPE-11	1	1	100%	1	1	1,000
7	OPE-14	4	2	50%	2	5	750–1,500
8	OPE-15	9	8	88.88%	5	21	500–2,500
9	OPE-16	7	6	85.71%	4	15	250–2,000
10	OPG-2	3	2	66.66%	2	4	750–1,500
11	OPG-5	8	8	100%	8	26	250–1,500
12	OPG-13	1	1	100%	2	2	1,500
13	OPI-7	9	7	77.77%	4	22	250–2,000
14	OPI-19	9	4	44.44%	4	17	250–2,000
15	OPJ-1	5	1	20%	1	5	500–1,500
16	OPJ-9	3	3	100%	4	7	750–1,500
17	OPJ-20	4	2	50%	6	21	250–1,000
18	OPK-9	2	2	100%	2	2	1,500–2,000
19	OPL-1	7	7	100%	4	13	500–1,500
20	OPL-2	9	9	100%	7	20	250–1,500
21	OPL-12	4	3	75%	6	15	750–1,000
22	OPL-20	8	5	62.5%	8	17	250–2,500
23	OPM17	8	8	100%	4	18	250–1,500
24	OPN-1	3	3	100%	2	3	1,000–2,000
25	OPN-2	7	7	100%	6	16	250–2,000
26	OPN-3	2	2	100%	3	4	1,500
27	OPN-4	6	4	66.66%	8	29	500–1,500
28	OPN-5	6	6	100%	7	17	250–1,500
29	OPN-6	7	7	100%	9	25	250–1,500
30	OPN-9	2	2	100%	2	3	250–1,500
31	OPN-12	4	1	25%	2	7	500–2,000
32	OPN-17	4	4	100%	3	9	500–1,000
33	OPP-16	3	3	100%	5	7	750–1,500
34	OPQ-5	4	4	100%	5	12	250–1,000
35	OPW-4	3	3	100%	3	6	750–2,000
36	OPW-5	1	1	100%	1	1	1,000
37	OPX-2	4	0	0%	1	4	750–1,500
38	OPX-12	4	4	100%	3	9	500–1,000
39	OPY-7	2	0	0%	2	4	1,000
40	OPY-8	3	0	0%	1	3	750-1500

Table 23. Molecular fingerprinting by simple sequence repeat (SSR) primers in the B-genome synthetic hexaploids (2n=6x=42; AABBSS).

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)	PIC
1	*Xgwm11-1B*	1	1	100%	1	1	50	0.00
2	*Xgwm18-1B*	4	4	100%	9	13	50–200	0.43
3	*Xgwm33-1B*	1	1	100%	7	7	50	0.00
4	*Xgwm124-1B*	2	2	100%	6	7	50–200	0.24
5	*Xgwm131-1B*	2	2	100%	3	4	150	0.27
6	*Xgwm140-1B*	1	1	100%	4	4	50	0.00
7	*Xgwm268-1B*	2	2	100%	9	12	100	0.65
8	*Xgwm273-1B*	4	2	50%	7	13	150–700	0.27
9	*Xgwm274-1B*	4	4	100%	8	16	150–250	0.66
10	*Xgwm403-1B*	2	0	0%	2	4	150–200	0.50
11	*Xgwm413-1B*	3	3	100%	8	10	200	0.47
12	*Xgwm550-1B*	4	2	50%	6	12	150–700	0.63
13	*Xgwm47-2B*	3	1	33.33%	6	8	50–200	0.25
14	*Xgwm16-2B*	5	3	60%	11	25	50–250	0.72
15	*Xgwm55.1-2B*	6	6	100%	5	13	50–150	0.81
16	*Xgwm55.2-2B*	3	3	100%	3	6	50–150	0.61
17	*Xgwm120-2B*	2	2	100%	7	11	50–150	0.40
18	*Xgwm129-2B*	1	1	100%	4	4	200	0.00
19	*Xgwm148-2B*	1	1	100%	1	1	150	0.00
20	*Xgwm191-2B*	2	2	100%	8	12	100	0.48
21	*Xgwm210-2B*	4	2	50%	8	11	150	0.31
22	*Xgwm257-2B*	8	5	62.5%	19	27	200–800	0.44
23	*Xgwm319-2B*	3	3	100%	19	33	50–250	0.58
24	*Xgwm374-2B*	4	4	100%	14	16	50–200	0.56
25	*Xgwm382-2B*	5	5	100%	12	33	50–200	0.75
26	*Xgwm388-2B*	3	1	33.33%	9	28	100–150	0.66
27	*Xgwm410-2B*	2	2	100%	5	6	50–250	0.61
28	*Xgwm429-2B*	4	4	100%	10	21	50–300	0.67
29	*Xgwm501-2B*	3	3	100%	8	10	50	0.38
30	*Xgwm526-2B*	5	3	60%	15	23	50–200	0.45
31	*Xgwm630-2B*	4	4	100%	17	39	50–200	0.66
32	*Xgwm72-3B*	2	2	100%	8	10	50–200	0.46
33	*Xgwm77-3B*	6	6	100%	10	23	50–250	0.78
34	*Xgwm112-3B*	3	3	100%	14	28	50–100	0.58
35	*Xgwm264-3B*	3	1	33.33%	3	5	150–200	0.66
36	*Xgwm285-3B*	2	2	100%	8	9	500	0.30
37	*Xgwm340-3B*	2	2	100%	13	22	100–200	0.45
38	*Xgwm376-3B*	3	3	100%	14	16	50–150	0.13
39	*Xgwm389-3B*	1	1	100%	6	6	100	0.00
40	*Xgwm493-3B*	5	5	100%	16	31	50–700	0.69
41	*Xgwm547-3B*	2	2	100%	3	4	50–200	0.27
42	*Xgwm566-3B*	2	2	100%	12	12	50–100	0.23
43	*Xgwm6-4B*	5	5	100%	15	47	50–1500	0.71
44	*Xgwm66-4B*	11	9	81.81%	14	34	50–600	0.78
45	*Xgwm113-4B*	2	2	100%	16	27	50–150	0.49
46	*Xgwm149-4B*	2	2	100%	15	25	50–150	0.48

Table 23. Molecular fingerprinting by simple sequence repeat (SSR) primers in the B-genome synthetic hexaploids (2n=6x=42; AABBSS).

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)	PIC
47	*Xgwm165-4B*	6	1	16.66%	10	29	50–250	0.71
48	*Xgwm368-4B*	5	5	100%	10	17	50	0.48
49	*Xgwm495-4B*	1	1	100%	2	2	50–200	0.00
50	*Xgwm513-4B*	3	3	100%	8	13	50–150	0.57
51	*Xgwm66-5B*	14	14	100%	8	50	50–700	0.91
52	*Xgwm67-5B*	4	4	100%	7	17	50–100	0.72
53	*Xgwm68-5B*	1	1	100%	12	22	50–250	0.56
54	*Xgwm159-5B*	5	5	100%	18	25	200–250	0.37
55	*Xgwm191-5B*	2	2	100%	15	25	150–250	0.37
56	*Xgwm213-5B*	15	15	100%	15	61	50–500	0.87
57	*Xgwm234-5B*	3	3	100%	12	22	50–250	0.64
58	*Xgwm335-5B*	5	5	100%	15	29	50–250	0.74
59	*Xgwm371-5B*	4	4	100%	15	34	50–200	0.70
60	*Xgwm408-5B*	5	5	100%	12	17	50–200	0.45
61	*Xgwm443-5B*	7	7	100%	13	25	50–150	0.76
62	*Xgwm499-5B*	7	7	100%	10	19	50–200	0.80
63	*Xgwm540-5B*	1	1	100%	1	8	100	0.32
64	*Xgwm544-5B*	2	2	100%	13	14	150	0.19
65	*Xgwm554-5B*	3	3	100%	19	30	150	0.46
66	*Xgwm604-5B*	3	3	100%	15	25	50–100	0.53
67	*Xgwm639-5B*	6	6	100%	10	28	50–200	0.69
68	*Xgwm70-6B*	3	3	100%	6	14	100–200	0.63
69	*Xgwm88-6B*	7	7	100%	7	16	50–500	0.77
70	*Xgwm191-6B*	3	3	100%	8	9	50	0.57
71	*Xgwm193-6B*	5	5	100%	13	29	150	0.59
72	*Xgwm219-6B*	3	3	100%	15	24	50–200	0.60
73	*Xgwm361-6B*	4	4	100%	13	31	100	0.74
74	*Xgwm508-6B*	5	5	100%	3	7	100–150	0.75
75	*Xgwm518-6B*	5	5	100%	7	16	50–200	0.17
76	*Xgwm16-7B*	2	2	100%	14	19	50–200	0.31
77	*Xgwm43-7B*	2	2	100%	12	13	50–250	0.44
78	*Xgwm68-7B*	2	2	100%	16	29	100–200	0.48
79	*Xgwm146-7B*	5	5	100%	16	30	50	0.61
80	*Xgwm274-7B*	2	2	100%	4	6	100	0.37
81	*Xgwm297-7B*	2	2	100%	16	17	50	0.05
82	*Xgwm302-7B*	5	5	100%	17	31	250	0.55
83	*Xgwm333-7B*	2	2	100%	4	5	50	0.21
84	*Xgwm344-7B*	2	2	100%	2	2	150	0.50
85	*Xgwm400-7B*	1	1	100%	13	13	150–200	0.16
86	*Xgwm537-7B*	7	7	100%	15	31	50–200	0.72
87	*Xgwm569-7B*	1	1	100%	1	1	150	0.00
88	*Xgwm573-7B*	3	3	100%	8	10	250	0.32
89	*Xgwm644-7B*	5	5	100%	16	18	100–150	0.57

Molecular and phenological study and disease screening of various Aegilops tauschii accessions in a similar durum wheat background.

Alvina Gul Kazi, Sadia Latif, Bilal Haider Abbasi, Awais Rasheed, Hadi Bux, Arsalan Ahmed, and Abdul Mujeeb-Kazi.

Diseased and insect pest resistance are the most readily exploited characters in wide hybridization. This also is true for synthetic hexaploid (SH) wheats, which have many genes for resistance to the three major rusts introgressed into bread wheat. A wide array of these wheats are being globally utilized for wheat improvement either at the SH or at the 'bread wheat/SH' advanced derivative level. The SH wheats built around the D genome are known to carry a good level of resistance to Karnal bunt, *S. tritici,* and *C. sativus*. The promise also exists for resistance and tolerance in this SH germ plasm for resistance to leaf rust, stripe rust, powdery mildew, loose smut, and cereal cyst nematode; mineral toxicities; drought; salinity; heat; cold; sprouting; water logging; high-molecular-weight (HMW)/low-molecular-weight (LMW) quality subunits; and yield and yield components. The least diversity observed so far in the D genome is for *F. graminearum* (less than 1.0 percent) but, under evaluation tests conducted at one location in Mexico, the observed FHB resistance is promising and superior than that of the leading bread wheat cultivars Frontana and Sumai 3 with their assemblage of four genes.

From the primary synthetics, an experimental set was made and categorized as having same durum cultivar as the female parent crossed with different *Ae. tauschii* accessions (89 entries, Table 24; Table 25, pp. 124-125; and Table 26, p. 124). This subset was designed to study the inheritance of different genes and also to identify the effect of cytoplasmic inheritance, if any. The 89 entries were screened against two biotic stresses (Karnal bunt and stripe rust), phenotypically characterized, and analyzed with RAPD and SSR markers.

Table 24. Synthetic entries numbered from combining same durum cultivars and different *Aegilops tauschii* accessions. Synthetic hexaploid (SH) numbers are similar to those maintained in the CIMMYT, Mexico, Wide Crosses Program with the pedigrees of the durum wheat cultivars detailed in Table 25.

Group number	D-genome synthetic hexaploid number	Durum parent nuber	Total number of SH entries
1	1, 18, 30, 63, 66, 78, 83, 89	4	8
2	2, 3, 14, 19, 26, 43, 49, 51, 65	8	9
3	4, 11, 20, 35, 37, 38, 42, 47, 64	13	9
4	5, 8, 9, 10, 12, 21, 23, 27, 58	12	9
5	6, 15, 22, 31, 45, 50, 56, 74, 75	23	9
6	7, 32, 48, 53, 68, 71, 72, 76, 86	26	9
7	13, 16, 17, 25, 36, 46, 57, 61, 88	5	9
8	24, 39, 40, 41, 60, 62, 73, 85, 90	1	9
9	28, 29, 33, 34, 55, 59, 69, 79, 82	11	9
10	44, 54, 67, 70, 77, 80, 81, 84, 87	22	9

Stripe rust studies. Seedling screening showed that 34 of 90 (37.7%) synthetics and 22 of 23 (95.6%) durum wheat parents were resistant (Table 27, pp. 126-127). These genotypes also were screened for APR under field conditions at NARC, which identified 37 of 89 (41.4%) synthetics and 20 of 23 (86.9%) durum wheat parents as resistant. Genotypes with both seedling resistance and APR were 19 of 90 (21.1%) synthetics (1, 13, 14, 17, 19, 20, 34, 37, 38, 42, 62, 63, 67, 72, 74, 80, 81, 87, and 89) and 19 of 23 (82.6%) durum wheat parents (1, 2, 4, 5, 8, 9, 11, 12, 13, 14, 17, 20, 21, 22, 26, 27, 28, 37, and 40). All this germ plasm represents the presence of major genes against stripe rust and can be exploited further in breeding programs.

Adult-plant resistance involving susceptibility at seedling stage and resistance only at the adult-plant stage is an indicator of minor genes, which are considered of great importance in acquiring durable resistance. Eighteen of 90 (20%) synthetics (2, 3, 5, 7, 11, 15, 22, 23, 31, 33, 53, 55, 58, 71, 73, 82, 83, and 84) and one of 23 (4.35%) durum wheat parents (45) had APR and are good candidates for providing durable resistance to wheat cultivars.

Karnal bunt (KB) studies. The KB evaluation was done by examining the grains following artificial inoculation. Grains from each entry were examined separately after hand threshing. The rating scale was from 0 to 5 (see Fig. 2, p. 86). Only a rating of 0 was considered acceptable and all others, from 1 to 5, as susceptible. In the first experiment, 30 of 90 entries (33.3%), including 4, 9, 10, 11, 13, 14, 16, 17, 29, 31, 32, 39, 40, 42, 45, 47, 49, 53, 59, 66, 67, 68, 81, 82, 83, 84, 86, 87, 89, and 90 were completely immune (Table 27, pp. 126-127).

Table 25. Pedigree/Parentage of the *Ae. tauschii* synthetic germ plasm used in this study.

Number	Pedigree
1	ALTAR 84/*Ae. tauschii* (191)
2	68.111/RGB-U//WARD/3/*Ae. tauschii* (328)
3	68.111/RGB-U//WARD/3/*Ae. tauschii* (321)
4	CETA/*Ae. tauschii* (540)
5	D67.2/P66.270/*Ae. tauschii* (213)
6	GARZA/BOY//*Ae. tauschii* (286)
7	GAN/*Ae. tauschii* (268)
8	D67.2/P66.270//*Ae. tauschii* (220)
9	D67.2/P66.270//*Ae. tauschii* (222)
10	D67.2/P66.270//*Ae. tauschii* (308)
11	CETA/*Ae. tauschii* (1016)
12	D67.2/P66.270//*Ae. tauschii* (221)
13	DVERD_2/*Ae. tauschii* (1027)
14	68.111/RGB-U//WARD/3/*Ae. tauschii* (329)
15	GARZA/ BOY//*Ae. tauschii* (467)
16	DVERD_2/*Ae. tauschii* (221)
17	DVERD_2/*Ae. tauschii* (214)
18	ALTAR 84/*Ae. tauschii* (220)
19	68.111/RGB-U//WARD/3/*Ae. tauschii* (452)
20	CETA/*Ae. tauschii* (327)
21	D67.2/P66.270//*Ae. tauschii* (633)
22	GARZA/BOY//*Ae. tauschii* (276)
23	D67.2/P66.270//*Ae. tauschii* (218)
24	CROC_1/*Ae. tauschii* (205)
25	DVERD_2/*Ae. tauschii* (295)
26	68.111/RGB-U//WARD/3/*Ae. tauschii* (463)
27	D67.2/P66.270//*Ae. tauschii* (257)
28	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (215)
29	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (223)
30	ALTAR 84/*Ae. tauschii* (333)
31	GARZA/ BOY//*Ae. tauschii* (265)
32	GAN/*Ae. tauschii* (182)
33	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. tauschii* (273)
34	CPI/GEDIZ/3/GOO// JO/CRA/4/*Ae. tauschii* (296)
35	CETA/*Ae. tauschii* (661)
36	DVERD_2/*Ae. tauschii* (402)
37	CETA/*Ae. tauschii* (174)
38	CETA/*Ae. tauschii* (1024)
39	CROC_1/*Ae. tauschii* (886)
40	CROC_1/*Ae. tauschii* (444)
41	CROC_1/*Ae. tauschii* (518)
42	CETA/*Ae. tauschii* (256)
43	68.111/RGB-U//WARD/3/*Ae. tauschii* (325)
44	DOY 1/*Ae. tauschii* (188)
45	GARZA/BOY//*Ae. tauschii* (307)

Table 26. Pedigrees of durum wheat parents in the *Ae. tauschii* synthetic germ plasm.

Number	Pedigree
D-1	CROC_1
D-4	ALTAR84
D-5	DVERD_2
D-8	68.111/RGB-U//WARD
D-11	CPI/GEDIZ/3/GOO//JO/CRA
D-12	D67.2/P66.270
D-13	CERCETA
D-22	DECOY1
D-23	GARZA/BOY
D-26	GAN

Molecular studies. ***Genetic diversity evaluation using random amplified polymorphic DNA (RAPD) primers.*** RAPD primers were used for genetic diversity evaluation of A-, B-, and D-genome synthetic hexaploids. All 520 RAPD primers of the Operon Series were screened and working primers were identified and applied to detect genetic polymorphism at DNA level. The samples which did not amplify were not included in the analysis.

Genetic analysis was performed only on the scorable bands. Every single band was considered as a single locus/allele. The loci were scored as present/absent. Bivariate data 1–0 were used to estimate genetic distances (GD). Unweighted Pair Group of Arithmetic Means (UPGMA) function estimated genetic distances between the genotypes as follows: $GD_{xy} = 1 - d_{xy}/d_x + d_y - d_{xy}$, where GD_{xy} = genetic distance between two genotypes, d_{xy} = total number of common loci (bands) in two genotypes, d_x = total number of loci (bands) in genotype 1, and d_y = total number of loci (bands) in genotype 2.

The efficiency of primers to amplify the genotypes ranged from a maximum of 36 genotypes (OPE-16) to four genotypes (OPE-6) in this experiment (Table 28, p. 128). The scorable bands ranged from six (OPE-6) to 86 (OPE-12) (Table 28, p. 128). The total number of loci was 197 with 186 polymorphic showing a percentage of 94.41% (Table 28, p. 128) and the range of scorable bands was from 250–3,000 bp.

Similarity matrix. A bivariate analysis was conducted to generate a similarity matrix and dendrogram using Nei and Li's coefficient to estimate genetic diversity. The value of the similarity matrix ranged from 73.9% (minimum) between entries 8 and 66 to 100% (maximum) between genotypes 48 and 18, 6 and 18, 2 and 18, 6 and 48, 2 and 6, and 2 and 48.

Dendrogram interpretation. The GD between genotypes were used to construct a dendrogram by UPGMA analysis for determining grouping of the lines on the basis of similarities and differences. Only one main cluster is in the dendrogram with two subclusters, A and B (Fig. 20, p. 129). In subcluster-A, 2, 6, 18, and 40 show 100% similarity and 8 is the most diverse line overall, followed by 13, 35, 36, 63, 65, and 87. The total number of genotypes in this cluster is 96. The B subcluster has only three genotypes in which D-4 represents the most diverse line.

Genetic diversity evaluation using simple sequence repeat (SSR) primers. SSR primers were used for genetic diversity evaluation of D-genome synthetic hexaploids. All 275 SSR primers were used to detect genetic polymorphism at the DNA level. Samples that did not amplify were not included in the analysis.

Genetic analysis was performed similar to that for the RAPD primers. The efficiency of primers to amplify the genotypes ranged from maximum 39 genotypes (*Xgwm129-5A*) to two (*Xgwm68-5B* and *Xgwm284-3B*) in this experiment (Table 29, p. 130). The scorable bands ranged from two (*Xgwm68-5B*) to 66 (*Xgwm129-5A*) in this experiment (Table 29, p. 130).

Genetic analysis of the population showed that the total number of alleles was 191, with 185 polymorphic showing a percentage of 96.85% (Table 29, p. 130). The range of scorable bands was 50–600 bp in this experiment.

Similarity matrix. The bivariate analysis was conducted to generate a similarity matrix and dendrogram using similar to that for the RAPD primers. The value of similarity matrix ranged from 75.5% (minimum) between 2 and 90 ans was 100% (maximum) in 33 different combinations.

Dendrogram interpretation. In this experiment, only one main cluster with two subclusters A and B; A has 38 and B has 61 genotypes (Fig. 21, p. 131). Subcluster A carries the most diverse line of the group, 27. Other highly diverse lines in this subcluster include 14, 54, and 61. In subcluster B, genotype 1 is the most diverse line and 4, 5, and 41 are other good examples.

The same durum wheats and different *Ae. tauschii* accessions. Other researchers have recognized that the interaction of the A and B genomes of durum wheat with the D genome of *Ae. tauschii* plays a significant role in gene expression and suppression for the traits under study. To delineate the genomic effects using same durum wheat cultivars with diverse D-genome accessions, we identified ten sets to study; 10 durum

Table 25. Pedigree/Parentage of the *Ae. tauschii* synthetic germ plasm used in this study.

Number	Pedigree
46	DVERD_2/*Ae. tauschii* (1022)
47	CETA/*Ae. tauschii* (796)
48	GAN/*Ae. tauschii* (236)
49	68.111/RGB-U//WARD/3/*Ae. tauschii* (326)
50	GARZA/BOY//*Ae. tauschii* (270)
51	68.111/RGB-U//WARD/3/*Ae. tauschii* (316)
52	ALTAR 84/*Ae. tauschii* (332)
53	GAN/*Ae. tauschii* (180)
54	DOY 1/*Ae. tauschii* (255)
55	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. tauschii* (453)
56	GARZA/BOY//*Ae. tauschii* (278)
57	DVERD_2/*Ae. tauschii* (333)
58	D67.2/P66.270//*Ae. tauschii* (217)
59	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (193)
60	CROC_1/*Ae. tauschii* (170)
61	DVERD_2/*Ae. tauschii* (1031)
62	CROC_1/*Ae. tauschii* (213)
63	ALTAR 84/*Ae. tauschii* (304)
64	CETA/*Ae. tauschii* (235)
65	68.111/RGB-U//WARD/3/*Ae. tauschii* (322)
66	ALTAR 84/*Ae. tauschii* (507)
67	DOY 1/*Ae. tauschii* (510)
68	GAN/*Ae. tauschii* (163)
69	CPI/GEDIZ/3/GOO// JO69/CRA/4/*Ae. tauschii* (633)
70	DOY 1/*Ae. tauschii* (349)
71	GAN/*Ae. tauschii* (408)
72	GAN/*Ae. tauschii* (201)
73	CROC_1/*Ae. tauschii* (333)
74	GARZA/BOY//*Ae. tauschii* (439)
75	GARZA/BOY//*Ae. tauschii* (350)
76	GAN/*Ae. tauschii* (285)
77	DOY 1/*Ae. tauschii* (333)
78	ALTAR 84/*Ae. tauschii* (219)
79	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (208)
80	DOY 1/*Ae. tauschii* (1030)
81	DOY 1/*Ae. tauschii* (515)
82	CPI/GEDIZ/3/GOO// JO69/CRA/4/*Ae. tauschii* (637)
83	ALTAR 84/*Ae. tauschii* (502)
84	DOY 1/*Ae. tauschii* (517)
85	CROC_1/*Ae. tauschii* (224)
86	GAN/*Ae. tauschii* (890)
87	DOY 1/*Ae. tauschii* (458)
88	DVERD_2/*Ae. tauschii* (1029)
89	ALTAR 84/*Ae. tauschii* (211)
90	CROC_1/*Ae. tauschii* (879)

Table 27. Phenotypic and disease characterization of D-genome synthetic hexaploids. FLOW = days-to-flowering, HT = plant height at maturity (cm), AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown), PMA = days to physiological maturity, TKW = 1,000-kernel weight (g), G/S = grains/spike, SL = spike length (cm), KB = Karnal bunt reaction (– = immune, + = susceptible), Yr (S) = reaction to stripe rust at the seedling stage, Yr (A) = reaction to stripe rust at the adult–plant stage (R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlapping of MR–MS types, MSS = moderately susceptible to susceptible, S = susceptible, and TS = trace susceptible).

Entry	FLOW	HT	AWN	PMA	TKW	G/S	SL	KB	Yr (S)	Yr (A)
1	66	73	LB	105	44.1	13	9.5	+	1	10MRR
2	60	105	LB	99	33.5	19	12.0	+	89	TR
3	66	77	LB	105	38.2	15	10.5	+	89	TR
4	60	90	LB	99	36.6	12	9.0	–	8	70S
5	66	113	LB	110	32.0	13	8.5	+	67	30MR
6	70	103	LB	109	29.7	19	12.0	+	8	70S
7	73	91	LB	112	39.2	18	12.0	+	78	10R
8	67	118	LB	107	32.2	14	10.5	+	8	90S
9	70	121	LB	109	35.4	14	12.0	–	8	90S
10	65	98	LB	105	31.0	13	9.0	–	89	70S
11	65	104	LB	105	27.0	13	9.5	–	89	0
12	70	104	LB	109	31.0	14	10.0	+	78	70S
13	66	96	LB	106	34.0	14	10.1	–	1	10R
14	70	120	LB	109	52.9	15	10.3	–	1	0
15	149	108	LB	179	28.8	17	9.0	+	56	10MR
16	71	102	LB	110	33.0	16	11.5	–	56	70S
17	70	110	LB	109	49.9	14	10.0	–	12	5R
18	70	103	LB	109	27.9	12	8.0	+	56	70S
19	70	86	LB	109	32.0	16	12.0	+	1	0
20	70	102	LB	108	49.8	15	11.5	+	1	0
21	70	115	LB	109	24.0	13	9.0	+	78	90S
22	70	90	LB	110	25.0	13	10.0	+	9	TR
23	75	90	LB	113	26.2	14	8.5	+	8	10R
24	74	88	LB	112	27.0	15	13.0	+	56	30MSS
25	69	90	LB	107	23.7	13	10.5	+	78	30MSS
26	73	90	LB	112	28.5	14	10.5	+	78	30MSS
27	141	117	LB	168	33.5	8	11.0	+	89	70S
28	66	73	LB	105	25.4	21	14.0	+	78	90S
29	92	103	LB	105	41.0	14	9.5	–	0	30MSS
30	70	82	LB	108	37.5	16	10.5	+	23	70S
31	73	75	LB	112	26.0	13	11.0	–	8	TR
32	62	100	LB	101	32.5	12	7.0	–	8	90S
33	59	100	LB	97	34.6	14	13.0	+	8	0
34	60	110	LB	100	35.5	13	13.0	+	0	0
35	62	110	LB	101	27.3	12	9.5	+	8	90S
36	65	90	LB	106	35.2	13	10.0	+	0	30MSS
37	146	90	LB	178	25.0	22	9.0	+	0	10MR
38	126	135	LB	175	30.0	20	13.0	+	0	10MRR
39	77	93	LB	113	37.0	13	10.0	–	8	30MSS
40	77	90	LB	113	35.2	13	10.5	–	7	40MS
41	68	83	LB	106	55.7	14	10.0	+	67	40MS
42	62	80	LB	101	46.2	12	8.5	–	0	10R
43	72	99	LB	109	49.8	14	8.5	+	78	10MS
44	65	109	LB	105	59.2	15	10.0	+	1	30MSS
45	64	85	LB	103	30.5	13	9.0	–	9	30MS
46	70	97	LB	106	44.0	14	9.5	+	9	70S

Table 27. Phenotypic and disease characterization of D-genome synthetic hexaploids**.** FLOW = days-to-flowering, HT = plant height at maturity (cm), AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown), PMA = days to physiological maturity, TKW = 1,000-kernel weight (g), G/S = grains/spike, SL = spike length (cm), KB = Karnal bunt reaction (– = immune, + = susceptible), Yr (S) = reaction to stripe rust at the seedling stage, Yr (A) = reaction to stripe rust at the adult–plant stage (R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlapping of MR–MS types, MSS = moderately susceptible to susceptible, S = susceptible, and TS = trace susceptible).

Entry	FLOW	HT	AWN	PMA	TKW	G/S	SL	KB	Yr (S)	Yr (A)
47	79	69	LB	114	31.0	14	10	–	78	90S
48	72	97	LB	109	34.6	15	11	+	78	90S
49	70	89	LB	109	35.7	12	7.5	–	3	90S
50	72	76	LB	109	39.2	13	11	+	89	30MSS
51	68	102	LB	106	45.0	12	9.5	+	1	70S
53	68	100	LB	106	37.2	14	9.5	–	67	10R
54	70	100	LB	110	37.0	12	8	+	0	90S
55	140	153	DB	182	40.0	13	12	+	78	10R
56	72	80	LB	111	43.2	15	10	+	9	90S
57	141	104	LB	177	40.0	15	12	+	56	90S
58	71	75	LB	110	31.0	15	13	+	78	10R
59	70	113	LB	108	32.5	19	12	–	1	30MRMS
60	65	97	LB	105	53.4	18	10	+	78	30MRMS
61	129	113	LB	177	40.0	19	10	+	89	90S
62	72	96	LB	112	39.8	20	11	+	1	10R
63	79	62	LB	116	37.9	12	7	+	1	10R
64	73	100	LB	109	35.5	14	10	+	89	30MSS
65	77	109	LB	115	32.2	15	12	+	1	90S
66	75	120	LB	113	35.2	16	12	–	78	90S
67	146	120	LB	172	41.0	30	13	–	0	0
68	68	113	LB	106	41.0	15	11	–	89	70S
69	63	104	LB	101	40.0	12	9	+	0	70S
70	149	100	LB	181	40.0	14	12	+	1	90S
71	66	100	LB	106	36.6	15	13	+	78	10R
72	140	95	LB	121	35.0	15	10	+	0	10R
73	142	72	LB	181	35.0	20	10	+	78	10R
74	144	128	LB	176	30.0	2	11	+	0	10R
75	150	97	LB	180	16.0	8	10	+	0	70S
76	141	102	LB	161	33.0	37	13	+	67	70S
77	133	114	LB	178	37.0	26	12	+	78	90S
78	136	99	LB	172	31.0	7	11	+	0	70S
79	74	119	LB	112	28.7	14	10	+	0	70S
80	139	110	LB	182	30.0	15	10	+	0	10MR
81	78	100	LB	116	35.9	15	11	–	0	0
82	72	109	LB	109	36.6	14	10	–	78	0
83	73	103	LB	110	26.1	12	9	–	45	10R
84	143	119	LB	175	40.0	12	13	–	45	0
85	74	112	LB	112	52.3	12	10	+	9	70MSS
86	67	86	LB	105	41.0	13	10	–	89	70MSS
87	76	72	LB	113	31.1	12	10	–	0	0
88	144	184	LB	184	30.0	13	10	+	89	90S
89	75	117	LB	112	27.3	14	10	–	0	10R
90	74	103	LB	112	32.1	16	13	–	78	90S

Table 28. Molecular fingerprinting pattern by RAPD primers in the D–genome synthetic hexaploids.

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)
1	OPA–3	12	12	100%	14	23	1,000–3,000
2	OPA-4	9	7	77.77%	5	21	750–2,500
3	OPA-13	5	5	100%	31	63	500–1,500
4	OPA-18	10	10	100%	7	26	1,000–2,000
5	OPB-1	9	9	100%	24	62	750–1,500
6	OPB-4	9	9	100%	15	35	500–2,500
7	OPB-5	7	7	100%	18	33	750–3,000
8	OPB-6	13	13	100%	15	59	500–3,000
9	OPB-12	7	5	71.42%	7	17	750–1,000
10	OPB-17	9	9	100%	10	33	50–1,500
11	OPC-5	8	8	100%	13	34	750–2,000
12	OPC-10	7	7	100%	6	14	500–2,000
13	OPC-15	9	9	100%	25	81	250–1,500
14	OPD-2	10	10	100%	13	46	750–2,500
15	OPD-5	8	5	62.5%	32	64	500–3,000
16	OPD-7	11	11	100%	14	41	250–2,000
17	OPD-13	5	3	100%	5	11	500–1,000
18	OPE-4	6	6	100%	11	23	500–1,500
19	OPE-6	5	3	60%	4	6	1,500
20	OPE-7	10	10	100%	35	76	500–2,000
21	OPE-12	12	12	100%	20	86	500–25,00
22	OPE-15	8	8	100%	6	22	1,000–2,500
23	OPE-16	8	8	100%	36	83	750–2,500
24	OPE-18	4	4	100%	11	15	250–1,500
25	OPE-19	2	2	100%	9	10	250–2,000

wheats with various *Ae. tauschii* accessions. The first durum wheat cultivar (Table 25, pp. 124-125), Altar 84, has a combination with eight D-genome accessions. Focussing on the major characters that play a key role in breeding aspects, plant height at maturity was 73–120 cm, days to physiological maturity was 105–172 days, and 1,000-kernel weight was 26.1–44.1 g (Table 27, pp. 126-127). Biotic stress data indicted susceptibility to Karnal bunt in all except in two entries (83 and 89). Stripe rust resistance (seedling and adult) was present in entries 63 and 89. The durum parent Altar 84 was immune to KB and also possessed stripe rust resistance. The observations across these parameters where eight different D-genome accessions were involved show a significant performance variation based upon the expression of the genomes influenced by accessions. The trend seen elucidates why appropriate synthetic entries should be selected in breeding, because trait masking across genomes is a common phenomenon and, hence, an extended analytical focus is helpful. Variable expression trends can be seen with other durum wheat cultivars and strengtens the view that accession diversity can be used to target the right synthetic for wheat improvement.

Wee selected the following synthetics to use: 27, 34, 44, 67 and 76 (Table 27, pp. 126-127). Entry 67 has both stripe rust resistance (seeding and adult) and also KB; hence the best line. The data in Table 27 (pp. 126-127) will identify accessions to be used for direct crossing, where exchanges will be restricted to only the D genome. Because the durum wheat parent Altar 84 was immune to KB and resistant to strip rust, varied results upon crossing with *Ae. tauschii* accessions indicated that the accessions were overriding the durum cultivars resistance. Hence, only selection of those accessions in which the corresponding synthetic showed KB and strip rust resistance would be ideal for use in direct crossing because their influence on the A and B genomes of bread wheat would, hopefully, not be penalizing. If the bread wheat is susceptible to KB and stripe rust, selecting resistant derivatives would unequivocally demonstrate that the *Ae. tauschii* carried the desired genes. This trend also is well expressed in other groups where other durum cultivars and *Ae. tauschii* accessions are used (Table 27, pp. 126-127).

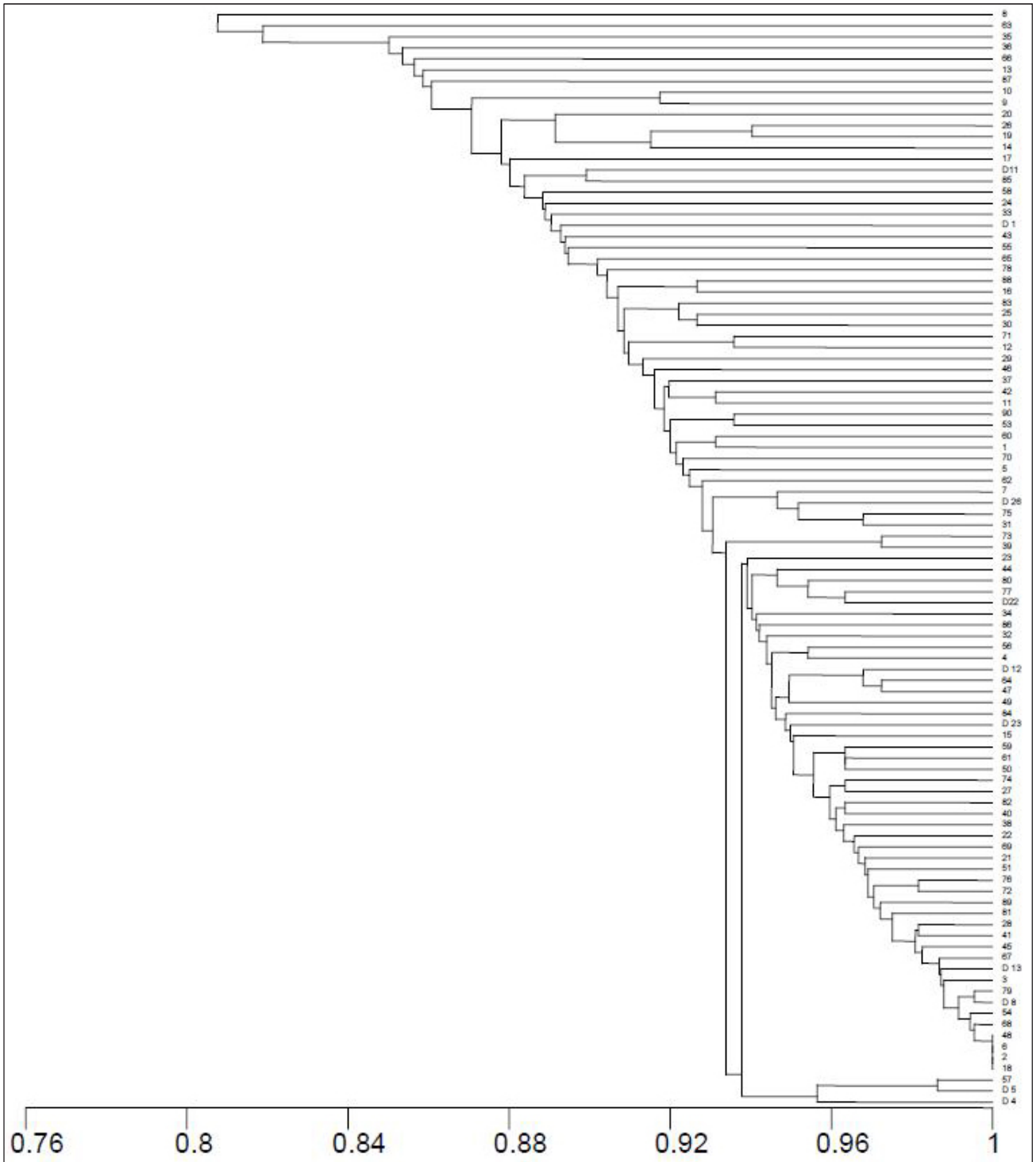


Fig. 20. A RAPD-based cluster formation of genotypes of various *Aegilops tauschii* accessions in a similar durum wheat background.

The synthetic combinations from group 1 involving Altar 84 and eight *Ae. tauschii* accessions showed diversity in molecular analysis also. Using RAPDs, 63 was most diverse line and next was 66. Using SSRs, entries 1, 63, 78, and 83 grouped together; next were entries 13 and 18, with entry 89 being the most diverse. Incorporating the stress resistance data, entries 63 and 89 possessed both seedling and adult-plant resistance, and entry 89 had KB resistance. Hence entry 89 possesses ideal KB and stripe rust resistance and also is the most diverse line based upon SSR-based polymorphism. This strategy enables tge integration of various components for adding efficiency to a breeding program.

Table 29. Molecular fingerprinting pattern by SSR primers in the D-genome synthetic hexaploids (experiment 1, same durum with different *Ae. tauschii* accessions).

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)	PIC
1	*Xgwm99-1A*	4	4	100%	15	19	50–150	0.34
2	*Xgwm666-1A*	3	3	100%	8	8	100–150	0.53
3	*Xgwm249-2A*	4	4	100%	7	13	50–150	0.64
4	*Xgwm294-2A*	4	4	100%	10	10	50–100	0.70
5	*Xgwm558-2A*	5	5	100%	11	12	50–200	0.76
6	*Xgwm614-2A*	4	4	100%	13	20	50–200	0.67
7	*Xgwm5-3A*	8	8	100%	16	33	50–300	0.78
8	*Xgwm30-3A*	3	3	100%	6	9	50–100	0.54
9	*Xgwm32-3A*	7	7	100%	22	46	50–200	0.67
10	*Xgwm666.2-3A*	4	4	100%	15	16	50–200	0.39
11	*Xgwm610-4A*	7	7	100%	34	62	50–200	0.86
12	*Xgwm129-5A*	6	6	100%	39	66	50–200	0.78
13	*Xgwm179-5A*	2	2	100%	4	4	50–150	0.37
14	*Xgwm617-5A*	7	5	71.42%	16	21	50–200	0.69
15	*Xgwm617-6A*	8	6	75%	13	18	50–200	0.68
16	*Xgwm63-7A*	4	4	100%	12	13	50–500	0.60
17	*Xgwm18-1B*	5	5	100%	21	26	50–200	0.64
18	*Xgwm33-1B*	4	4	100%	4	8	50–100	0.67
19	*Xgwm124-1B*	7	7	100%	24	38	50–200	0.78
20	*Xgwm550-1B*	7	7	100%	14	23	50–200	0.55
21	*Xgwm16-2B*	7	7	100%	17	27	50–200	0.50
22	*Xgwm610-2B*	3	3	100%	11	13	50–100	0.36
23	*Xgwm257-2B*	6	6	100%	9	11	50–200	0.77
24	*Xgwm131-3B*	2	2	100%	2	2	50–150	0.50
25	*Xgwm284-3B*	3	3	100%	5	8	50–600	0.62
26	*Xgwm66-4B*	6	6	100%	9	14	50–100	0.72
27	*Xgwm149-4B*	8	8	100%	8	16	50–250	0.73
28	*Xgwm66-5B*	2	2	100%	9	13	50–150	0.34
29	*Xgwm68-5B*	1	1	100%	2	2	50	0.00
30	*Xgwm213-5B*	4	4	100%	12	14	50–150	0.64
31	*Xgwm132-6B*	4	4	100%	6	8	100–150	0.58
32	*Xgwm232-1D*	4	2	50%	4	5	50–150	0.65
33	*Xgwm157-2D*	5	5	100%	26	31	50–300	0.63
34	*Xgwm212-2D*	3	3	100%	20	20	50–150	0.60
35	*Xgwm455-2D*	6	6	100%	19	34	50–100	0.65
36	*Xgwm3-3D*	1	1	100%	20	20	50	0.00
37	*Xgwm183-3D*	2	2	100%	21	21	100–150	0.69
38	*Xgwm314-3D*	4	4	100%	10	19	100–200	0.67
39	*Xgwm608-4D*	1	1	100%	9	9	100	0.00
40	*Xgwm16-5D*	6	6	100%	28	47	50–150	0.69
41	*Xgwm192-5D*	7	7	100%	23	40	150–250	0.77
42	*Xgwm55-6D*	6	6	100%	9	23	50–150	0.75

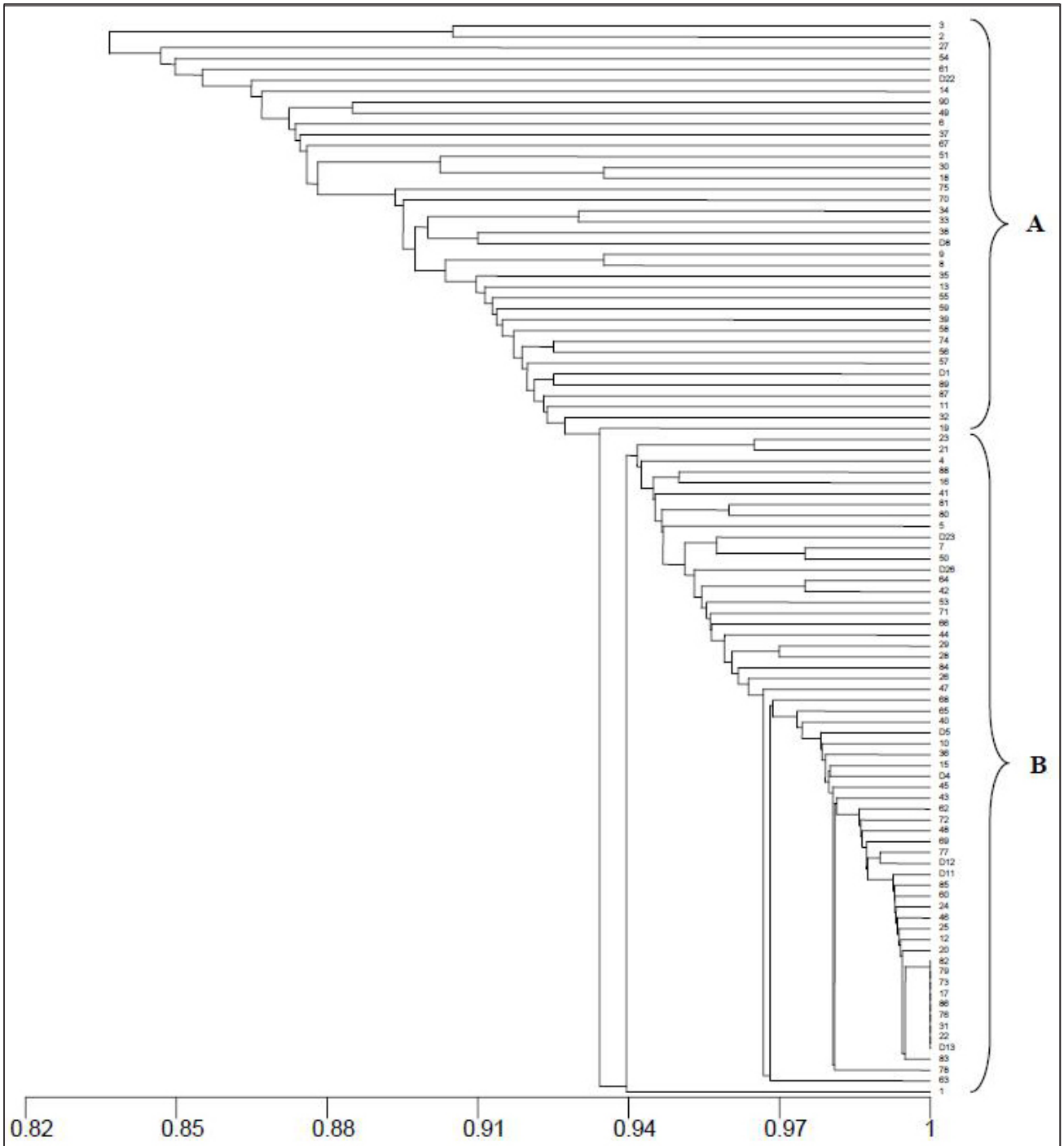


Fig. 21. An SSR-based cluster formation of genotypes of various *Aegilops tauschii* accessions in a similar durum wheat background.

Phenotypic and molecular characterization of synthetic hexaploids derived from the same Ae. tauschii accessions and diverse durum cultivars.

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This study with same *Ae. tauschii* accession used as the female parent in crosses with different durum cultivars as the pollen parent (78 entries) is designed to study the inheritance of different genes and also identify the effect of cytoplasmic inheritance if any. The total of 78 entries was screened against two biotic stresses (Karnal bunt and stripe rust), phenotypically characterized, and analyzed with RAPD and SSRs for molecular characterization (Table 30 and Table 31, pp. 132-133).

Stripe rust studies. Seedling screening showed that 51 of 78 (65.4%, Table 32, pp. 134-135) exhibited resistance. These genotypes also were screened for APR under field conditions at NARC, which identified 51 of the 95 (65.4%) as resistant genotypes. Genotypes with both seedling and adult-plant resistance were 15 of 78 (19.2%), including entries 9, 11, 12, 13, 14, 15, 16, 31, 32, 33, 39, 42, 50, 54, and 66. All this germ plasm represents the presence of major genes against stripe rust and can be exploited further in breeding programs.

Adult-plant resistance involving susceptibility at seedling stage and resistance only at the adult-plant stage is an indicator of presence of minor genes, which are considered of great importance against rust diseases in acquiring durable resistance. Nine of 78 entries (11.5%) had APR, including entries 21, 22, 25, 29, 36, 49, 62, 63, and 64 and are good candidates for providing durable resistance to wheat cultivars.

Karnal bunt studies. Karnal bunt (KB) evaluation was done by examining the grains following artificial inoculation. Grains from each entry were examined separately after hand threshing. The rating scale was from 0 to 5; only a rating of 0 was considered acceptable and all others as susceptible (See Fig. 2, p. 86). In this experiment, 29 of the 78 genotypes (37.2%) were found to be immune, including 2, 5, 7, 8, 9, 13, 17, 20, 21, 22, 23, 25, 26, 28, 29, 30, 41, 42, 43, 44, 45, 48, 49, 53, 54, 68, 72, 73, and 77 (Table 32, pp. 135-136).

Molecular studies. ***Genetic diversity evaluation using random amplified polymorphic DNA (RAPD) primers.*** RAPD primers were used for genetic diversity evaluation of A-, B-, and D-genome synthetic hexaploids. All 520 RAPD primers of the Operon Series were screened and working primers were identified and applied to detect genetic polymorphism at DNA level. The samples which did not amplify were not included in the analysis.

Genetic analysis was performed only on the scorable bands. Every single band was considered as a single locus/allele. The loci were scored as present/absent. Bivariate data 1–0 were used to estimate genetic distances (GD). The unweighted pair group of arithmetic means (UPGMA) func-

Table 30. Synthetic hexaploid entries derived from combining durum wheat cultivars with *Aegilops tauschii* accessions and their respective reciprocal cross combinations. Entry numbers are similar to data base maintained in CIMMYT Wide Crosses program in Mexico.

Group number	D-genome synthetic hexaploid entry	Total number of entries
1	1, 39, 45, 49	4
2	2, 20, 78	3
3	3, 10, 47	3
4	4, 19, 30	3
5	5, 27, 46	3
6	6,17, 24	3
7	7, 41, 68	3
8	8, 15, 33	3
9	9, 26, 28, 43, 59, 74	6
10	11, 40, 63	3
11	12, 50, 76	3
12	13, 69, 75	3
13	14, 16, 52	3
14	18, 60, 73	3
15	21, 25, 66	3
16	22, 29, 42, 56	4
17	23, 35, 38, 71	4
18	31, 61, 67, 77	4
19	32, 48, 65	3
20	34, 44, 57	3
21	36, 51, 54, 58	4
22	37, 53, 55, 62, 64, 70, 72	7

Table 31. Pedigrees of the genotypes used in this study that combined durum wheat cultivars with *Aegilops tauschii* accessions.

Synthetic number	Pedigree
1	DVERD_2/*Ae. tauschii* (1026)
2	ARLIN/*Ae. tauschii* (665)
3	ARLIN/*Ae. tauschii* (295)
4	ALTAR 84/*Ae. tauschii* (221)
5	RASCON/*Ae. tauschii* (314)
6	D67.2/ P66.270//*Ae. tauschii* (633)
7	D67.2/ P66.270//*Ae. tauschii* (223)
8	STY-US/CELTA//PALS/3/SRN_5/4/*Ae. tauschii* (174)
9	CROC_1/*Ae. tauschii* (507)

Table 31. Pedigrees of the genotypes used in this study that combined durum wheat cultivars with *Aegilops tauschii* accessions.

Synthetic number	Pedigree
10	ROK/KML//*Ae. tauschii* (295)
11	RABI//GS/CRA/3/*Ae. tauschii* (457)
12	GAN/*Ae. tauschii* (446)
13	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (205)
14	CROC_1/*Ae. tauschii* (215)
15	CETA/*Ae. tauschii* (174)
16	SORA/*Ae. tauschii* (215)
17	SNIPE/YAV79//DACK/TEAL/3/*Ae. tauschii* (633)
18	YAV_2/TEZ//*Ae. tauschii* (170)
19	D67.2/P66.270//*Ae. tauschii* (221)
20	6973/WARD.7463//74110/3/*Ae. tauschii* (665)
21	ALTAR 84/*Ae. tauschii* (211)
22	ARLIN_1/*Ae. tauschii* (1018)
23	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (629)
24	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (633)
25	SORA/*Ae. tauschii* (211)
26	LARU/*Ae. tauschii* (507)
27	SCOT/MEXI_1//*Ae. tauschii* (314)
28	ALTAR 84/*Ae. tauschii* (507)
29	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. tauschii* (1018)
30	DVERD_2/*Ae. tauschii* (221)
31	YAR/*Ae. tauschii* (783)
32	LCK59.61/*Ae. tauschii* (308)
33	ALTAR 84/*Ae. tauschii* (174)
34	DVERD_2/*Ae. tauschii* (1031)
35	SNIPE/YAV79//DACK/TEAL/3/*Ae. tauschii* (629)
36	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. tauschii* (1029)
37	DVERD_2/*Ae. tauschii* (333)
38	68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (629)
39	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. tauschii* (1026)
40	YAV79//DACK/RABI/3/SNIPE/4/*Ae. tauschii*(457)
41	ALTAR 84/*Ae. tauschii* (223)

Table 31. Pedigrees of the genotypes used in this study that combined durum wheat cultivars with *Aegilops tauschii* accessions.

Synthetic number	Pedigree
42	DOY 1/*Ae. tauschii* (1018)
43	DOY 1/*Ae. tauschii* (507)
44	CETA/*Ae. tauschii* (1031)
45	CETA/*Ae. tauschii* (1026)
46	KAPUDE/*Ae. tauschii* (314)
47	DVERD_2/*Ae. tauschii* (295)
48	D67.2/ P66.270//*Ae. tauschii* (308)
49	DOY 1/*Ae. tauschii* (1026)
50	DOY 1/*Ae. tauschii* (446)
51	DVERD_2/*Ae. tauschii* (1029)
52	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (215)
53	ARLIN_1/*Ae. tauschii* (333)
54	DOY 1/*Ae. tauschii* (1029)
55	ALTAR 84/*Ae. tauschii* (333)
56	CETA/*Ae. tauschii* (1018)
57	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. tauschii* (1031)
58	CETA/*Ae. tauschii* (1029)
59	ROK/ KML//*Ae. tauschii* (507)
60	CROC_1/*Ae. tauschii* (170)
61	CETA/*Ae. tauschii* (783)
62	LARU/*Ae. tauschii* (333)
63	YAV_2/ TEZ//*Ae. tauschii* (457)
64	CROC_1/*Ae. tauschii* (333)
65	ARLIN/*Ae. tauschii* (308)
66	D67.2/ P66.270//*Ae. tauschii* (211)
67	68.111/RGB-U//WARD RESEL/3/STIL/4/*Ae. tauschii* (783)
68	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (223)
69	ALTAR 84/*Ae. tauschii* (205)
70	DOY 1/*Ae. tauschii* (333)
71	CIT71/ CPT//*Ae. tauschii* (629)
72	ROK/ KML//*Ae. tauschii* (333)
73	CETA/*Ae. tauschii* (170)
74	DVERD_2/*Ae. tauschii* (507)
75	CROC_1/*Ae. tauschii* (205)
76	SRN/*Ae. tauschii* (446)
77	LCK59.61/*Ae. tauschii* (783)
78	CETA/*Ae. tauschii* (665)

Table 32. Phenotypic and disease characterization of D-genome synthetic hexaploids that combined durum wheat cultivars with *Aegilops tauschii* accessions. FLOW = days-to-flowering, HT = plant height at maturity (cm), AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown), PMA = days to physiological maturity, TKW = 1,000-kernel weight (g), G/S = grains/spike, SL = spike length (cm), KB = Karnal bunt reaction (– = immune, + = susceptible), Yr (S) = reaction to stripe rust at the seedling stage, Yr (A) = reaction to stripe rust at the adult–plant stage (R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlapping of MR–MS types, MSS = moderately susceptible to susceptible, S = susceptible, and TS = trace susceptible).

Entry	FLOW	HT	AWN	PMA	TKW	G/S	SL	KB	Yr (S)	Yr (A)
1	75	92	LB	112	30.0	15	12	+	1	10MRMS
2	76	90	LB	114	30.0	17	13	-	1	10MRMS
3	74	88	LB	112	31.2	14	11	+	1	30MRMS
4	79	100	LB	118	29.5	12	9	+	45	30MSS
5	72	109	LB	109	23.6	14	10	-	0	90S
6	79	115	LB	115	55.7	13	9	+	23	90S
7	75	113	LB	113	69.2	14	11	-	89	30MSS
8	89	91	LB	123	38.6	13	11	-	1	90S
9	92	93	LB	101	31.5	15	12	-	0	30MR
10	84	107	LB	101	25.4	18	14	+	89	30MSS
11	70	112	LB	112	39.7	12	11	+	1	0
12	76	102	LB	113	29.4	13	11	+	1	0
13	78	100	LB	116	31.2	12	11	-	1	10R
14	76	109	LB	113	32.5	14	11	+	1	0
15	70	123	LB	107	35.2	15	13	+	89	5MR
16	72	100	LB	108	36.6	16	13	+	89	10MR
17	72	99	LB	109	55.0	14	9	-	34	90S
18	68	124	LB	106	39.5	16	11	+	89	70S
19	71	112	LB	109	26.0	13	11	+	89	50S
20	73	114	LB	112	37.0	14	11	-	1	50S
21	75	107	LB	113	36.2	16	12	-	78	10R
22	73	104	LB	111	32.5	12	8	-	78	10R
23	73	110	LB	101	17.6	12	9	-	78	90S
24	70	111	LB	108	25.6	16	13	+	78	90S
25	77	92	LB	116	39.9	14	12	-	98	10R
26	79	75	LB	118	33.5	13	11	-	45	90S
27	128	112	LB	181	37.0	9	10	+	34	90S
28	144	82	LB	180	35.0	20	15	-	89	90S
29	83	109	LB	123	37.6	23	18	-	89	0
30	79	109	LB	118	25.0	15	11	-	89	90S
31	80	105	LB	113	26.0	15	12	+	12	10R
32	82	98	LB	112	26.0	15	11	+	12	10R
33	81	99	LB	118	27.0	14	10.5	+	12	5R
34	75	110	LB	112	31.0	16	11	+	34	90S
35	74	112	LB	112	29.5	14	10	+	89	30MRMS
36	87	85	LB	123	39.0	13	10	+	89	10R
37	70	109	LB	105	31.0	14	10	+	1	90S
38	70	110	LB	106	32.5	13	9.5	+	1	90S
39	69	84	LB	105	42.0	12	8	+	34	5R
40	73	104	LB	112	40.6	18	14	+	1	90S
41	129	128	LB	173	33.0	15	11	+	34	10MSS
42	83	102	LB	120	37.6	15	11	-	34	5R
43	83	107	LB	118	29.8	13	9.5	-	0	30MSS
44	84	76	LB	119	25.3	12	8.5	-	0	90S
45	83	122	LB	115	27.3	15	10.5	-	1	90S

Table 32. Phenotypic and disease characterization of D-genome synthetic hexaploids that combined durum wheat cultivars with *Aegilops tauschii* accessions. FLOW = days-to-flowering, HT = plant height at maturity (cm), AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown), PMA = days to physiological maturity, TKW = 1,000-kernel weight (g), G/S = grains/spike, SL = spike length (cm), KB = Karnal bunt reaction (– = immune, + = susceptible), Yr (S) = reaction to stripe rust at the seedling stage, Yr (A) = reaction to stripe rust at the adult–plant stage (R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlapping of MR–MS types, MSS = moderately susceptible to susceptible, S = susceptible, and TS = trace susceptible).

Entry	FLOW	HT	AWN	PMA	TKW	G/S	SL	KB	Yr (S)	Yr (A)
46	72	98	LB	112	25.5	14	10	+	1	90S
47	72	96	LB	109	38.5	16	12	+	1	90S
48	74	120	LB	112	29.6	15	10	-	34	90S
49	76	103	LB	114	29.5	12	9	-	89	0
50	76	99	LB	113	30.5	14	10.5	+	0	0
51	72	100	LB	109	31.5	13	11.5	+	0	70S
52	66	106	LB	106	36.5	12	9.5	+	0	70S
53	70	98	LB	106	37.0	14	11	-	78	90S
54	83	108	LB	118	36.5	16	11	-	0	10R
55	70	104	LB	106	31.5	14	10	+	34	90S
56	138	114	LB	167	30.5	14	10	+	78	90S
57	72	111	LB	106	32.6	15	10.5	+	0	90S
58	83	92	LB	115	32.5	13	9.5	+	0	30MSS
59	66	111	LB	108	31.2	12	9	+	34	70S
60	66	98	LB	105	30.5	13	9	+	0	30MRMS
61	83	92	LB	114	29.5	16	12	+	23	90S
62	69	96	LB	106	37.3	15	10	+	89	10R
63	72	111	LB	109	35.3	13	9.5	+	89	10R
64	72	121	LB	113	34.5	12	10	+	89	10R
65	73	120	LB	112	40.0	11	10	+	1	90S

tion estimated genetic distances between the genotypes as follows: $GD_{xy} = 1 - d_{xy}/d_x + d_y - d_{xy}$, where GD_{xy} = genetic distance between two genotypes, d_{xy} = total number of common loci (bands) in two genotypes, d_x = total number of loci (bands) in genotype 1, and d_y = total number of loci (bands) in genotype 2. The efficiency of primers to amplify the genotypes ranged from maximum from 34 (OPC-5) to one (OPA-9) (Table 33, p. 136). Scorable bands ranged from four (OPG-18, OPI-11, OPJ-9, OPJ-13) to 185 (OPC-15) (Table 33, p. 136). Genetic analysis of the population showed that the total number of loci reached 194, out of which 164 were polymorphic, and the percentage of polymorphism was 84.53% (Table 33, p. 136). The range of scorable bands was from 250-3,000 bp.

Similarity matrix. Bivariate analysis was conducted to generate a similarity matrix and dendrogram using Nei and Li's coefficient to estimate genetic diversity. The value of similarity matrix ranged from 70.3% (minimum) between genotypes 6 and 49 and was 100% (maximum) in 33 combinations.

Dendrogram interpretation. The GD between genotypes were used to construct a dendrogram by UPGMA analysis for determining grouping of the lines on the basis of similarities and differences. The dendrogram shows only one main cluster with two subclusters, A and B (Fig. 22, p. 137). Subcluster A has 66 genotypes of which 16 is the most diverse line and 2, 18, 50, 54, 61, 69, 71, 72, and 73 have 100% similarity to each other. Some other good lines in subcluster A include 3, 13, 15, 19, 23, 24, 57, and 76. Subcluster B has 12 genotypes of which 1, 25, and 41 are the best lines.

Evaluation of genetic diversity using simple sequence repeats (SSR) primers. SSR primers were used for genetic diversity evaluation of A-, B-, and D-genome synthetic hexaploids. All 275 SSR primers were applied to detect genetic polymprphism at the DNA level. Samples that did not amplify were not included in the analysis, The genetic analysis was similar to that for RAPD primers. The efficiency of primers to amplify the genotypes ranged from a maximum of 60 (*Xgwm437-7D*) to two (*Xgwm18-1B*, *Xgwm210-2B*, *Xgwm257-2B*, *Xgwm285-3B*, and *Xgwm102-2D*) (Table 34, pp. 139-140).

Table 33. Molecular fingerprinting pattern by RAPDs in D–genome wynthetic hexaploids (Same *Ae. tauschii* accessions with different durums)

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples qmplified	Scorable bands	Amplification product 4ange (bp)
1	OPA–2	4	4	100%	13	26	500–1,500
2	OPA-3	7	5	71.42%	9	21	1,000–2,000
3	OPA-4	1	1	100%	5	5	7,50–1,000
4	OPA-7	8	6	75%	8	18	1,000–2,500
5	OPA-9	5	0	0%	1	5	750–3,000
6	OPA-13	8	6	75%	16	40	750–3,000
7	OPB-5	2	2	100%	13	14	1,000–2,500
8	OPC-1	3	3	100%	7	13	1,000–2,000
9	OPC-2	10	10	100%	20	59	750–3,000
10	OPC-5	12	12	100%	34	137	750–2,500
11	OPC-15	12	12	100%	33	185	500–2,500
12	OPC-18	12	8	66.66%	22	45	500–3,000
13	OPD-19	8	0	0%	1	8	500–2,500
14	OPE-19	9	9	100%	12	98	250–2,000
15	OPE-20	9	7	77.77%	28	52	750–2,000
16	OPF-1	10	10	100%	7	25	250–2,500
17	OPG-9	6	6	100%	7	14	750–2,000
18	OPG-15	7	7	100%	11	21	1,000–3,000
19	OPG-18	4	1	25%	2	4	500–2,000
20	OPH-4	6	6	100%	4	12	500–2,500
21	OPH-15	9	9	100%	11	28	500–2,500
22	OPI-4	10	10	100%	11	27	250–2,000
23	OPI-11	3	1	33.33%	2	4	500–2,000
24	OPJ-9	4	4	100%	4	4	1,000–1,500
25	OPJ-12	6	6	100%	4	18	250–15,00
26	OPJ-13	3	3	100%	3	4	500–750
27	OPK-19	6	6	100%	14	34	500–2,000
28	OPK-20	10	10	100%	11	25	500–2,500

The scorable bands ranged from two (*Xgwm210-2B*, *Xgwm257-2B*, and *Xgwm157-2D*) to 120 (*Xgwm192-5D*) (Table 34, pp. 138-139). A genetic analysis of the population showed that the total number of alleles reached 208, of which 204 were polymorphic, and the percent polymorphism was 98.07% (Table 34, pp. 138-139). The range of scorable bands was from 50–500 bp.

Similarity matrix. Bivariate analysis was conducted to generate a similarity matrix and dendrogram using Nei and Li's coefficient to estimate genetic diversity. The value of similarity matrix ranged from 60.5% (minimum) between genotypes 7 and 1 and 7 and 49 and 98.6% (maximum) between 75 and 78 and 74 and 75.

Dendrogram interpretation. The dendrogram (Fig. 23, p. 137) shows one main cluster with subclusters A and B. Subcluster A can further be divided into two groups A1 and A2, with 10 and 57 genotypes, respectively. Group A1 has the most diverse genotype, 7, and other good lines include 9, 53, and 64. In group A2, 5, 39, 46, 63, and 77 are highly diverse genotypes. Subcluster B has 11 genotypes with 1 as the most diverse and 8 and 15 as highly diverse lines.

Same *Ae. tauschii* accession and different durum wheat cultivars. Contrary to the previous study (this issue: pp. 123-131), the influence of the same *Ae. tauschii* accession across diverse durum cultivars was observed across some group categories. This set of materials involved 22 groups with a total number of 78 entries that were studied for phenotypic, biotic stress, and molecular parameters. Across the entire group, days-to-flowering ranged from 66 days (entries 52, 59, 60, 68, and 71), spike length was satisfactory (line 71 was 14 cm), and a 1,000-kernel weight of 69.2 g was observed for entry 7. The best entries on the basis of overall phenotype were 7, 40, 71, and 77 with the most desirable being 71, because it is the earliest flowering and has the longest spike length. Karnal bunt resistance was found in entries 7 and 77.

The molecular diversity of these 78 entries was deduced from RAPD and SSR analysis. Entries 1, 3, 13, 15, 16, 19, 23, 24, 25, 41, 57, and 76 exhibited higher levels of diversity when analyzed by RAPDs. The most diverse were 1, 5, 7, 8, 9, 15, 39, 46, 53, 63, 64, and 77. Entries 1 and 15 were highly diverse for both RAPDs and SSRs. For biotic stress, entries 7 and 77 were the best for KB resistance, possessed good phenotypic traits, and are good candidates for future breeding efforts.

For some specific same *Ae. tauschii*/ durum wheat cultivar groups, synthetic groups 1 of 4 (entries 1, 39, 45, and 49) involved accession 1026. Entries 45 and 49 have KB resistance, entry 1 has stripe rust resistance at the seedling and adult-plant level and also is the most diverse genetically (RAPD/SSR). Entries 39 and 49 have APR and with the overall picture it is concluded that entry 49 possessing KB, APR for stripe rust, and good phenotype will be the ideal candidate for breeding.

Group 2, where the alien accession was 665, was comprised of three synthetics (2, 20, and 78). Of these entries, 2 and 20 are immune to KB and 2 has seedling and adult-plant strip rust resistance. Entry 2 is a desirable breeding parent with highest genetic diversity in the group based on SSR analysis.

Group 9 has six synthetics structured on the alien accession number 507, including SH 9, 26, 28, 43, 59, and 74. All entries except 59 and 74 are immune to KB; none are resistant to stripe rust. The diversity was high in entries 26 and 43 through RAPDs and 9 and 26 through SSRs. Because of its diversity, entry 26 is a preferred candidate for breeding utilization with KB resistance as an added positive attribute.

In group 22, seven synthetics involved *Ae. tauschii* accession 333, including 37, 53, 55, 62, 64, 70, and 72. All except 53 and 72 were susceptible to KB and entries 62, 64 had APR for stripe rust. Entry 64 had the maximum molecular diversity based upon RAPD and SSR analysis and possesses more positive attributes to give it high priority in breeding.

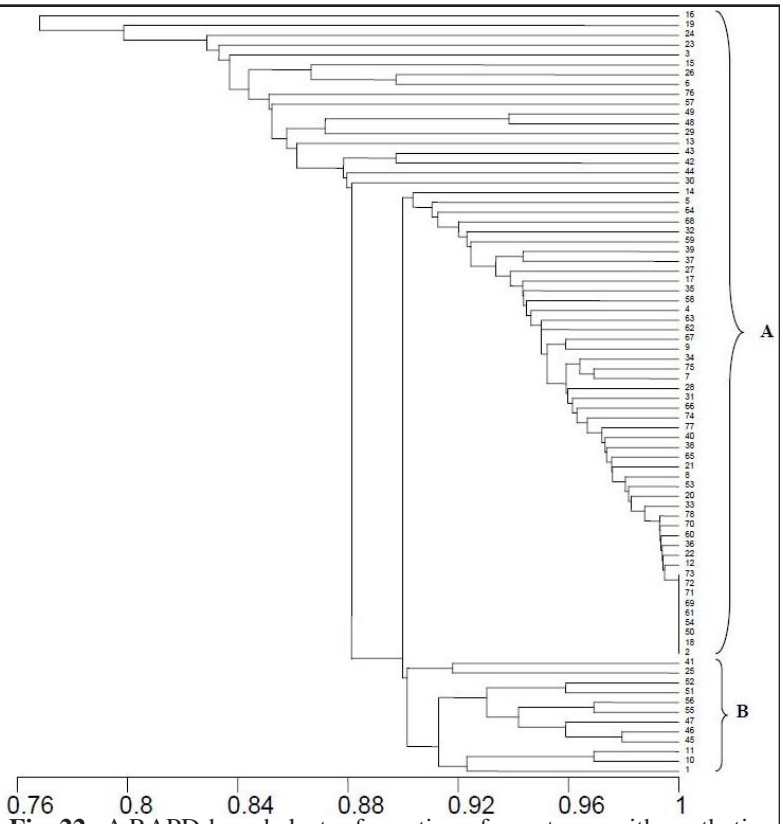

Fig. 22. A RAPD-based cluster formation of genotypes with synthetic hexaploids derived from the same *Ae. tauschii* accessions and diverse durum cultivars.

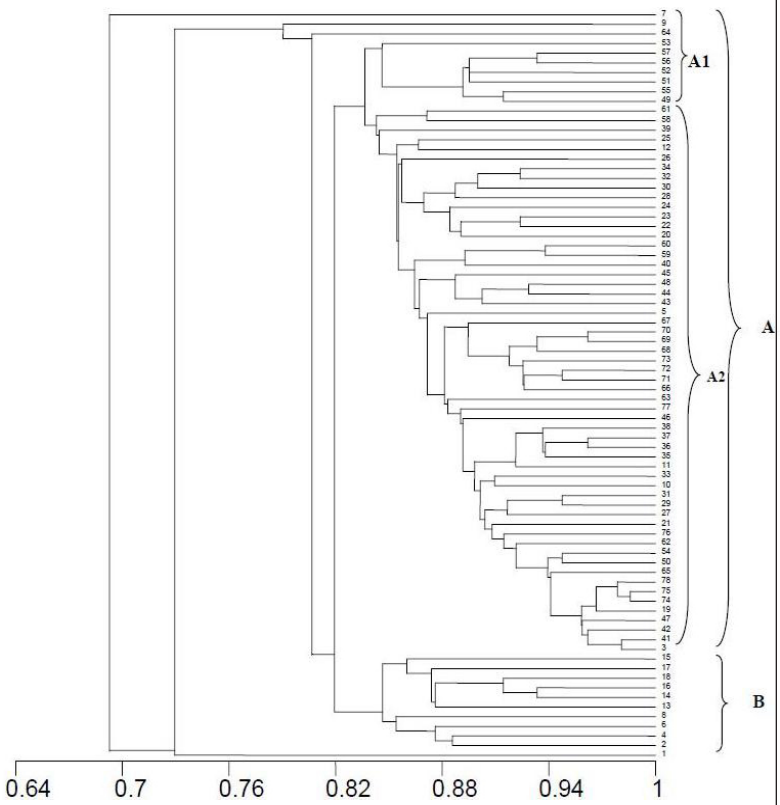

Fig. 23. An SSR-based cluster formation of genotypes with synthetic hexaploids derived from the same *Ae. tauschii* accessions and diverse durum cultivars.

Table 34. Molecular fingerprinting pattern by SSRs in D-genome synthetic hexaploids derived from the same *Ae. tauschii* with different durums).

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)	PIC
1	*Xgwm99-1A*	3	3	100%	5	7	50-150	0.31
2	*Xgwm10-2A*	4	4	100%	8	11	50-150	0.44
3	*Xgwm47.1-2A*	4	4	100%	20	23	50-200	0.41
4	*Xgwm47.2-2A*	2	2	100%	4	5	50-200	0.21
5	*Xgwm71.1-2A*	18	18	100%	23	60	50-100	0.78
6	*Xgwm95-2A*	3	2	66.67%	6	7	50-100	0.84
7	*Xgwm296-2A*	6	6	100%	17	28	50-200	0.75
8	*Xgwm312-2A*	1	1	100%	12	12	200	0.00
9	*Xgwm558-2A*	2	2	100%	5	5	50-200	0.32
10	*Xgwm205-5A*	1	1	100%	7	7	150	0.00
11	*Xgwm291-5A*	3	3	100%	4	4	100-150	0.62
12	*Xgwm169-6A*	2	2	100%	10	11	100-200	0.09
13	*Xgwm494-6A*	4	4	100%	17	20	50-200	0.42
14	*Xgwm11-1B*	5	4	80%	8	11	50-150	0.47
15	*Xgwm18-1B*	5	5	100%	2	6	100-200	0.77
16	*Xgwm124-1B*	4	4	100%	3	6	50-150	0.69
17	*Xgwm131-1B*	2	2	100%	3	3	200	0.00
18	*Xgwm140-1B*	3	3	100%	8	10	50-150	0.21
19	*Xgwm210-2B*	1	1	100%	2	2	200	0.54
20	*Xgwm257-2B*	1	1	100%	2	2	100	0.00
21	*Xgwm319-2B*	6	6	100%	4	11	50-200	0.78
22	*Xgwm284-3B*	1	1	100%	7	7	500	0.00
23	*Xgwm285-3B*	5	5	100%	2	6	50-200	0.73
24	*Xgwm66-4B*	5	5	100%	12	18	50-200	0.43
25	*Xgwm113-4B*	1	1	100%	4	4	50	0.00
26	*Xgwm149-4B*	1	1	100%	4	4	50	0.00
27	*Xgwm102-2D*	2	2	100%	2	2	150-200	0.50
28	*Xgwm157-2D*	5	5	100%	13	21	50-150	0.73
29	*Xgwm249-2D*	6	6	100%	8	23	50-150	0.76
30	*Xgwm261-2D*	6	6	100%	26	33	50-200	0.54
31	*Xgwm296-2D*	5	5	100%	47	53	150-200	0.62
32	*Xgwm301-2D*	4	4	100%	50	51	50-150	0.45
33	*Xgwm320-2D*	3	3	100%	24	24	200-250	0.55
34	*Xgwm455-2D*	3	2	66.67%	16	17	100-200	0.06
35	*Xgwm484-2D*	7	7	100%	17	35	50-250	0.80
36	*Xgwm539-2D*	5	5	100%	58	65	50-150	0.47
37	*Xgwm608-2D*	4	4	100%	23	30	100-200	0.61
38	*Xgwm3-3D*	3	3	100%	57	62	50-100	0.29
39	*Xgwm383-3D*	4	4	100%	51	51	200-250	0.45
40	*Xgwm456-3D*	2	2	100%	43	56	50-150	0.28
41	*Xgwm190-5D*	5	5	100%	35	37	200-250	0.48
42	*Xgwm192-5D*	4	4	100%	54	120	100-250	0.70
43	*Xgwm269-5D*	6	6	100%	35	70	100-250	0.73
44	*Xgwm272-5D*	3	3	100%	6	6	50-150	0.50
45	*Xgwm292-5D*	4	4	100%	28	28	150-500	0.56
46	*Xgwm358-5D*	7	7	100%	37	43	50-500	0.49

Table 34. Molecular fingerprinting pattern by SSRs in D-genome synthetic hexaploids derived from the same *Ae. tauschii* with different durums).

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)	PIC
47	*Xgwm565-5D*	5	4	80%	51	101	150-250	0.66
48	*Xgwm55-6D*	5	5	100%	51	86	100-200	0.74
49	*Xgwm325-6D*	4	4	100%	13	13	50-200	0.48
50	*Xgwm469-6D*	7	7	100%	48	68	150-250	0.73
51	*Xgwm295-7D*	2	2	100%	3	3	50-250	0.44
52	*Xgwm350-7D*	2	2	100%	8	8	100-150	0.37
53	*Xgwm428-7D*	1	1	100%	25	25	150	0.00
54	*Xgwm437-7D*	1	1	100%	60	60	100	0.00

In the absence of the involved *Ae. tauschii* accession in each synthetic, the precise answers of genomic interactions cannot be obtained. From each group, it is apparent that this intergenomic phenomenon is present because the KB immunity of the durum wheat cultivars is differentially expressed in each group's derived synthetic combination. Those giving susceptible SHs indicate that the D genome has masked the expression of the durum genomes. This experiment allows the selection of SH parents for use in breeding and at the same time has opened up avenues that will be interesting to follow in the future to unravel how the D genome acts in different durum wheat backgrounds. For greater precision, it may be appropriate to purify each *Ae. tauschii* accession and then design a study that targets the purified accessions influence upon trait expression.

Analysis of cytoplasmic influence across durum and Ae. tauschii pairs for diversity and other traits.

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From the primary set of synthetics an experimental set was made consisting of crosses 'durum wheat cultivar / *Ae. tauschii* accession' and their reciprocal cross combinations, which comprised four combinations and eight entries. This subset was designed to study the inheritance of different genes and also to identify the effect of any cytoplasmic inheritance. The eight entries were screened for Karnal bunt and stripe rust resistance, phenotypically characterized, and analyzed with RAPD and SSR primerss for molecular characterization (Tables 35 and 36).

Table 35. The primary set of synthetics consisting of crosses 'durum wheat cultivar/*Ae. tauschii* accession' and their reciprocal cross combinations.

Group number	D-genome synthetic hexaploid entry	Total number of entries
1	1, 6	2
2	2, 5	2
3	3, 4	2
4	7, 8	2

Stripe rust studies. Seedling screening showed that five of the eight (62.5%) were resistant (Table 37, p. 140). These genotypes were also screened for APR under field conditions at NARC; again, five of eight (62.5%) lines were resistant. Two of the eight genotypes (entries 4 and 5, 255) possess both seedling and adult-plant resistance. This germ plasm represents presence of major genes against stripe rust and can be exploited further in breeding programs.

Adult plant resistance involving susceptibility at seedling stage and resistance only at the adult-plant stage is an indicator of the presence of minor genes that are considered of great importance against rust diseases in acquiring durable resistance. Three genotyps (1, 2, and 3) out of eight (37.5%) had APR and are good candidates for providing durable resistance to wheat cultivars.

Table 36. Pedigrees of the *Ae. tauschii* lines used in the crosses with durum wheats.

Entry number	Pedigree
1	*Ae. tauschii* (1026)/DOY 1
2	*Ae. tauschii* (1018)/DOY 1
3	*Ae. tauschii* (1029)/DVERD_2
4	DVERD_2/*Ae. tauschii* (1029)
5	DOY 1/*Ae. tauschii* (1018)
6	DOY 1/*Ae. tauschii* (1026)
7	DVERD_2/*Ae. tauschii* (1031)
8	*Ae. tauschii* (1031)/ DVERD_2

Karnal bunt studies. Karnal bunt (KB) evaluation was done by examining the grains following artificial inoculation. Grains from each entry were examined separately after hand threshing and rated on a scale was from 0 to 5 (see Fig. 2, p. 87). Only rating scale of 0 was considered acceptable and all others as susceptible. We found that five of the eight entries (62.5%), including lines 1, 2, 3, 5 and 8, were completely immune (Table 37).

Table 37. Phenotypic and disease characterization of D-genome synthetic hexaploids involved in crosses 'durum wheat cultivar/*Ae. tauschii* accession' and their reciprocals (see Table 36, p. 139 for pedigree information). FLOW = days-to-flowering, HT = plant height at maturity (cm), AWN = awn color (LB = light brown, AW = amery white, Y = yellow, and DB = dark brown), PMA = days to physiological maturity, TKW = 1,000-kernel weight (g), G/S = grains/spike, SL = spike length (cm), KB = Karnal bunt reaction (– = immune, + = susceptible), Yr (S) = reaction to stripe rust at the seedling stage, Yr (A) = reaction to stripe rust at the adult–plant stage (R = resistant, TR = trace resistant, MR = moderately resistant, MS = moderately susceptible, M = overlapping of MR–MS types, MSS = moderately susceptible to susceptible, S = susceptible, and TS = trace susceptible).

Entry	FLOW	HT	AWN	PMA	TKW	G/S	SL	KB	Yr (S)	Yr (A)
1	85	119	LB	117	32.5	15	13	-	78	10R
2	91	116	LB	123	36.6	13	13	-	67	5R
3	85	125	LB	119	35.3	12	10	-	89	10MR
4	76	95	LB	112	45.8	14	10	+	0	10MR
5	65	100	LB	105	37.5	15	12	-	0	10MR
6	70	123	LB	109	33.5	15	12	+	0	30S
7	75	102	LB	113	29.5	12	11	+	0	30MS
8	88	110	LB	122	34.9	15	12	-	0	90S

Molecular studies. ***Genetic diversity evaluation using random amplified polymorphic DNA (RAPD) primers.*** RAPD primers were used for genetic diversity evaluation of D-genome synthetic hexaploids. All 520 RAPD primers of Operon Series were screened and working primers were identified and applied to detect genetic polymorphism at DNA level. Samples that did not amplify were not included in the analysis.

Genetic analysis was performed only on the scorable bands. Every single band was considered as a single locus/allele. The loci were scored as present/absent. Bivariate data 1–0 were used to estimate genetic distances (GD). The unweighted pair group of arithmetic means (UPGMA) function was used to estimate the GD between the genotypes as follows: $GD_{xy} = 1 - d_{xy}/d_x + d_y - d_{xy}$, where GD_{xy} = genetic distance between two genotypes, d_{xy} = total number of common loci (bands) in two genotypes, d_x = total number of loci (bands) in genotype 1, and d_y = total number of loci (bands) in genotype 2.

Efficiency of primers to amplify the genotypes ranged from maximum from eight (OPD-13 and OPI-19) to one (OPA-7, OPA-11, OPA-13, OPB-3, OPB-12, OPB-13, OPB-16, OPC-14, OPE-4, OPG-7, OPH-12, OPI-17, OPM-9, OPM-12, OPQ-7, OPQ-8, OPR-11, OPS-5, OPS-7, OPT-14, OPT-15, OPW-16, OPW-17, and OPX-12) (Table 38, pp. 142-144). Scorable bands ranged from one (OPA-7, OPA-11, OPA-13, OPB-3, OPB-12, OPC-14, OPE-4, OPI-17, OPM-9, and OPV-14) to 33 (OPQ-9) (Table 38, pp. 141-143).

Genetic analysis of the population showed that the total number of loci was 419, of which only 217 were polymorphic; 51.78% polymorphism (Table 38, pp. 141-143). The range of scorable bands was from 500–3,000 bp.

Similarity matrix. A bivariate analysis was conducted to generate a similarity matrix and dendrogram using Nei and Li's coefficient to estimate genetic diversity. In this experiment, the minimum value of 41.9% was found between genotypes 3 and 5 and the maximum value of 73.8% was present between genotypes 7 and 8.

Dendrogram interpretation. The genetic distances between genotypes were used to construct a dendrogram by UPGMA analysis for determining grouping of the lines on the basis of similarities and differences. In case of this experiment, the dendrogram shows one main cluster with two more sub-clusters A and B (Fig. 24, p. 144). Subcluster A has only two genotypes 5 and 6 and subcluster B is divided into two groups with three genotypes each.

Genetic diversity evaluation using simple sequence repeat (SSR) primers. SSR primers were used for genetic diversity evaluation of A-, B-, and D-genome synthetic hexaploids. All 275 SSR primers were applied to detect genetic poly-

Table 38. Molecular fingerprinting pattern by RAPDs in D-genome synthetic hexaploids (durum/D-genome accessions and reciprocal)

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)
1	OPA-3	2	2	100%	2	3	1,000–1,500
2	OPA-7	1	1	100%	1	1	30,00
3	OPA-9	2	2	100%	3	3	1,500–2,000
4	OPA-11	1	1	100%	1	1	2,000
5	OPA-13	1	1	100%	1	1	1,500
6	OPB-3	1	1	100%	1	1	500
7	OPB-12	1	1	100%	1	1	1,000
8	OPB-13	3	0	0%	1	3	1,000–2,000
9	OPB-16	2	0	0%	1	2	250–500
10	OPC-10	3	1	33.33%	2	3	1,000–2,000
11	OPC-12	1	1	100%	5	5	3,000
12	OPC-13	1	1	100%	5	5	3,000
13	OPC-14	1	1	100%	1	1	3,000
14	OPC-15	1	1	100%	6	6	3,000
15	OPC-16	1	1	100%	6	6	3,000
16	OPC-17	1	1	100%	5	5	3,000
17	OPC-18	1	1	100%	7	7	3,000
18	OPC-19	1	1	100%	2	2	3,000
19	OPC-20	2	2	100%	7	8	1,000–3,000
20	OPD-1	2	2	100%	7	8	1,000–3,000
21	OPD-2	1	1	100%	5	5	3,000
22	OPD-3	2	2	100%	4	4	2,500–3,000
23	OPD-4	1	1	100%	3	3	3,000
24	OPD-5	1	1	100%	2	2	3,000
25	OPD-9	1	1	100%	5	5	3,000
26	OPD-10	1	1	100%	5	5	3,000
27	OPD-12	1	1	100%	7	7	3,000
28	OPD-13	1	1	100%	8	8	3,000
29	OPE-4	1	1	100%	1	1	3,000
30	OPE-5	1	1	100%	5	5	3,000
31	OPE-7	6	2	33.33%	3	14	500–2,000
32	OPE-11	3	0	0%	2	6	750–2,000
33	OPF-10	1	1	100%	5	5	3,000
34	OPF-11	1	1	100%	3	3	3,000
35	OPF-12	1	1	100%	3	3	3,000
36	OPF-13	1	1	100%	4	4	3,000
37	OPF-14	1	1	100%	3	3	3,000
38	OPF-16	1	1	100%	4	4	3,000
39	OPF-17	1	1	100%	5	5	3,000
40	OPG-2	4	1	25%	4	13	3000
41	OPG-7	8	0	0%	1	8	750–2,000
42	OPG-8	4	4	100%	6	16	750–2,000
43	OPG-13	5	5	100%	3	8	750–2,000
44	OPG-16	1	1	100%	6	6	500–1,500
45	OPG-17	1	1	100%	3	3	3,000
46	OPH-2	3	3	100%	6	10	3,000

Table 38. Molecular fingerprinting pattern by RAPDs in D-genome synthetic hexaploids (durum/D-genome accessions and reciprocal)

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)
47	OPH-3	2	2	100%	4	4	2,500–30,00
48	OPH-4	3	1	33.33%	2	4	1,000–3,000
49	OPH-12	3	0	0%	1	3	500–1,000
50	OPH-15	1	1	100%	2	2	750–1,500
51	OPI-13	1	1	100%	2	2	3,000
52	OPI-15	6	3	50%	4	12	1,000
53	OPI-17	1	1	100%	1	1	750–2,000
54	OPI-18	1	1	100%	2	2	1,000
55	OPI-19	10	7	70%	8	32	750–3,000
56	OPI-20	1	1	100%	3	3	3,000
57	OPJ-5	1	1	100%	4	4	3,000
58	OPJ-7	1	1	100%	4	4	3,000
59	OPJ-8	1	1	100%	4	4	3,000
60	OPJ-20	3	0	0%	2	6	750–1,500
61	OPK-1	1	1	100%	4	4	3,000
62	OPK-2	1	1	100%	6	6	3,000
63	OPK-3	1	1	100%	5	5	3,000
64	OPK-4	1	1	100%	6	6	3,000
65	OPK-16	1	1	100%	2	2	3,000
66	OPK-17	3	0	0%	2	6	250–500
67	OPL-4	1	1	100%	2	2	1,000
68	OPL-11	6	3	50%	2	10	250–1,000
69	OPL-12	7	5	71.42%	5	25	250–1,500
70	OPL-15	7	2	28.57%	5	32	250–1,500
71	OPL-17	6	0	0%	3	12	500–1,500
72	OPL-18	5	3	60%	3	10	1,000–2,000
73	OPL-20	8	4	50%	5	22	250–2,000
74	OPM-1	5	3	60%	6	23	500–1,500
75	OPM-2	6	6	100%	6	23	250–1,500
76	OPM-9	1	1	100%	1	1	750–1,500
77	OPM-10	4	3	75%	3	11	1,500
78	OPM-11	1	1	100%	3	3	1,500
79	OPM-12	5	0	0%	1	5	750–1,500
80	OPM-13	7	5	71.42%	4	10	250–1,500
81	OPM-15	5	2	40%	2	8	250–1,500
82	OPM-16	6	3	50%	3	12	500–1,500
83	OPM-19	1	1	100%	3	3	3,000
84	OPN-19	4	2	50%	3	7	500–1,500
85	OPN-20	6	1	16.66%	3	16	500–1,500
86	OPP-15	4	2	50%	2	7	750–2,000
87	OPP-16	6	3	50%	6	24	500–2,500
88	OPP-17	4	0	0%	2	6	750–1,500
89	OPQ-3	2	0	0%	1	2	1,000–2,000
90	OPQ-7	2	0	0%	1	2	750–1,500
91	OPQ-8	3	3	100%	6	10	250–1,000
92	OPQ-9	8	1	12.5%	5	33	250–1,500

Table 38. Molecular fingerprinting pattern by RAPDs in D-genome synthetic hexaploids (durum/D-genome accessions and reciprocal)

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)
93	OPQ-10	6	0	0%	2	10	250–1,500
94	OPQ-11	4	1	25%	6	23	250–1,500
95	OPQ-12	4	2	50%	5	12	250–2,000
96	OPQ-13	8	3	37.5%	5	30	250–1,500
97	OPQ-14	9	4	44.44%	3	25	500–2,000
98	OPQ-18	4	0	0%	2	8	750–1500
99	OPR-1	2	2	100%	3	4	250–750
100	OPR-2	5	5	100%	3	9	250–1,500
101	OPR-8	1	1	100%	3	3	500
102	OPR-11	2	0	0%	1	2	500–750
103	OPR-12	3	3	100%	4	8	500–1,000
104	OPR-16	5	3	60%	3	6	250–1,500
105	OPR-17	4	4	100%	6	17	250–1,000
106	OPR-19	8	8	100%	5	18	250–2,000
107	OPR-20	3	3	100%	5	5	500–1,000
108	OPS-5	2	0	0%	1	2	1,000
109	OPS-7	5	0	0%	1	5	500–2,000
110	OPT-10	3	0	0%	3	9	750–1,500
111	OPT-12	2	2	100%	3	6	1,000–1,500
112	OPT-13	3	3	100%	3	6	500–1,500
113	OPT-14	4	0	0%	1	4	750–1,500
114	OPT-15	4	0	0%	1	4	1,000–2,000
115	OPU-13	2	2	100%	5	8	1,000–1,500
116	OPU-18	3	0	0%	4	12	500–2,000
117	OPU-20	3	3	100%	6	16	500–1500
118	OPV-3	6	3	50%	3	13	750–2,000
119	OPV-5	2	0	0%	3	6	750–2,000
120	OPV-8	2	2	100%	3	4	500–750
121	OPV-14	1	1	100%	1	1	500–750
122	OPV-15	2	0	0%	3	6	500–750
123	OPW-11	4	1	25%	2	7	250–1,000
124	OPW-12	4	2	50%	3	8	250–1,000
125	OPW-13	5	4	80%	4	13	500–2,500
126	OPW-16	4	0	0%	1	4	500–1,500
127	OPW-17	3	0	0%	1	3	500–3,000
128	OPW-18	3	3	100%	4	5	500–3,000
129	OPW-19	4	1	25%	3	6	500–1,500
130	OPX-1	3	0	0%	2	6	1,000–1,500
131	OPX-4	6	3	50%	4	15	250–2,000
132	OPX-9	4	2	50%	5	17	750–2,000
133	OPX-11	3	3	100%	6	12	750–1,500
134	OPX-12	2	0	0%	1	2	500–1,500
135	OPX-13	5	0	0%	4	18	250–1,500
136	OPX-14	1	1	100%	2	2	2,000
137	OPY-7	3	3	100%	3	6	750–2,000
138	OPY-8	4	0	0%	3	12	500–2,000

morphism at DNA level. Samples that did not amplify were not included in the analysis. Genetic analysis was similar to that for the RAPD primers.

Primer efficiency to amplify the genotypes ranged from a maximum of eight (82 primers in total; 41 in A, 30 in B, and 11 in D) to one (*Xgwm445-2A*, *Xgwm512-2A*, *Xgwm160-4A*, *Xgwm459-6A*, *Xgwm210-2B*, *Xgwm325-6D*, and *Xgwm469-6D*) in this experiment (Table 39, pp. 145-149). Scorable bands ranged from one (*Xgwm445-2A*, *Xgwm459-6A*, *Xgwm325-6D*, and *Xgwm469-6D*) to 33 (*Xgwm63-7A*) (Table 39, pp. 144-148).

Genetic analysis of the popluation showed that the total number of alleles was 502, of which only 399 were polymorphic; 79.48% (Table 39, pp. 144-148). The range of scorable bands was from 50–1,000 bp.

Similarity matrix. A bivariate analysis was conducted to generate a similarity matrix and dendrogram using Nei and Li's coefficient to estimate genetic diversity. A minimum value of 18.7% was found between genotypes

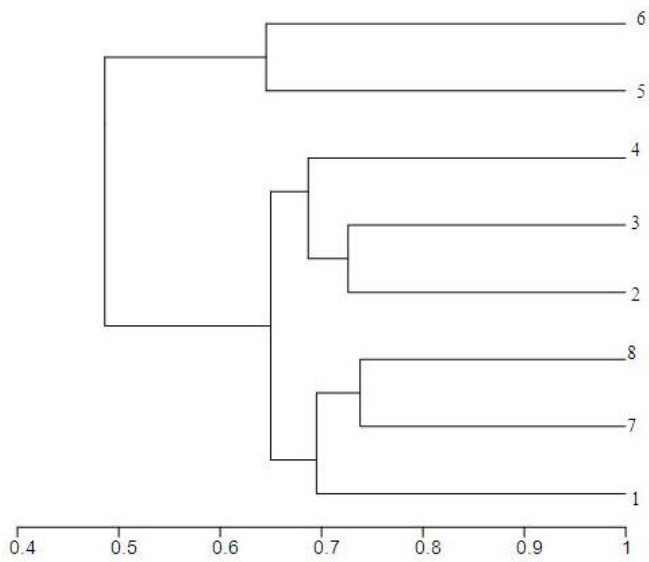


Fig. 24. SSR-based cluster formation of eight synthetic hexaploid genotypes of the same durum wheat cultivar with different *Aegilops tauschii* and the reciprocal crosses.

Table 39. Molecular fingerprinting pattern by SSRs in the D-genome synthetic hexaploids of 'durum wheat/D-genome accessions' and their reciprocal crosses.

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)	PIC
1	*Xgwm33-1A*	4	4	100%	8	17	50	0.66
2	*Xgwm99-1A*	5	3	60%	8	18	50–150	0.64
3	*Xgwm135-1A*	3	1	33.33%	8	17	100–150	0.55
4	*Xgwm164-1A*	4	2	50%	7	14	100–250	0.57
5	*Xgwm357-1A*	1	1	100%	8	8	100	0.00
6	*Xgwm497-1A*	2	0	0%	8	16	100–150	0.50
7	*Xgwm666-1A*	2	2	100%	4	4	100–150	0.37
8	*Xgwm10-2A*	1	1	100%	3	8	150	0.00
9	*Xgwm47.1-2A*	2	2	100%	7	7	50–200	0.40
10	*Xgwm47.2-2A*	1	1	100%	4	4	50–200	0.00
11	*Xgwm71.1-2A*	3	1	33.33%	7	16	50–150	0.64
12	*Xgwm71.2-2A*	3	1	33.33%	7	20	50–150	0.67
13	*Xgwm95-2A*	2	2	100%	8	10	50–150	0.50
14	*Xgwm122-2A*	2	2	100%	6	6	100–200	0.44
15	*Xgwm249-2A*	2	2	100%	7	12	100–150	0.45
16	*Xgwm265-2A*	4	2	50%	8	22	50–150	0.68
17	*Xgwm275-2A*	2	2	100%	8	9	100–150	0.11
18	*Xgwm294-2A*	1	1	100%	8	8	150	0.00
19	*Xgwm311-2A*	2	2	100%	6	11	100–150	0.48
20	*Xgwm312-2A*	2	2	100%	8	13	50–250	0.42
21	*Xgwm328-2A*	1	1	100%	8	8	100	0.00
22	*Xgwm339-2A*	2	2	100%	6	6	150–200	0.27
23	*Xgwm356-2A*	1	1	100%	8	8	150	0.00
24	*Xgwm359-2A*	1	1	100%	8	8	150–200	0.11
25	*Xgwm382-2A*	4	0	0%	8	32	50–200	0.75

Table 39. Molecular fingerprinting pattern by SSRs in the D-genome synthetic hexaploids of 'durum wheat/D-genome accessions' and their reciprocal crosses.

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)	PIC
26	*Xgwm425-2A*	3	0	0%	8	24	50–150	0.67
27	*Xgwm445-2A*	1	1	100%	1	1	200	0.37
28	*Xgwm473-2A*	1	1	100%	3	3	200	0.00
29	*Xgwm497-2A*	2	2	100%	7	7	200–250	0.24
30	*Xgwm512-2A*	1	1	100%	1	1	150	0.00
31	*Xgwm515-2A*	3	0	0%	3	24	50–150	0.67
32	*Xgwm558-2A*	4	2	50%	7	16	50–150	0.64
33	*Xgwm614-2A*	2	2	100%	8	12	50–150	0.37
34	*Xgwm2-3A*	1	1	100%	4	4	100	0.00
35	*Xgwm5-3A*	3	3	100%	8	11	100–500	0.41
36	*Xgwm32-3A*	2	2	100%	6	9	150–200	0.37
37	*Xgwm155-3A*	2	2	100%	8	12	100–250	0.37
38	*Xgwm162-3A*	2	2	100%	3	3	50–200	0.44
39	*Xgwm391-3A*	3	3	100%	7	13	50–800	0.65
40	*Xgwm462-3A*	1	1	100%	6	6	100–250	0.47
41	*Xgwm666.2-3A*	2	2	100%	4	4	50–100	0.37
42	*Xgwm4-4A*	1	1	100%	8	8	250	0.00
43	*Xgwm160-4A*	1	1	100%	1	7	200	0.33
44	*Xgwm397-4A*	1	1	100%	8	8	200	0.00
45	*Xgwm601-4A*	3	3	100%	7	15	50–200	0.58
46	*Xgwm610-4A*	2	2	100%	8	9	50–200	0.42
47	*Xgwm637-4A*	2	2	100%	8	15	500–800	0.49
48	*Xgwm126-5A*	3	3	100%	8	20	600–1000	0.64
49	*Xgwm129-5A*	1	1	100%	2	2	900	0.00
50	*Xgwm154-5A*	2	0	0%	8	16	50–100	0.50
51	*Xgwm156-5A*	2	2	100%	8	10	150–300	0.21
52	*Xgwm179-5A*	2	2	100%	8	10	150	0.21
53	*Xgwm186-5A*	3	3	100%	5	8	50–700	0.62
54	*Xgwm205-5A*	2	2	100%	5	5	50–150	0.32
55	*Xgwm291-5A*	1	1	100%	4	4	150	0.00
56	*Xgwm293-5A*	1	1	100%	8	8	200	0.00
57	*Xgwm304-5A*	1	1	100%	4	4	200	0.00
58	*Xgwm410-5A*	4	0	0%	7	28	200–300	0.75
59	*Xgwm415-5A*	1	1	100%	8	8	100	0.00
60	*Xgwm617-5A*	5	5	100%	8	28	50–800	0.80
61	*Xgwm639-5A*	2	0	0%	6	12	150	0.50
62	*Xgwm666-5A*	2	0	0%	8	16	50–100	0.50
63	*Xgwm169-6A*	2	2	100%	8	13	50–200	0.42
64	*Xgwm334-6A*	1	1	100%	3	3	150	0.00
65	*Xgwm459-6A*	1	1	100%	1	1	50	0.00
66	*Xgwm494-6A*	2	2	100%	8	10	50–200	0.21
67	*Xgwm570-6A*	1	1	100%	8	8	100	0.00
68	*Xgwm617-6A*	4	2	50%	8	22	50–400	0.66
69	*Xgwm60-7A*	2	0	0%	2	4	150–200	0.67
70	*Xgwm63-7A*	5	2	40%	8	33	300–1000	0.67
71	*Xgwm130-7A*	3	3	100%	8	11	50–100	0.53

Table 39. Molecular fingerprinting pattern by SSRs in the D-genome synthetic hexaploids of 'durum wheat/D-genome accessions' and their reciprocal crosses.

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)	PIC
72	*Xgwm233-7A*	1	1	100%	8	8	50	0.00
73	*Xgwm276-7A*	3	3	100%	8	17	50–100	0.62
74	*Xgwm332-7A*	3	1	33.33%	7	20	50–400	0.66
75	*Xgwm471-7A*	2	2	100%	8	8	100–150	0.46
76	*Xgwm635-7A*	1	1	100%	8	8	100	0.00
77	*Xgwm666-7A*	3	1	33.33%	8	23	50–300	0.66
78	*Xgwm11-1B*	2	2	100%	5	6	50–250	0.18
79	*Xgwm18-1B*	4	4	100%	8	14	50–300	0.58
80	*Xgwm33-1B*	3	3	100%	8	15	50–100	0.53
81	*Xgwm107-1B*	3	1	33.33%	6	16	200–300	0.65
82	*Xgwm124-1B*	3	3	100%	8	20	50–1000	0.65
83	*Xgwm131-1B*	3	1	33.33%	8	21	100–300	0.65
84	*Xgwm140-1B*	2	2	100%	5	5	50–400	0.32
85	*Xgwm153-1B*	4	4	100%	8	18	50–600	0.66
86	*Xgwm259-1B*	1	1	100%	2	2	100	0.00
87	*Xgwm264-1B*	3	1	33.33%	8	20	50–200	0.64
88	*Xgwm268-1B*	2	2	100%	2	2	200–250	0.50
89	*Xgwm274-1B*	2	2	100%	3	4	150–800	0.27
90	*Xgwm413-1B*	1	1	100%	3	3	100	0.00
91	*Xgwm498-1B*	4	4	100%	4	10	50–150	0.67
92	*Xgwm550-1B*	4	2	50%	4	8	150–250	0.68
93	*Xgwm582-1B*	2	2	100%	6	7	150–1000	0.48
94	*Xgwm16-2B*	3	3	100%	7	13	50–200	0.53
95	*Xgwm47-2B*	2	2	100%	8	11	50–200	0.29
96	*Xgwm55.1-2B*	3	3	100%	8	20	50–150	0.64
97	*Xgwm55.2-2B*	1	1	100%	6	6	100	0.00
98	*Xgwm129-2B*	2	2	100%	4	4	50–250	0.37
99	*Xgwm210-2B*	3	0	0%	1	3	50–150	0.66
100	*Xgwm257-2B*	3	3	100%	5	6	50–150	0.61
101	*Xgwm319-2B*	3	1	33.33%	3	6	50–200	0.61
102	*Xgwm374-2B*	3	3	100%	8	17	150–500	0.62
103	*Xgwm382-2B*	3	3	100%	8	11	50–250	0.75
104	*Xgwm388-2B*	2	2	100%	8	15	50–200	0.49
105	*Xgwm429-2B*	1	1	100%	5	5	250	0.00
106	*Xgwm501-2B*	2	2	100%	8	8	50–200	0.37
107	*Xgwm526-2B*	1	1	100%	3	3	150	0.00
108	*Xgwm630-2B*	3	1	33.33%	7	13	50–200	0.64
109	*Xgwm77-3B*	2	0	0%	7	14	50–150	0.50
110	*Xgwm112-3B*	3	1	33.33%	5	12	50–300	0.65
111	*Xgwm114-3B*	2	2	100%	7	11	50–150	0.48
112	*Xgwm131-3B*	3	3	100%	7	20	100–300	0.66
113	*Xgwm181-3B*	2	2	100%	7	8	50–100	0.45
114	*Xgwm247-3B*	2	2	100%	4	4	50–150	0.37
115	*Xgwm264-3B*	4	2	50%	7	17	50–500	0.74
116	*Xgwm284-3B*	2	2	100%	7	8	50–500	0.33
117	*Xgwm299-3B*	4	2	50%	4	7	50–300	0.67

Table 39. Molecular fingerprinting pattern by SSRs in the D-genome synthetic hexaploids of 'durum wheat/D-genome accessions' and their reciprocal crosses.

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)	PIC
118	*Xgwm340-3B*	4	4	100%	7	14	50–500	0.71
119	*Xgwm376-3B*	3	3	100%	7	11	150–400	0.56
120	*Xgwm389-3B*	2	2	100%	8	8	50–150	0.37
121	Xgwm493-3B	3	3	100%	7	10	50–200	0.64
122	Xgwm547-3B	2	2	100%	8	9	50–200	0.30
123	Xgwm566-3B	4	4	100%	7	12	50–300	0.64
124	Xgwm6-4B	3	3	100%	6	15	50–250	0.64
125	Xgwm66-4B	4	1	25%	8	18	50–300	0.69
126	Xgwm113-4B	1	1	100%	5	5	50	0.00
127	Xgwm149-4B	1	1	100%	5	5	50	0.00
128	Xgwm165-4B	4	1	25%	4	9	150–500	0.64
129	Xgwm251-4B	4	4	100%	7	9	50–250	0.65
130	Xgwm368-4B	1	1	100%	8	8	50	0.00
131	Xgwm495-4B	2	2	100%	8	8	50–150	0.37
132	Xgwm513-4B	2	2	100%	7	10	50–150	0.33
133	Xgwm538-4B	2	2	100%	8	14	50–150	0.50
134	Xgwm66-5B	5	5	100%	8	28	50–300	0.78
135	Xgwm68-5B	2	2	100%	8	11	50	0.49
136	Xgwm159-5B	2	2	100%	8	9	50–150	0.30
137	Xgwm191-5B	4	4	100%	8	18	50–300	0.70
138	Xgwm234-5B	2	2	100%	5	5	150	0.48
139	Xgwm335-5B	3	3	100%	5	6	50–150	0.46
140	Xgwm371-5B	9	7	77.77%	6	31	50–700	0.87
141	Xgwm499-5B	2	2	100%	8	13	50–200	0.49
142	Xgwm408-5B	2	0	0%	5	10	150–200	0.50
143	Xgwm540-5B	3	3	100%	7	9	50–250	0.25
144	Xgwm544-5B	3	3	100%	4	8	50–150	0.64
145	Xgwm554-5B	2	0	0%	8	16	50–200	0.50
146	Xgwm604-5B	2	2	100%	5	7	100–200	0.32
147	Xgwm639-5B	2	3	100%	4	7	50–400	0.46
148	Xgwm70-6B	4	2	50%	8	12	50–100	0.37
149	Xgwm88-6B	3	3	100%	8	12	50–150	0.40
150	Xgwm132-6B	4	4	100%	8	19	50–300	0.72
151	Xgwm191-6B	2	2	100%	7	11	100–800	0.40
152	Xgwm193-6B	1	1	100%	7	7	100	0.00
153	Xgwm219-6B	4	1	25%	8	26	50–900	0.73
154	Xgwm361-6B	3	3	100%	8	20	50–250	0.64
155	Xgwm508-6B	4	2	50%	7	17	50–200	0.74
156	Xgwm518-6B	5	5	100%	5	14	50–400	0.75
157	Xgwm613-6B	1	1	100%	6	6	150	0.00
158	Xgwm644-6B	2	2	100%	2	3	150–900	0.37
159	Xgwm43-7B	6	1	16.66%	5	10	150–1000	0.56
160	Xgwm46-7B	1	1	100%	4	4	150	0.00
161	Xgwm68-7B	1	1	100%	5	5	100	0.00
162	Xgwm146-7B	2	2	100%	8	13	50–150	0.42
163	Xgwm297-7B	4	4	100%	6	14	50–250	0.70

Table 39. Molecular fingerprinting pattern by SSRs in the D-genome synthetic hexaploids of 'durum wheat/D-genome accessions' and their reciprocal crosses.

Primer number	Primer	Total loci	Polymorphic loci	% polymorphism	Samples amplified	Scorable bands	Amplification product range (bp)	PIC
164	*Xgwm302-7B*	1	1	100%	5	5	50–250	0.00
165	*Xgwm333-7B*	2	2	100%	4	5	200	0.21
166	*Xgwm344-7B*	1	1	100%	3	3	150	0.00
167	*Xgwm400-7B*	1	1	100%	3	3	150	0.00
168	*Xgwm537-7B*	2	2	100%	3	3	50–200	0.44
169	*Xgwm577-7B*	3	1	33.33%	5	13	50–250	0.65
170	*Xgwm611-7B*	2	2	100%	3	3	50–200	0.44
171	*Xgwm644-7B*	2	2	100%	3	5	200–700	0.44
172	*Xgwm33-1D*	3	1	33.33%	8	23	50–100	0.64
173	*Xgwm232-1D*	2	2	100%	4	4	50–200	0.50
174	*Xgwm458-1D*	2	2	100%	8	9	50–100	0.49
175	*Xgwm642-1D*	2	2	100%	8	14	50–100	0.46
176	*Xgwm30-2D*	2	2	100%	3	4	150–700	0.00
177	*Xgwm102-2D*	2	2	100%	8	8	150–200	0.50
178	*Xgwm157-2D*	2	2	100%	5	5	150–200	0.32
179	*Xgwm210-2D*	3	3	100%	6	8	50–150	0.50
180	*Xgwm249-2D*	6	6	100%	6	18	50–1000	0.79
181	*Xgwm261-2D*	4	4	100%	8	21	50–200	0.72
182	*Xgwm296-2D*	1	1	100%	8	8	200	0.00
183	*Xgwm320-2D*	1	1	100%	2	2	100	0.00
184	*Xgwm455-2D*	2	2	100%	6	8	200–400	0.44
185	*Xgwm515-2D*	3	3	100%	8	17	50–200	0.62
186	*Xgwm539-2D*	4	2	50%	8	21	50–100	0.71
187	*Xgwm608-2D*	2	2	100%	5	5	150	0.48
188	*Xgwm2-3D*	2	2	100%	8	9	50–250	0.11
189	*Xgwm52-3D*	1	1	100%	4	4	100	0.00
190	*Xgwm71-3D*	2	2	100%	3	5	150–300	0.44
191	*Xgwm497-3D*	1	1	100%	6	6	100	0.00
192	*Xgwm645-3D*	2	2	100%	5	6	100–150	0.18
193	*Xgwm664-3D*	2	2	100%	6	6	150	0.18
194	*Xgwm194-4D*	4	2	50%	5	7	50–800	0.73
195	*Xgwm609-4D*	2	2	100%	5	8	50–1000	0.50
196	*Xgwm174-5D*	3	3	100%	7	11	50–150	0.65
197	*Xgwm292-5D*	1	1	100%	4	4	150	0.00
198	*Xgwm358-5D*	6	6	100%	7	22	150–900	0.79
199	*Xgwm565-5D*	1	1	100%	5	5	150	0.00
200	*Xgwm583-5D*	4	2	50%	8	24	50–150	0.71
201	*Xgwm654-5D*	3	3	100%	7	10	50–200	0.64
202	*Xgwm325-6D*	1	1	100%	1	1	100	0.00
203	*Xgwm469-6D*	1	1	100%	1	1	100	0.00
204	*Xgwm37-7D*	4	4	100%	8	17	50–250	0.63
205	*Xgwm44-7D*	2	2	100%	4	6	50–200	0.37
206	*Xgwm111-7D*	1	1	100%	3	3	100	0.00
207	*Xgwm295-7D*	4	4	100%	7	14	50–200	0.69

3 and 4 and the maximum value of 68.4% was present between genotypes 2 and 7. Only in one cluster so 1 and 2 represent the most diverse lines (Fig. 25); genotype 1 forms a group with 2, 3 with 6, 4 with 5 and 7 with 8.

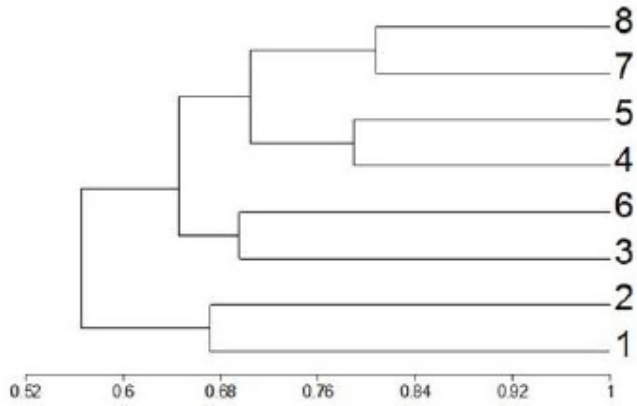


Fig. 25. SSR-based cluster formation of eight synthetic hexaploid genotypes of (same durum wheat cultivar with different *Aegilops tauschii* and the reciprocal crosses).

'Durum wheat / *Ae. tauschii*' and the reciprocal cross combinations. Four durum wheat cultivars were involved with four alien accessions; the pairs were entries 6 and 1, 5 and 2, 4 and 3, and 7 and 8. Individual pairs were structured around the durum wheat cultivar as the female parent and the *Ae. tauschii* accession as the male parent to allow cytoplasmic influences to be analyzed across each pair for diversity and other observed traits.

The overall comparison of each set was based upon eight traits; five phentypic (days-to-flowering, days to physiological maturity, 1,000-kernel weight, number of grains/spike, and spike length) and three biotic stress (Karnal bunt and seedling and adult-plant stripe rust resistance. For combination 6 and 1. no remarkable differences were seen phenotypically even though a cytoplasmic difference was introduced into the synthetic pair. Entry 1 was immune to KB but entry 6 was susceptible. Entry 1 was seedling susceptible to stripe rust, but 6 was immune. Entry 1 had APR, but 6 was susceptible. These differences indicate differential trait response around genomic interactions in which the cytoplasm may be a factor. The susceptibility of entry 6 suggests that the durum wheat attribute is suppressed by the *Ae. tauschii* parent. In entry 1, the KB resistance appears to be a consequence of expression of the durum wheat genomes and the inability of the D genome to mask the resistance effect or also contribute towards the resistance in a manner not well identified. For the 5 and 2 combination, entry 2, with the alien cytoplasm flowered 26 days later and days to physiological maturity (18 day delay); clear cytoplasmic effects. The 1,000-kernel weight, grains/spike, and spike length did not differ within the two categories. Both entries were KB resistant. Stripe rust resistance was seen in entry 5 at the seedling and adult-plant stage, but in entry 2, only APR was exhibited.

These trends were scattered across the other two sets that with entries 4 and 3 and, 7 and 8 (Table 37, p. 140). Remarkable in these sets, and similar to set 6 and 1, was KB resistance. Field resistance of the durum wheats is the normal expectation in synthetics derived from their parentage. However, when resistance is suppressed and the SH becomes susceptible is a clear indication to suggest that the expression is suppressed, which was seen in entries 6, 4, and 7. Their reciprocal crosses in entries 1, 3, and 8 are all KB resistant, suggesting that trait expression with the alien diploid parent as the female is preferred for breeding. Across all the eight combinations, lines 1, 5, and 6 are suitable for phenotypic attributes and KB and sripe rust resistance. Entry 1 was the most diverse based upon RAPD and SSR analysis and is a good candidate for breeding.

It would be logical to expect the diversity profiles to place both combinations of each set in close proximity. The trends do not seem to follow this perfectly. Sets 7 and 8 and 4 and 3 are linked as expected according to RAPD analysis. Entries 6 and 1 form a set that is the most diverse, and entries 5 and 2 fell in different clusters. Based upon SSR analysis, entries 7 and 8 were linked, but the other three sets were unrelated. In 3 and 4, entry 3 was more diverse, entry 1 was more diverse than entry 6, and entry 2 was more diverse than 5.

The question now arises as to why do reciprocal combinations show varied trends. A plausible answer may be due to structural genomic modifications that are ongoing during the maintenance of the individual SH entries. This implies that when the SH is first produced (C_0), its molecular profile would provide the relationship attribute similarity within its partners; e.g., 6 and 1. After generation advance, structural changes occur and consequently variations emerge to give the trend that is seen and which separates the set partners from each other. If the SH set variation occurs at the C_0 stage, then the variation is undoubtedly from the *Ae. tauschii* utilized in SH production is a population seed and not individual purified single accession seed used to develop the SHs. Variation in individual seedlings of an accession for traits have offered evidence that variation within an accession is prevalent and for precision experimentation, seed from individual alien seedlings must be obtained, utilizedc and used for comparative deductions.

The practicality of transferring alien genes to wheat.

Alvina Gul Kazi, Rabia Sultan, Awais Rasheed, Farrukh Bashir, Hadi Bux, Arsalan Ahmed, and Abdul Mujeeb-Kazi.

Diploid and polyploid species with nonhomologous genomes to those of wheat are included in the tertiary gene pool. Alien genetic transfers require complex cytogenetic manipulation protocols that facilitate homoeologous exchanges. Irradiation or tissue culture is another option when homoeologous exchanges are not possible. Protocols promoting homoeologous exchanges are preferred, because the introduced alien segments would be placed in the best location in the recipient chromosomes. Wheat–alien chromosome translocations are the general output of irradiation or tissue/callus culture and are less favored because compensating exchange products are rarely obtained. Hybridization requires embryo rescue and the frequency of success is low with practical agricultural output being time consuming unless modified approaches are incorporated. In essence, the choice of the parental alien species is crucial for successful wheat improvement programs that require several prerequisites beyond just producing novel hybrid combinations.

Genes can be transferred from primary and secondary gene pools to *T. aestivum* directly by hybridization, back crossing, and selection via chromosome recombination. From the tertiary gene pool, genetic transfers cannot be made easily by recombination and to obtain practical end-products, some transfer prerequisites include hybrid production, embryo rescue, plant regeneration, cytological diagnostics, breeding methodology, and stress screening, culminating in stability of the advanced derivatives as contributed by homozygosity. Wide hybridization, including both interspecific and intergeneric hybridization, is the first step to introduce alien variation and transfer desirable traits from wild species into cultivated wheat. The strategies encompass diversity resources across all gene pools.

Translocations have contributed significantly to disease resistance transfers with the major impact from the T1A·1R and T1B·1R translocations (presumably greater for T1B·1R because it influences *T. aestvum* cultivar yields). For the transfer of whole chromosome arms, the centric breakage-fusion mechanism of univalent at meiotic metaphase can be exploited. Univalents have a tendency to break at the centromere, followed by fusion of the broken arms. When an alien target chromosome and its homoeologous wheat chromosome are simultaneously univalent, compensating, whole-arm translocations can be recovered at fairly high frequencies. The first stable cytological transfer of a T1BL·1RS translocation from the bread wheat was from th eadvanced line Veery to the durum wheat Cando. The noncompensating translocations are genetically unbalanced and lead to reduced agronomic performance, whereas all wheat–alien translocations produced by induced homoeologous recombination are of compensating type and thus have greater agronomic potential.

The two categories of investigations on translocations are

1. Tapping existing global resources. Following the success of the T1BL·1RS spontaneous translocation in bread wheat, some such wheats were utilized to have a well documented base in Pakistan. In addition, a thrust was made to transfer some translocations globally available into elite bread wheat background with wide adaptation. The translocations selected include TT1AL·1RS, T5AL·5RS, T4BS·4BL-2R, T7DS·7DL-7Ag, and some Thatcher-based leaf rust stocks that include translocations carrying the *Lr* genes. The limited backcross route was used and germ plasm in each category advanced to BC_2. Translocation validation was aided by Giemsa C-banding.
2. New translocations focussed on the 'bread wheat/*Th. bessarabicum*' amphiploid where manipulating the *Ph* genetic control was exploited. This collaborative effort is still underway with partners in CIMMYT, Mexico. Eight translocation lines were produced that are monosomic or disomic euploid for a wheat–*Th. bessarabicum* translocation product. Our focus in Pakistan has been on the cytological validation of the new translocation lines, their phenotypic evaluation, and seed increase for subsequent biotic/abiotic stress analyses.

Exploiting translocations in wheat improvement. Translocations fall into two categories for utilization in wheat improvement and these are separated according to their origin.

1. Old translocations that are predominantly in genetic backgrounds that prevent their global exploitation readily, because the wheat involved is not widely adapted. Very few old translocation stocks are in extensive use. Thus, there is a need to transfer the ill-adapted translocations into good agronomic wheat backgrounds. Of the translocations that have been utilized here, some in extensive use and others are not in general use: T1AL·1RS, T5AL·5RS, T1BL·1RS, T4BS·4BL-2R, T7DS·7DL-7Ag, T2BS·2RL, T4BS·4BL-5RL, T6BS·6RL, and T2AS-2RS·2RL.

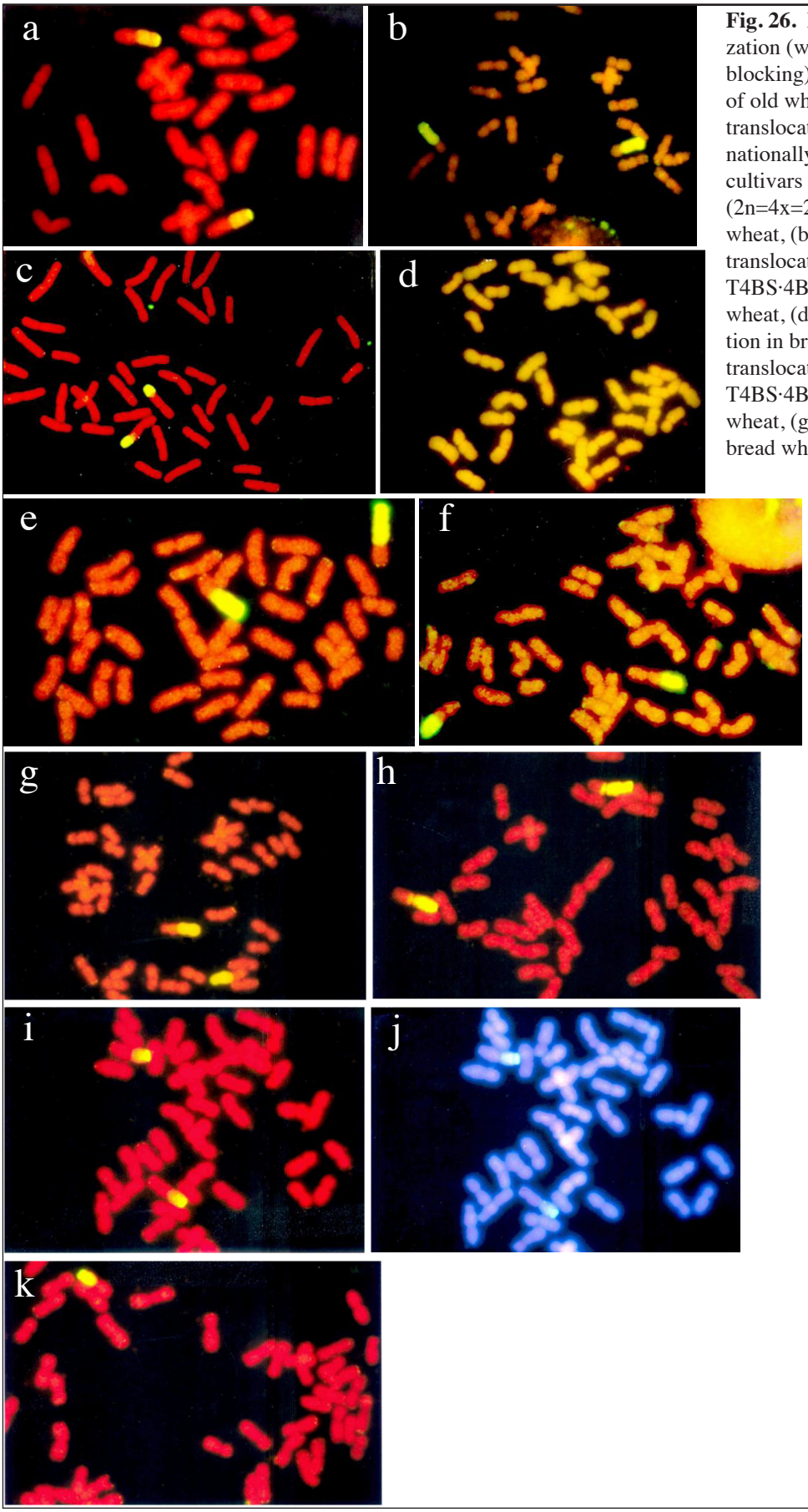


Fig. 26. Fluorescent *in situ* hybridization (wheat DNA and E^b DNA for blocking) demonstrating the usage of old wheat–alien chromosome translocations incorporated into nationally adapted, elite bread wheat cultivars and/or stocks: (a) T1AL·1RS (2n=4x=28) translocation in durum wheat, (b) T5AS·5RL (2n=4x=28) translocation in durum wheat, (c) T4BS·4BL-2R translocation in bread wheat, (d) T7DS·7DL-7Ag translocation in bread wheat, (e)T2BS·2RL translocation in bread wheat, (f) T4BS·4BL-6RL translocation in bread wheat, (g) T6BS·6RL translocation in bread wheat, (h) T2AS-2RS·2RL translocation in bread wheat, (i) T1BL·1RS translocation in bread wheat, (j) T1BL·1RS translocation in bread wheat (DAPI), and (k) T1AL·1RS (heterozygous) translocation in bread wheat.

Their status after backcrossing to elite wheats either durum or bread wheat has yielded advanced derivatives of which adequate seed amounts are available for exploitation in breeding for various stress factors after characterization. Details of the translocations transferred following reciprocal backcrossing to commercial wheats are elucidated (Figs. 26, p. 151; T1AL·1RS (a) and T5AL·5RS in durum wheat (b), T4BS·4BL-2R (c) and T7DS·7DL-7Ag in bread wheat (d), T2BS·2RL in bread wheat (e), T4BS·4BL-4RL in bread wheat (f), T6BS·6RL in bread wheat (g), T2AS-2RS·2RL in bread wheat (h), T1BL·1RS in bread wheat (i and j; two filters), and T1AL·1RS in bread wheat (a heterozygote (k) from which a homozygous form was obtained).

2. New Translocations. By utilizing the recessive *ph1b* genetic stock the CS–*Th. bessarabicum* amphiploid with the *Ph1bPh1b* locus was manipulated cytogenetically to set up a wheat–alien recombination system that would induce homoeologous translocations.

The generation of new translocations is schematically shown (Fig. 27). The protocol is elucidated in the steps described below accompanied by characterization. The use of the molecular diagnostics identifies the selfed BC_1 derivatives that are *ph1bph1b* homozygous (Fig. 28a, p. 153). These plants are then selfed to maximize the chances of obtaining wheat–alien translocation events. Once these are observed, the dominant *Ph1b* system is restored by backcrossing the translocation plants to any elite wheat cultivar. The process ends with the recovery of euploid, 42-chromosome derivatives that carry the translocation. This stock is maintained for subsequent use in agriculture via trait identification.

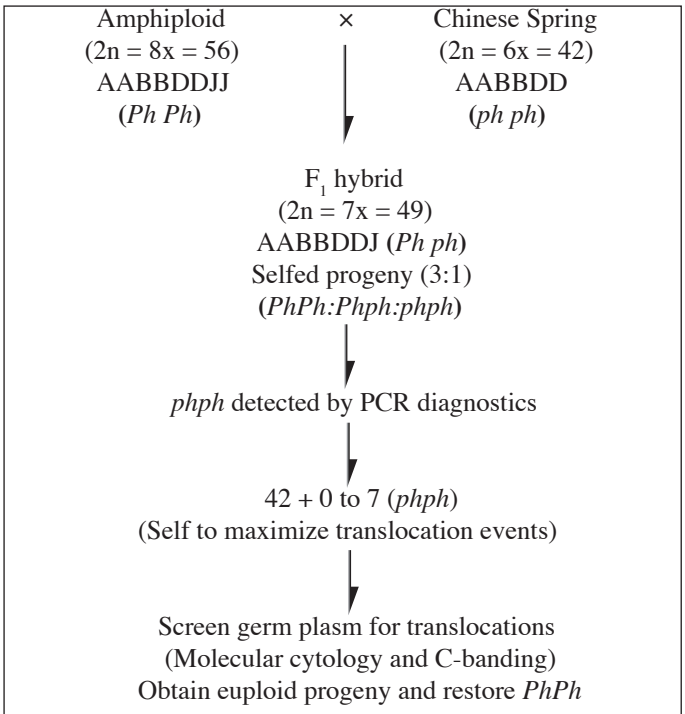


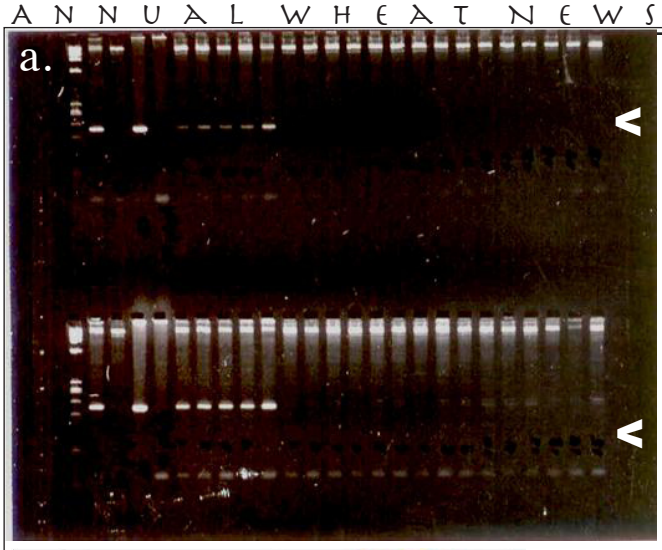
Fig. 27. Schematic elucidating the recurrent backcrossing steps used to transfer the existent wheat/alien chromosome translocations into agronomically superior wheat cultivars.

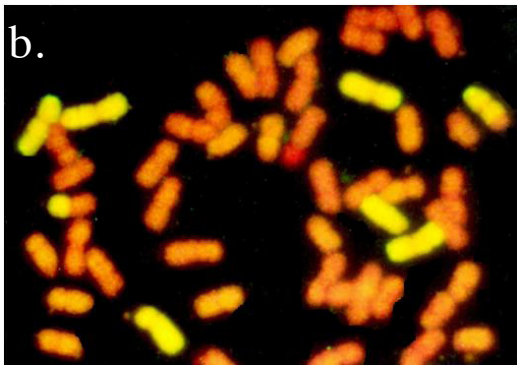
In the initiation of the translocation, six E^b chromosomes are visible in FISH and two are translocated; one is a Robertsonian and one has a terminal exchange with more of an E^b constitution (Fig. 28b, p. 153).

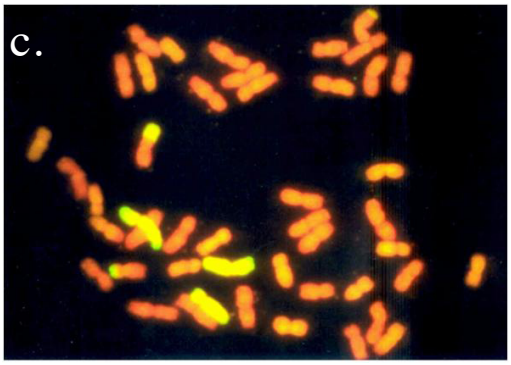
Additional plants where a varying number of translocations are present need to be separated as single events and turned into euploids for subsequent screening and practical utilization; two translocated chromosomes and three complete alien chromosomes (Fig. 28c, p. 153) and a desirable alien exchange (Fig. 28d, p. 153). Further backcrossing of such plants will give rise to derivatives with reduced chromosome number. Ultimately, plants with one alien and one translocation chromosome(Fig 28e, p. 153) and one translocation chromosome (Fig. 28f, p. 153) are obtained. These plants are ideal for sources of restoring the *Ph* system by backcrossing and obtaining euploids that will have 40 wheat chromosomes and a disomic pair that is translocated (Fig. 28g, p. 153). Translocations that are undesirable also are obtained and discarded as they have too much alien chromatin (Fig. 28h, p. 153).

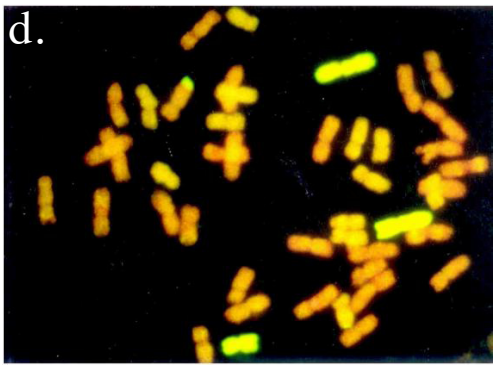
Over the course of producing these new translocations, some euploid derivatives have been obtained that are *Ph1bPh1b* homozygous (Table 40, p. 154). All plants have 42 chromosomes and the translocations have been identified by the conventional Giemsa C-banding (Fig. 29, p. 154) for T6BS·6BL-6E^b(J), T5DL·5DS-5JS, T1DS·1JS, and T3BL·3JS. The translocations advanced to the 42-chromosome, euploid level are characterized as homoeologous, nonhomoeologous, Robertsonian, and smaller exchanges. Some characteristics of these translocations also are of good fertility and acceptable agronomic plant type.

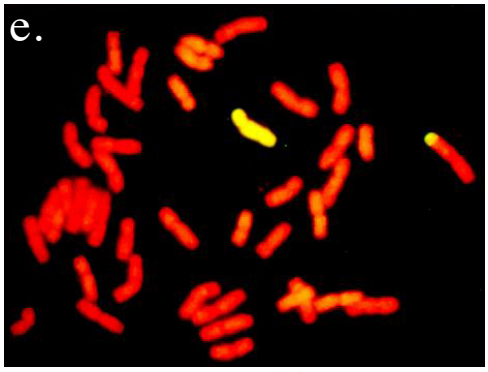
Translocations in wheat breeding. Cytogeneticists have produced wheat–alien chromosome translocations over past several decades that hold the tremendous potential of utilization in wheat improvement programs. Globally, The main translocation in practical use is the spontaneous T1BL·1RS Robertsonian exchange that occurred as a consequence of a centric breakage-fusion event. CIMMYT put this translocation to maximum use in spring habit wheat emanating from

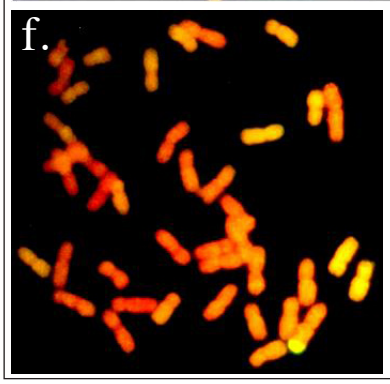


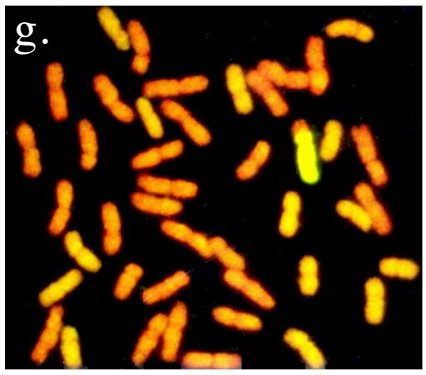












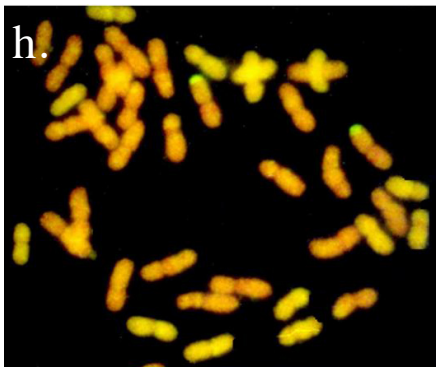


Fig. 28. Steps for producing new translocations generated by exploiting the *ph1b* cytogenetic system: (a) a PCR marker identifies the *ph1b* plants for use in promoting wheat/alien chromosomal exchanges (arrows show *Ph* derivatives with presence and absence of the band are derivatives with the *ph1b* locus; (b) BC_1 plants homozygous for the *ph1bph1b* locus, 50 chromosomes (6 E^b + 1 Robertsonian translocation + 1 terminal alien segment); (c) BC_1 plant derivative with 47 chromosomes (3 E^b + 2 with terminal exchanges on the long and short arms; (d) BC_1 plant derivative with 45 chromosomes (3 E^b + 1 with a terminal exchange); (e) a selfed BC_1 plant with 42 chromosomes (1 E^b + 1 translocation), a source for restoring the *PhPh* composition by backcrossing with Ph and for selecting euploid 42 derivatives with the translocation homozygote; (f) a selfed BC_1 plant with 42 chromosomes (1 translocation), a source for restoring the *PhPh* composition and generating homozygous euploid translocation derivatives; (g) derivatives with an excess of alien material translocated; and (h) a 42-chromosome translocation homozygote derivative with a small alien segment transferred.

the initial spring/winter wheat crossing protocol taken up in mid-1970s. The next translocation in practical use is the T1AL·1RS Robertsonian exchange linked with the cultivar Amigo for greenbug resistance. This translocation could be a consequence of univalent misdivision and/or irradiation. Amigo has entered into wheat breeding programs but to a lesser degree than T1BL·1RS. The cultivar TAM200 charaterizes this translocation. Both of these translocations are present in good agronomic type wheat and, thus, are ready for their rapid exploitation. Other translocations involving

Table 40. Details of some new translocation euploid derivatives with 44 chromosomes involving Bread wheat/*Thinopyrum bessarabicum* exchanges

Sample	Translocation	Phenotypic detail (Mean)				
		Spike length (cm)	Days-to-flowering	Nodes/spike	Plant height at maturity (cm)	Grains/spike
WWX-1	T7DS·7DL-4J	14.2	105	26	82	58
WWX-2	T6BS·6BL-6J	14.5	100	27	78	44
WWX-3	T6JS·7DL	18.0	108	26	84	27
WWX-4	T5JS-5DS·5DL	13.5	105	24	72	40
WWX-5	T1DS·1JS	15.5	98	26	80	40
WWX-6	T1AS·1AL-1JL	15.0	100	27	75	33
WWX-7	T3JS·3BL	12.5	95	24	80	54

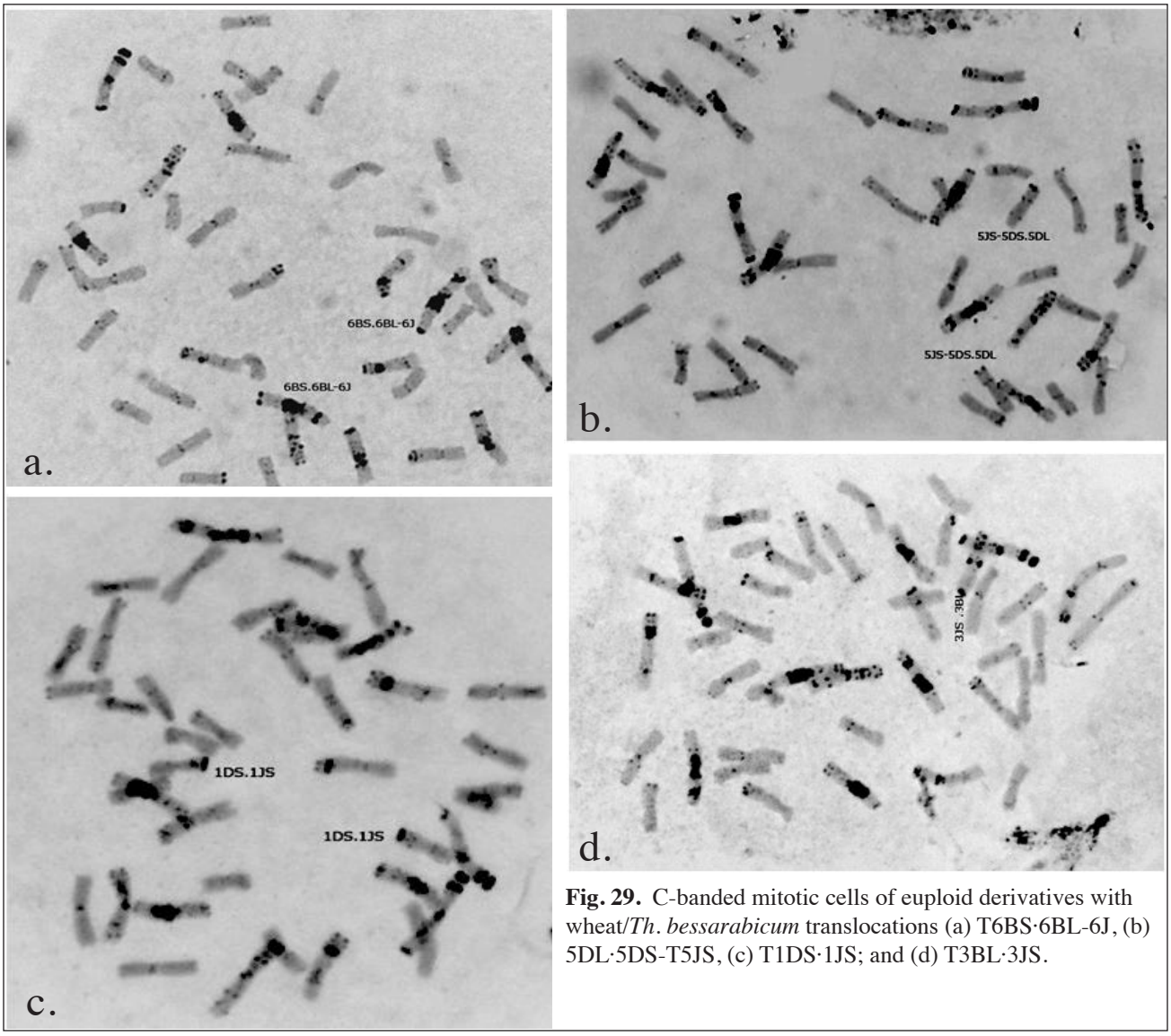


Fig. 29. C-banded mitotic cells of euploid derivatives with wheat/*Th. bessarabicum* translocations (a) T6BS·6BL-6J, (b) 5DL·5DS-T5JS, (c) T1DS·1JS; and (d) T3BL·3JS.

T5AL·5RS, T4BS·4BL-2R, T7DS·7DL-7Ag, T2BS·2RL, T6BS·6RL, and T2AS-2RS·2RL as some examples have been slow to exploit as they are present in wheat backgrounds that are poorly adapted globally. Several rust genes associated with translocations are either present in the cultivars Chinese Spring or Thatcher both of those are not amenable for appropriate field screening. A strategy is to transfer these translocations into well adapted wheat cultivars and was an effort taken on in this study. Backcrossing elite widely adapted national wheat onto the globally available translocations twice

followed by a selfing has given a usable stock of all the above translocations in an improved wheat phenotype that can be screened for various stresses under field situations. With an additional two crosses, the phenotype would be significantly improved and resemble the recurrent wheat cultivar used in backcrosses around 93%. The possibility of enriching the durum wheat was explored and, from a pentaploid F_1 followed by backcrosses with durum, recovering a durum wheat with the translocation was routine T1AL·1RS and T5AS·5RL.

Production of new wheat–alien chromosome translocations. With the success of the existing translocations, and the anticipated promise of exploiting those that are being transferred into elite agronomically superior widely adapted wheat cultivars during the last decade efforts have emerged that target wheat genetic stocks using cytogenetic manipulation systems to promote chromosomal exchanges. The options available for targeting on homoeologous exchanges are favoured by the use of *Ph*^{I} or the *ph1b* stocks. The focus in this study has been on the latter and exploited has been the CS/*Th. bessarabicum* amphiploid combination that is *PhPh* in its genetic control structure. We have shown how the crossing of this amphiploid with the *phph* stock could become a source of new translocations. In Pakistan, this directional exploitation was maximized around a collaboration with CIMMYT over the last five years. The various steps for the initiation of multiple translocations (Fig. 28, p. 153) culminate with disomic translocation stocks that are identified after C-banding (Table 40, Fig. 29, p. 154). The protocol is ideal for improvement of plant type and also for forcing exchanges within homoeologous groups. When the *Ph1bph1b* derivatives are produced, options are to either produce polyhaploids or self the heterozygote. In the first case, haploids will be obtained that are *Ph1b:ph1b* in a 1:1 ratio. In the *Ph1bph1b* selfs, the derivatives will have a 1:2:1 segregation progeny of plants that are *Ph1bPh1b* ; *Ph1bph1b* : *ph1bph1g*. Only the *ph1b* or *ph1bph1b* derivatives will be the candidate plants to give rise to translocated progeny upon advance (Fig. 27, p. 152).

The *ph1b* haploids upon doubling give derivatives that are *phph* and these upon several selfings will generate progeny that have translocations (Fig. 27, p. 152). The progress is similar with the *ph1bph1b* plants from selfing of the *Ph1bph1b* source. Once the translocations are detected, a backcross with an elite *Ph1bPh1b* wheat cultivar that is the first step to restore the *Ph1bPh1b* system. Backcrosses and cytology are required to deliver the final euploid product that is 2n=6x=42 (AABBDD) and has the translocation as a disomic homozygote. The most advanced euploid derivatives generated are validated with Giemsa C-banding (Table 40, p. 154).

Of the numerous wheat–alien translocations that were initiated during the selfing of the *ph1bph1b*-derived constitutions, seven euploid events are described that have various homozygous exchanges, are euploid and possess acceptable seed fertility plus plant types. These are:

1. T7DS·7DL-4E^{b} is a nonhomoeologous translocation where the 4E^{b} presence may possibly disturb the group 7 wheat homoeology,
2. T6BS·6BL-6E^{b} is an ideal homoeologous euploid exchange derivative with good compensation anticipated for group 6,
3. T7DL·6E^{b}S is a nonhomoeologous Robertsonian event and not preferred as it possesses a full alien arm that may add to negative aspects when used in wheat breeding, however, fortuitous benefits are possible and screening for stress factors will surely occur,

4-5. T5DL·5DS-5E^{b}S and T1AS·1AL-1E^{b}L are both highly desirable homoeologous derivatives that involve groups 5 and 1, respectively, fitting the alien chromosome exchange trait advantage value expectation, and

6-7. two other Robertsonian translocations T1DS·1 E^{b}S and T3BL·3E^{b}S are advantageous over T7DL·6E^{b}S in that both are homoeologous in nature, which may support their usage in practical agriculture for trait benefits.

All the translocations have satisfactory phenotypes that support each to be exploited in breeding programs. Plant height at maturity renders them as good donor candidates for derivatives to be targeted for irrigated agriculture with a height range of 72–84 cm. Complimenting height are spike length, satisfactory with 17–58 grains/spike coupled with 98–108 days to maturity and a nodal number range of 24–27. All alien exchanges were terminal and no interstitial exchanges were generated.

Using the amphiploid base of intergeneric hybrids is ideal for generating a high frequency of translocation events that may address traits not well defined for their inheritance or those of polygenic nature, e.g., salinity, drought, and heat. This basic effort has defined the route of how translocations can be obtained and for the future leaves behind the option to target the strategy for greater efficiency by exploiting the wheat/maize haploidy system.

Targeting individual alien chromosomes that are trait positive, each of the seven addition lines of *Th. bessarabicum* could be crossed with the CS *phbph1b* stock BC_1 progenies for each chromosome; 2n=6x=42 + 1 E^b1E^b (*Ph1bPh1b*) / CS (*ph1bph1b*) = 42 (*Ph1bph1b*) + 1 E^b to 7 E^b.

The various 43-chromosome *Ph1bph1b* derivatives would be the future candidates that lead to generating haploids that are 21 (*Ph1b*), 21 + 1E^b (*Ph1b*), 21 (*ph1b*), and 21 + 1E^b (*ph1b*). The 21+ 1E^b (*ph1b*) plants would be doubled, selfed, and progeny screened for expected translocations between 1E^b and 1A, 1B, or 1D, after which the line would be restored to *Ph1bPh1b* to recover euploid products that have 42 chromosomes, have the homoeologous exchange, are cytologically characterized, and trait positive as identified earlier in the alien disomic addition line.

Alien genetic transfers are the easiest to achieve via homologous exchanges and such are the preferred means where closely related sources are used that reside in the primary gene pool. Tapping into the secondary pool has some complexity and is the next option. The tertiary resource, due to its most complex nature of genetic transfer, reflects a challenge that researchers have pursued consistently. From producing the intergeneric hybrids to the end product are all complex steps, but the potential value of tertiary gene pool diversity are ranked extremely high. Progress is generally slow, but practical benefits are very promising. So far, the maximum benefits have been through the spontaneous T1BL·1RS translocation, but others are being incorporated. For maximizing the use of these combinations, it is imperative that they are in good agronomic backgrounds so they fit the field cropping cycles appropriately and are easier to screen and then have a days to flowering duration that is similar to that of the wheat crop. Using a reciprocal backcrossing protocol, some of the major globally available translocations have been placed in good agronomic type spring wheat that are good candidates for further practical utilization. The existing translocations have been made user friendly.

The merit of such interest on producing new forms opens up a huge arena of investigations that can enrich wheat genetic diversity. This one combination (wheat–*Th. bessarabicum*) has just scratched the surface of what lies ahead. Seven translocation products and numerous others in the research pipe line is extremely encouraging. We envision the possibility of enhancing the output, developing markers for the exchange sites, and using the gene bank wheat–alien reservoir for initiating further translocation output efforts.

Cytological and morphological characterization of a Thinopyrum bessarabicum amphiploid and its addition lines.

Alvina Gul Kazi, Rabia Sultan, Awais Rasheed, Hadi Bux, Usman Rahim, Abdul Aziz Napar, and Abdul Mujeeb-Kazi.

The three wheat genomes are believed to have been derived from single ancestral diploid species each having seven pairs of chromosomes. The haploid chromosome complement of bread wheat is made up of seven groups of three related chromosomes. These genetically related chromosomes from different genomes are referred to as homoeologous chromosomes.

Intergeneric crosses involve alien species that are extremely diverse genomically and their hybridization with wheat is complex. When hybridized, the combinations exhibit meiotic details that suggest little to no intergenomic chromosomal associations. Despite these constraints, research interest has remained high since the late 1980s and hybridization progress remained a major obstacle until the mid-1990s. Some intergeneric hybrids were easy to produce whereas others were more difficult. Subtle manipulations and careful choice of parents were a solace and widened the hybridization categories. The manuipulation categories were influenced by various factors and have been extensively reported and reviewed.

Disomic addition lines, in which single pair of homologous chromosomes from a related species is added to the wheat complement, are used to identify alien chromosomes carrying useful genes and are the starting point for the cytogenetic transfer of alien genetic material to wheat. In general, such lines have been agronomically inferior to wheat, are not entirely stable, and require cytological maintenance at each selfing generation. Stocks can degenerate quickly once the added chromosome has been lost in even a small percentage of the population, because male gametes carrying an alien chromosome are less competitive than normal wheat gametes. The method for the addition of a single, alien, chromosome pair into a recipient cultivar involves hybridization followed by backcrossing the hybrid or amphiploid to the recipient species and selecting addition lines from the backcross progeny. This procedure is now regarded as the standard method of producing alien chromosome addition lines. Using this technique, alien addition lines have been

produced in wheat with single added chromosome pairs from *Aegilops, Agropyron, Dasypyrum, Secale*, and *Hordeum*. None of the alien chromosome addition lines has become popular as a commercial cultivar, because of the instability of the alien chromosomes and the incorporation of undesirable characteristics associated with them. They are, however, a crucial starting point for allowing cytogenetic-based segmental transfers and use in wheat breeding.

Three wheat–*Th. intermedium* addition lines were developed to BC_3F_3 and showed good resistance to barley yellow dwarf virus. *Th. bessarabicum* addition lines were developed in *T. aestivum* for chromosomes of groups 1 to 7 and were identified through biochemical diagnostic markers with the help of FISH.

The diploid grass *Th. bessarabicum* (2n=2x=14 JJ or E^bE^b) is an excellent source of salt tolerance and crossing with the cultivar Chinese Spring (CS) (2n=8x=56; $AABBDDE^bE^b$) yielded an amphiploid from which seven disomic chromosome addition lines were developed. The first six were developed at CIMMYT, Mexico, and the seventh addition line in Pakistan. Our specific studies were

cytological characterization of the amphiploid and its disomic addition lines using conventional cytological protocols,

morphological characterization and documentation of the germ plasm, and

seed increase for the on-going projects and biotic and abiotic stress evaluations.

The parents, CS, diploid *Th. bessarabicum*, the six disomic addition lines in a Genaro or CS background, the CS–*Th. bessarabicum* amphiploid, the *ph1b* genetic stock, and selfed derivatives were the source of new translocations initiated at the 49-chromosome selfed level. Studies in Pakistan focussed on seed increase of the amphiploid and parental seed and the amphiploid was cytologically characterized. The F_1 perennial hybrid was studied in a living herbarium that is maintained at CIMMYT through exchange of F_1 clones that gave the option to characterize the F_1 hybrid in Pakistan. All acquired disomic additions and a multiple disomic addition for group 6 and 7 enabled the generation of the group-7 disomic addition. All addition liness were backcrossed with the Pakistani wheat Inquilab twice, followed by selfing to extract the respective disomic additions across all seven groups, i.e., $1E^b$ to $7E^b$. In the process, new BC_1 derivatives also emerged that are reported.

The spike morphology of various phases of exploiting the *Th. bessarabicum* variation in a CS background and the incorporation of other elite wheats in the cross combinations shows the co-dominant phenotype in all cross derivatives where the parental wheat phenotype is modified, giving evidence of alien genetic expression in a wheat background (Fig. 30). The spike structure of CS wheat and *Th. bessarabicum*, dorsal and ventral views of the F_1 hybrid (2n=4x=28, $ABDE^b$), the 56-chromosome amphiploid, are compared to two spikes at the BC_1 stage with cultivars Pavon and Pak-81 (CS/*Th. bessarabicum*//Pavon or Pak-81 with 2n-7x=49, $AABBDDE^b$ chromosomes, Fig. 30f and 30g).

Fig. 30. Spike morphology of prebreeding germ plasm involving bread wheat (2n=6x=42, AABBDD) and *Thinopyrum bessarabicum* (2n=2x=14; E^bE^b): (a) Chinese Spring (CS) wheat (2n=6x=42, AABBDD), (b) *Th. bessarabicum* (2n=2x=14, E^bE^b), (c) CS/*Th. bessarabicum* F_1 hybrid (2n=4x=28, $ABDE^b$, dorsal view), (d) CS/*Th. bessarabicum* F_1 hybrid (2n=4x=28, $ABDE^b$, ventral view), (e) CS/*Th. bessarabicum* amphiploid (2n=8x=56, $AABBDDE^bE^b$), (f) CS/*Th. bessarabicum*//Pavon selfed BC_1 (n, 2n=7x=49; $AABBDDE^b$), and (g) CS/*Th. bessarabicum*//Pak81 selfed BC_1 (n, 2n=7x=49; $AABBDDE^b$).

Cytological validation. The amphiploid with a normal 56-chromosome complement, which was generally the case in C_0 and C_1 seed, was somatically analyzed (Fig. 31a, p. 158). All seven C_0 seed had 56 chromosomes (Fig. 31a, p. 158). Five of the C_0 derivatives bred true and had 56 chromosomes. Two C_0 derivatives gave aneuploid progeny that were hypoploid (54 and 55 chromosomes, Figs. 31b

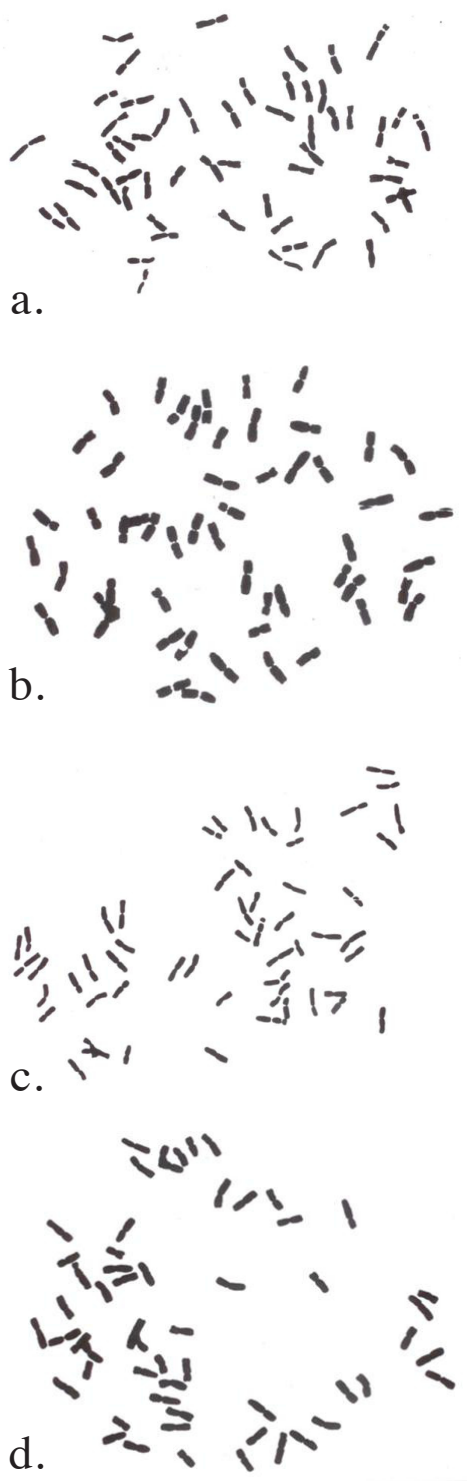


Fig. 31. Somatic chromosome numbers of various amphiploid plants of the 'bread wheat/*Thinopyrum bessarabicum*' intergeneric combination: (a) a mitotic cell with the normal chromosome number of 2n=8x=56 ($AABBDDE^bE^b$), (b) a hypoploid cell with 54 chromosomes, (c) a hypoploid cell with 55 chromosomes, and (d) a hypoploid cell with 57 chromosomes.

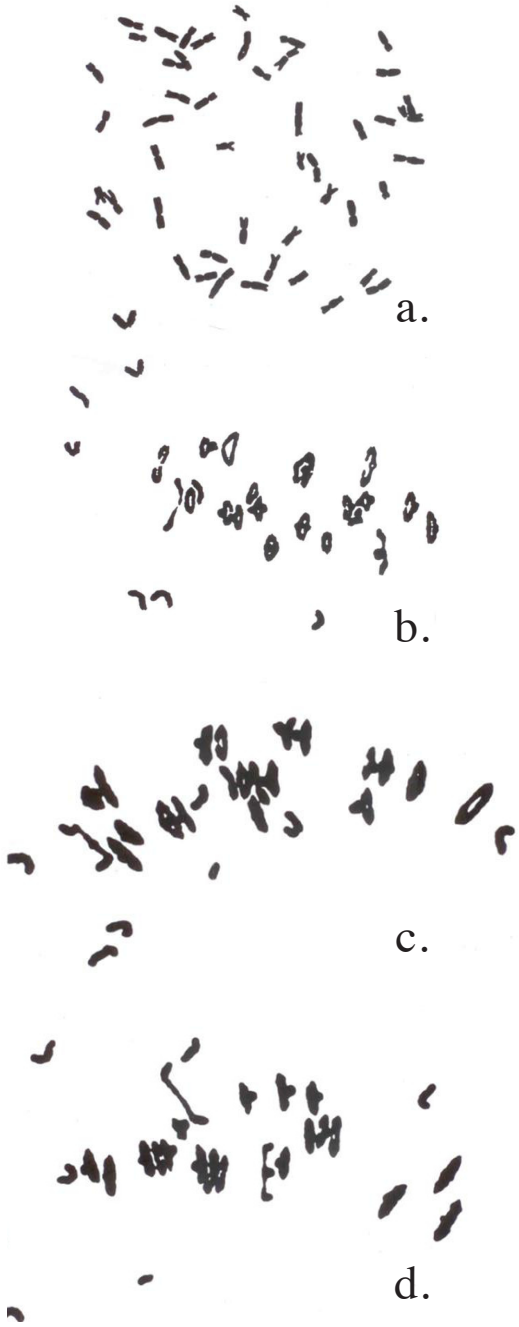


Fig. 32. Somatic and meiotic details of a BC_1 derivative from 'bread wheat/*Thinopyrum bessarabicum*//bread wheat' with 2n=7x=49 chromosomes ($AABBDDE^b$): (a) a somatic BC_1 cell with 49 chromosomes, (b) a BC_1 meiotic cell with 49 chromosomes associated at metaphase 1 as 2 rod bivalents + 19 ring bivalents + 7 univalents, (c) a BC_1 meiotic cell with 49 chromosomes associated at metaphase I as 1 rod bivalent + 20 ring bivalents + 7 univalents, and (d) a BC_1 meiotic cell with 49 chromosomes associated at metaphase I as 2 rod bivalents + 19 ring bivalents + 7 univalents.

and c, p. 158), or hyperploid (57 chromosomes), (Fig. 31d).

Crossing the CS–*Th. bessarabicum* amphiploid with Pavon or Pak-81 gave BC_1 derivatives that generally were normal, but in some cases aneuploid. In the normal derivatives, the somatic count is 49 chromosomes (Fig. 31a) and at meiosis, upto 21 bivalents (varying rods and rings) plus seven univalents (Fig. 31b, c, and d).

The aneuploid BC_1 derivatives are cytologically possess 47, 48, and 50 chromosomes (Fig. 33a, b, and c, p. 159), which were less frequent and less than 3% (6 of the 200 BC_1 observed). The aneuploid trend however became more pronounced when the aneuploid and normal BC_1s were selfed several times, but univalent retention was very high.

Exploiting the

Fig. 33. Somatic details of a selfed BC_1(n) from 'bread wheat/*Thinopyrum bessarabicum*//bread wheat' (n) with retention of a high number of alien chromosomes: (a) BC_1(n) with 47 chromosomes; (b) BC_1(n) with 48 chromosomes, and (c) BC_1(n) with 50 chromosomes.

BC_1 derivatives. The normal BC_1 derivatives were advanced in a dual manner. When selfed several times, a unique gametic transmission behavior was observed where instead of a progressive loss of the alien univalents, they were retained and counts of the selfed progenies were much closer to the expected 49 chromosomes (Fig. 34a, a somatic BC_1n cell with 48 chromosomes and a meiotic association at metaphase 1 of various univalents and bivalents that characterize alien chromosome retention (Fig. 34b and c).

Fig. 34. Somatic and meiotic details of a 'bread wheat/*Thinopyrum bessarabicum*//bread wheat' BC_1(n) selfed derivative: (a) with 48 somatic chromosomes, (b) a meiocyte at metaphase I with 6 univalents + 1 rod bivalent + 20 ring bivalents, and (c) a meiocyte at metaphase I with 6 univalents + 2 rod bivalents + 19 ring bivalents.

Further backcrossing of the BC_1 individuals with Pavon or Pak-81 or even of the acquired disomic additions to extract new disomic addition lines with 44 chromosomes that represent the group 1–7 addition series (Fig. 35a, a somatic cell with 44 chromosomes, (b) a meiocyte with 22 bivalents associated as 3 rod and 19 ring bivalents, which separate at anaphase I normally in a 22 + 22 split (c)).

Fig. 35. Advanced BC_1(n) derivatives from 'bread wheat/*Thinopyrum bessarabicum*//bread wheat' (n) showing a disomic *Th. bessarabicum* chromosome addition line at mitosis and meiosis (a) with 44 somatic chromosomes, (b) with 44 chromosomes associated at metaphase I as 3 rod + 19 ring bivalents, and (c) with 44 chromosomes at anaphase I showing a normal 22/22 separation.

Identification and maintenance of addition lines. With the backcrossing approach, a double disomic addition stock for group-7 was extracted. The homoeology of other six groups was identified and these were backcrossed onto local cultivars from which modified phenotypic additions could be extracted for groups 1 to 6 in a nationally adapted background of Inquilab. The addition lines all had 44 chromosomes and with 22 bivalents at meiosis. Seed set was uniformally good across all the lines and from each addition line, five plants were selected as base material for subsequent advance and maintenance. The details of the seven disomic addition lines relative to plant height at maturity, days to maturity, spike length, and seed/spike are in Table 41. Homoeologous groups $2E^b$, $3E^b$, $4E^b$, and $5E^b$ are easily identified by morphology, such as tapering spike (Fig. 36a), solid stem (Fig. 36b), blue aleurone (Fig. 36c), and club-shaped spike (Fig. 36a). The status of each of the disomic lines after two cycles of increase and at the end of the current 2009–10 crop cycle is given in Table 42, including the number of individual plants that were somatically counted and found to contain 44 chromosomes.

Table 41. Mean characteristics of the *Thinopyrum bessarabicum* disomic addition lines (2n=6x=42 + $1E^bE^b$ to $7E^bE^b$).

Addition line	Characteristics			
	Height at maturity (cm)	Days to maturity	Spike length (cm)	Number of seeds/spike
$1E^bE^b$	66	110	11.0	65
$2E^bE^b$	72	110	11.0	70
$3E^bE^b$	82	113	14.0	55
$4E^bE^b$	78	114	12.0	50
$5E^bE^b$	72	112	10.0	37
$6E^bE^b$	58	104	6.5	28
$7E^bE^b$	55	114	12.0	22

Fig. 36. Morphological markers identifying some wheat/*Thinopyrum bessarabicum* disomic addition lines (2n=6x=44): (a) Chinese Spring and homoeologous group 2 and 5 disomic addition lines with a tapering ($2E^b$) and club ($5E^b$) shaped spikes; (b) a group-3 disomic addition line ($3E^b$) with a solid stem (left) versus a normal hollow stem (right), and (c) a group-4 disomic addition line ($4E^b$) with blue aleurone (right) versus normal (left) colored wheat seed

The complete cytological details at the *in situ* hybridization level are shown (Fig. 37, p. 161). The F_1 hybrid with 28 chromosomes (Fig. 37a, p. 161) and the seven alien E^b chromosomes are differentiated due to their yellow color in the fluorescent *in situ* hybridization preparation. Wheat DNA was the blocking source and the E-genome DNA was used for blocking (Fig. 37a, p. 161). In the amphiploid somatic cell, the 14 E^b chromosomes are similarly differentiated (Fig. 37b, p. 161). The seven disomic addition lines are similarly identified ($1E^b$ (Fig. 37c, p. 161) a DAPI filter is used; $2E^b$ (Fig. 37d, p. 161), $3E^b$ (Fig. 37e, p. 162), $7E^b$ (Fig. 37f, p. 161), $4E^b$ (Fig. 37g, p. 161), $5E^b$ (Fig. 37h, p. 161), and $6E^b$ (Fig. 37i, p. 161). In all, the 42 wheat chromosomes are evident and the two alien E^b pair for each group clearly differentiated.

The germ plasm resource that is present at CIMMYT, Mexico, was investigated at NARC, Pakistan, from 2005 to 2010. The environmental conditions in Mexico were amenable for maintaining all perennial hybrids and the initial F_1 hybrid (bread wheat/*Th. bessarabicum)* was available from which clones were brought to NARC for analysis. Spike samples of the alien species from the source were used in Fig. 36. The CS spike shows a modified phenotype in the F_1 and the variation was inherited by the 2n=8x=56 $AABBDDE_bE_b$ amphiploid. The two BC_1 derivatives also have carried the modified phenotype, an important characteristic because it indicates modified expression (co-dominance) suggesting that alien characteristics can be transferred with a high chance of getting practical benefits towards improving wheat. Maintaining aneuploidy is

Table 42. Status of seed amounts produced from each *Thinopyrum bessarabicum* disomic addition lines at the end of the 2009–10 crop cycle at NARC, Pakistan. The seed number is the mean number of spikes multiplied by number of plants.

Addition line	Total plants	Seed number 2009–10	Reserve from 2008–09
$1E^bE^b$	305	19,500	500
$2E^bE^b$	90	6,300	500
$3E^bE^b$	52	2,860	500
$4E^bE^b$	310	15,500	500
$5E^bE^b$	285	10,545	500
$6E^bE^b$	4	112	100
$7E^bE^b$	173	3,806	100

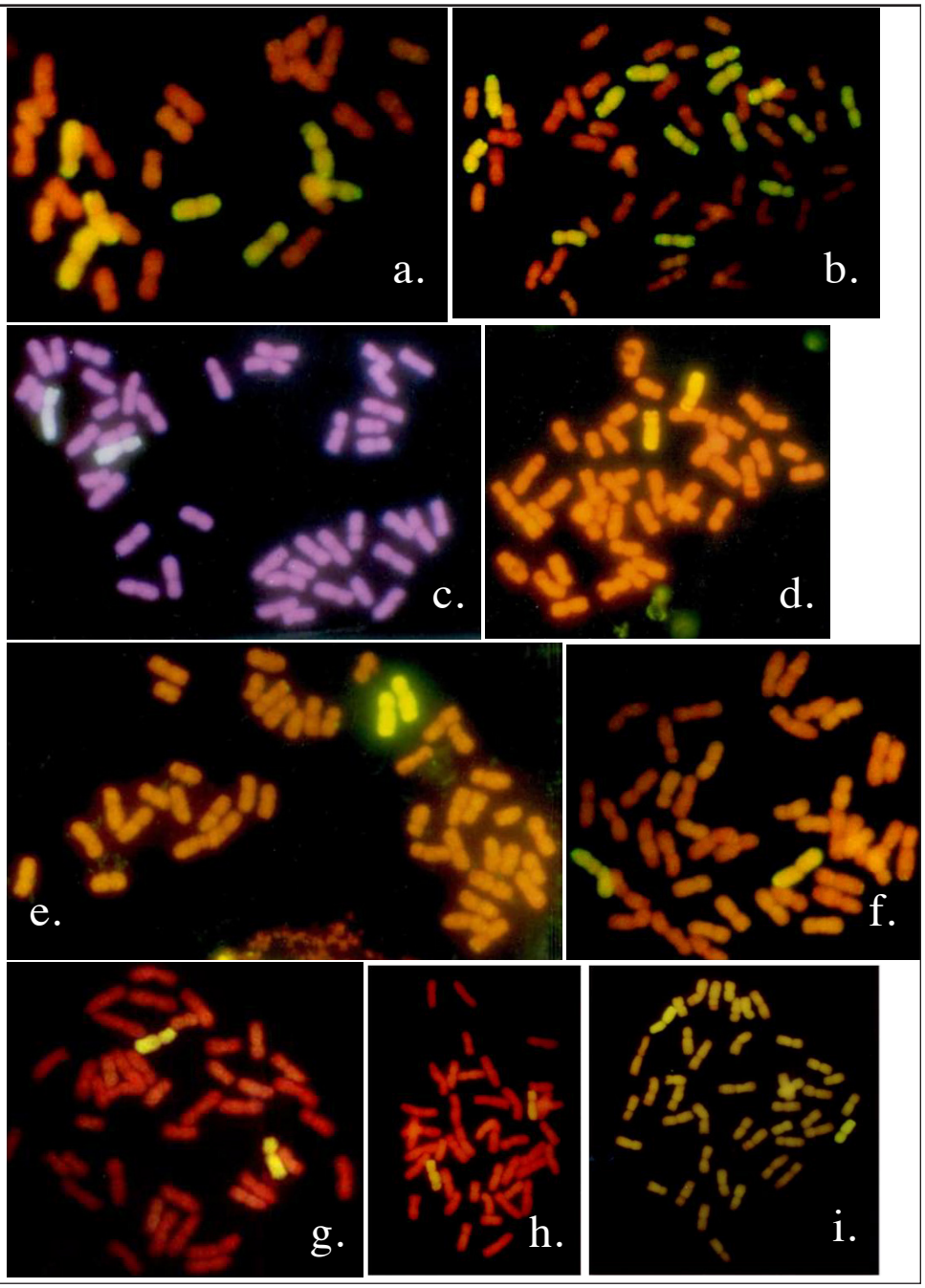


Fig. 37. Fluorescent *in situ* hybridization (wheat DNA for blocking and E^b DNA for blocking where E^b chromosomes are yellow) of somatic cells from an intergeneric hybrid combination of bread wheat/*Thinopyrum bessarabicum* at various stages of progeny development: (a) an F_1 hybrid with 2n=4x=28, $ABDE^b$ chromosomes; (b) an amphiploid somatic cell with 2n=8x=56, $AABBDDE^bE^b$ chromosomes, (14 E^bE^b-genome chromosomes are yellow); (c) a homoeologous group-1 E^bE^b disomic addition line; (d) a homoeologous group-2 E^bE^b disomic addition line; (e) a homoeologous group-3 E^bE^b disomic addition line; (f) a homoeologous group-7 E^bE^b disomic addition line; (g) a homoeologous group-4 E^bE^b disomic addition line; (h) a homoeologous group-5 E^bE^b disomic addition line; and (i) a homoeologous group-6 E^bE^b disomic addition line.

common and this trend prevailed. Hypo- and hyperploid plants were seen but euploid plants with 56 chromosomes were advanced after they were validated via C-banding and FISH (normal (Fig. 31a, p. 158) and aneuploid (Fig. 31b, c, and d, p. 158).

The utilization of the amphiploid has a two-fold advance scenario; production of addition lines and cytologically manipulating homoeologous transfer by the *ph1b* system. Systematic backcrossing and cytology (conventional and differential staining) is used to produce alien disomic alien chromosome addition lines, followed by seed increase and trait characterization. Having the addition lines in a nationally adapted wheat cultivar is an advantage for all future studies, and was incorporated in this program through the use of the high-yielding, widely adapted cultivar Inquilab. Addition lines 1 to 6 were backcrossed twice and then selfed from which plants with 44 chromosomes were increased. Addition line $7E^b$ was developed in Pakistan. The protocol was conventional and achieving the target goals was routine. The phenotype still requires more input using Inquilab for at least 2–3 additional backcrosses. Normal development is gauged at the BC_1 stage when the derivatives possess 2n=7x=49 chromosomes and at meiosis associate as 21 pairs (wheat) plus seven univalents (*Th. bessarabicum*) (Fig. 33, p. 159). When the disomics are generated, those showing

greater stability have normal metaphase I with 22 bivalents and anaphase I with a 22/22 split; the case in all seven addition lines (Fig. 35, p. 159). Their characterization has utilized various means covering giemsa C-banding, biochemical differentiation, molecular inputs and morphological parameters. *In situ* hybridization is an excellent, rapid means to validate the alien chromosome presence in the F_1 (Fig. 37a, p. 161), the amphiploid (Fig. 37b, p. 161), and in each disomic addition where two alien (homologous pair) chromosomes are observed (Fig. 37c–i, p. 161).

Crossing an amphiploid (2n=8x=56) with bread wheat generates a BC_1 with 2n=7x=49 chromosomes and this BC_1 is highly self-fertile with the capacity to retain alien chromosomes in a high frequency. This uniqueness allows the BC_1 derivatives to be used in stress evaluation. The two BC_1 combinations in the stocks in Pakistan have elite wheat cultivars Pavon and Pak-81 that are widely adapted wheat and amenable to field screening across the country.

Production of new bread wheat/synthetic hexaploid advanced derivatives.

Alvina Gul Kazi, Farrukh Bashir, Hadi Bux, Awais Rasheed, and Abdul Mujeeb-Kazi.

The International Maize and Wheat Improvement Center (CIMMYT), Mexico, produced 1,014 synthetic hexaploid wheats (SH) by artificially crossing elite, tetraploid wheat cultivars or their advanced breeding lines (2n=2x=28, AABB) with different accessions of *Ae. tauschii* (2n=4x=14, DD). The F_1 hybrids (2n=3x=21, ABD) were treated with colchicine, which caused chromosome doubling, and formed fertile SH wheats also known primary synthetics. All SH wheats were cytologically validated, increased, and screened against different biotic and abiotic stresses at CIMMYT. Due to varied trait diversity present in these SHs, different subsets were identified that showed resistance against diseases such as Karnal bunt, *Fusarium* head blight, *Septoria* leaf spot, *Helminthosposium* spot blotch, leaf rust, stripe rust, and abiotic stresses such as drought, water logging, and salinity and heat tolerance. We used these SHs to incorporate useful genetic traits into elite bread wheat cultivars around a major Pakistani focus to enrich and widen the narrow genetic pool of bread wheat and to combat different biotic and abiotic stresses faced by wheat production within and outside Pakistan. The desirable SHs were grown in the field at the Institute of Biotechnology and Genetic Engineering (IBGE), NWFP Agricultural University, Peshawar, in 2004–05. The IBGE planting followed cytological validation of somatic euploidy (2n=6x=42), increased the seed of all lines, and served for the production of F_1 cross combinations of elite international and nationally adapted wheat cultivars. The best bread wheats and SHs were identified and crossed, ultimately resulting in F_1 hybrid seed.

The F_1 hybrid seed was field planted in National Agricultural Research Centre (NARC), Islamabad, during the regular wheat crop cycle November 2005–May 2006. The F_2 seed produced was bulked and planted in the NARC fields in 2006–07 under artificial stripe rust stress. Only the resistant adult plants were harvested as individual spikes from selected plants and bulked. The resulting F_3 seed was planted in 2007–08 and, after artificial inoculation with stripe rust, the best plants both in terms of agronomy and disease resistance were selected (F_4 seed). These progenies were planted in the off-season at the Pakistan Agricultural Research Council (PARC) station in Kaghan (2,666 masl) for increase. The F_5 seed was harvested in September, 2008, and then planted in the NARC fields during the normal 2008–09 wheat cycle. The population was once again screened after artificial inoculation with stripe rust and single-plant selections were made on the basis of stripe rust resistance and agronomic type. The F_6 seed obtained was categorized for threshability, 1,000-kernel weight, and seed color and subsequently planted at the off-season at Kaghan near the end of May, 2009. These plants were harvested at the end of September, 2009, and then planted at NARC in '6 row × 5 m' plots for observation and seed increase and subsequently a source for national varietal testing based upon data to be gathered in May, 2010. These lines were planted again at Kaghan for seed increase. Representative samples of each combination have also been sent to the Plant Breeding Institute, Cobbity, Australia, for gene determination, a study of quality parameters, and characterization of drought tolerance. Pedigrees of these genotypes are given (Table 43, pp. 163-164).

Across all the synthetics that were increased, a natural infection of stripe rust enabled screening in the field and allowed the selection for superior agronomic performance at Peshawar. The selection criteria included plant height at maturity, no lodging, days-to-maturity, a closed crown, and spike details related mainly with spike length, grain fill, and erectness. Such synthetics from all the sets were crossed with elite, adapted, bread wheat cultivars yielding F_1 seed.

The first cycle in 2004–05 at IBGE, Peshawar. Seed increase of all synthetic sets with 25–30 grams seed/entry and crosses between SHs and elite adapted bread wheats with good agronomic traits in the various subsets in international/national cultivation to give F_1 seed.

Table 43. Pedigrees of the stripe rust-resistant F_7 produced from the CIMMYT, Mexico, collection of 1,014 synthetic hexaploid wheats.

Combination	Pedigree	Number of sister lines
1	68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (878)/6/CETA/… x 68.111/RGB-U//WARD RESEL/3/STIL/4/*Ae. tauschii* (783)	3
2	OPATA x 68.111/RGB-U//WARD RESEL/3/STIL/4/*Ae. tauschii* (783)	2
3	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (208)/5/OAPTA x 68.111/RGB-U//WARD RESEL/3/STIL/4/*Ae. tauschii* (783)	107
4	MAYOOR//TK SN1081/*Ae. tauschii*(222)/3/OPATA x 68.111/RGB-//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (878)	22
5	149 CHAPIO/INQALAB 91 x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (878)	1
6	CPI/GEDIZ/3/GOO//JO69/CRA/4/*Ae. tauschii* (208)/5/OAPTA x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (878)	2
7	OPATA x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (878)	6
8	MAYOOR//TK SN1081/AE.SQUARROSA)(222)/3/OPATA x GAN/*Ae. tauschii* (248)	6
9	ALTAR 84/*Ae. tauschii* (221)//YACO x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (878)	3
10	MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/OPATA x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (878)	4
11	MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/OPATA x CETA/*Ae. tauschii* (895)	1
12	68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (878)/6/CETA/… x CETA/*Ae. tauschii* (895)	2
13	CROC-1/*Ae. tauschii* (224)//KAUZ x CETA/*Ae. tauschii* (895)	1
14	TURACO/5/CHIR3/4/SIREN//ALTAR 84/*Ae. tauschii* (205)/3/3*BUC/6/OPATA x SCA/*Ae. tauschii* (518)	31
15	87 INQALAB 91/TSAPKI x SCA/*Ae. tauschii* (518)	1
16	162 CHAPIO/ INQALAB 91 x 68.111/RGB-U//WARD/3/*Ae. tauschii* (452)	6
17	YS/PASTOR x DOY1/*Ae. tauschii* (458)	5
18	CNDO/R143//ENTE/MEXI_2/3/*Ae. tauschii* (TAUS)/4/WEAVER/5/2*KAUZ x DOY1/*Ae. tauschii* (458)	20
19	MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/PASTOR x GAN/*Ae. tauschii* (259)	2
20	MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/OPATA x DOY1/*Ae. tauschii* (372)	7
21	OPATA/PASTOR x DOY1/*Ae. tauschii* (1024)	2
22	TURACO/5/CHIR3/4/SIREN//ALTAR 84/*Ae. tauschii* (205)/3/3*BUC/6/CNO x CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. tauschii* (273)	4
23	TURACO/5/CHIR3/4/SIREN//ALTAR 84/*Ae. tauschii* (205)/3/3*BUC/6/CNO x CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. tauschii* (227)	32
24	ALTAR 84/*Ae. tauschii* (224)//2*YACO/3/MAYOOR/TK SN1081/*Ae. tauschii* (222)/4/KUKUN x GAN/*Ae. tauschii* (248)	77
25	SERI x 68.111/RGB-U//WARD RESEL/3/STIL/4/*Ae. tauschii* (392)	1
26	BAKHTAWAR 94 x 68.111/RGB-U//WARD RESEL/3/STIL/4/*Ae. tauschii* (431)	3
27	OPATA x 68.111/RGB-U//WARD RESEL/3/STIL/4/*Ae. tauschii* (1038)	6
Using A-genome synthetic hexaploids		
28	MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/CBRD x CPI/GEDIZ/3/GOO//JO/CRA/4/T.MONO-COCCUM (101)	23
29	144 ALTAR 84/*Ae. tauschii* (221)//YACO/3/ INQALAB 91 x D67.2/P66.270//T.BOEOTICUM (66)	1
30	MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/CBRD x ARLIN_1/T.MONOCOCCUM (95)	3
31	MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/CBRD x CPI/GEDIZ/3/GOO//JO/CRA/4/T.MONO-COCCUM (101)	15
Drought tolerant and stripe-rust resistant F_7 produced.		
32	MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/FCT x YAV_3/SCO//JO69/CRA/3/YAV/79/4/*Ae. tauschii* (498)/5/OPATA	3
33	CHIR3/CBRD x GAN/*Ae. tauschii* (897)//OPATA	2
34	GAN/*Ae. tauschii* (897)//OPATA x D67.2/P66.270//*Ae. tauschii* (223)	1
35	MAYOOR//TK SN1081/*Ae. tauschii* (222)/4/SABUF/3/BCN//CETA/*Ae. tauschii* (895) x GAN/*Ae. tauschii* (897)//OPATA	2

Table 43. Pedigrees of the stripe rust-resistant F_7 produced from the CIMMYT, Mexico, collection of 1,014 synthetic hexaploid wheats.

Combination	Pedigree	Number of sister lines
36	MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/OPATA x DOY1/*Ae. tauschii* (515)	1
37	MAYOOR/TK SN1081/*Ae. tauschii* (222)/3/PASTOR x CROC_1/*Ae. tauschii* (444)	16
38	TURACO/5/CHIR3/4/SIREN//ALTAR 84/*Ae. tauschii* (205)/3/3*BUC/6/CNO x CROC_1/*Ae. tauschii* (444)	2
39	URES/PRL//BAV92 x YAV_2/TEZ//*Ae. tauschii* (249)	1
40	OPATA/PASTOR	5
41	OPATA x ALTAR 84/*Ae. tauschii* (J BANGOR)	9
42	OPATA x GAN/*Ae. tauschii* (408)	2
43	OPATA x CROC_1/*Ae. tauschii* (886)	2
44	OPATA x ROK/KML//*Ae. tauschii* (214)	1
45	OPATA x 68.112/WARD//*Ae. tauschii* (369)	1
46	OPATA x ALTAR 84/ *Ae. tauschii* (J BANGOR)	2
47	OPATA x DOY 1/*Ae. tauschii* (517)	11
48	OPATA x 68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (629)	14
49	OPATA x CETA/*Ae. tauschii* (895)	2
50	OPATA x DOY 1/*Ae. tauschii* (255)	4
51	OPATA x DOY 1/*Ae. tauschii* (1026)	2
52	OPATA x ALTAR 84/*Ae. tauschii* (205)	7
53	OPATA x INQALAB 91/AC8528	4
54	OPATA x INQALAB 91/FISCAL	1
55	OPATA x CETA/*Ae. tauschii* (1031)	8
56	OPATA x 74 INQALAB 91/TSAPKI	7
F_7 produced for bread wheat improvement using exotic germ plasm.		
57	182 SAAR/INQALAB 91 x MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/CBRD	15
58	MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/PASTOR x SARSABZ	1
59	MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/CBRD x KAMBARA	11
60	KAUZ x MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/CBRD	3
61	ALTAR 84/*Ae. tauschii* (224)//2*YACO/7/OPATA/6/68.111RGB-U//WARD/3/FGO/4/… x 162 SAAR/INQALAB 91	6
62	ALTAR 84/*Ae. tauschii* (224)//2*YACO/3/MAYOOR//TK SN1081/*Ae. tauschii* (222)/4/KUKUN x ALTAR 84/*Ae. tauschii* (221)//YACO	1
63	SARSABZ x CHIR3/CBRD	13
64	MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/PASTOR x MH-97	2
65	KAUZ x MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/CBRD	14
66	PASTOR x MAYOOR//TK SN1081/*Ae. tauschii* (222)/3/CBRD	15
67	BAV x (MAYOOR//TK SN1081/AE.SQUARROSA) (222)/3/CBRD	2
68	139 CHAPIO/INQALAB 91 x PICUS/3/KAUZ*2/BOW//KAUZ	100

Bread wheat/SH derivatives via advancing growth cycles. All F_1 crosses made during the 2004–05 crop cycle in Peshawar were moved to the NARC, Islamabad, as follows:

– F_1 grown at NARC during the winter 2005–06 cycle yielding F_2 seed,

– F_2 seed grown at NARC during the winter of 2006–07 yielding F_3 seed (a modified bulk procedure was adopted and plants selected for all superior agronomic traits resistant to stripe rust (artificial inoculation) and seed filling was observed prior to selection),

– F_3 seed grown at NARC during the winter of 2007–08 yielding F_4 seed (same selection criteria as in the previous section),

– F_4 modified bulk population planted at the Kaghan hill station during the summer 2008 (June to October) from which F_5 products were harvested and brought to NARC,

– F_5 progenies planted in the winter cycle of 2008–09 at NARC and observed for phenotype, stripe rust resistance (APR), and superior agronomic plant type leading to individual plant selections (F_6, the F_6 seed was

characterized for threshability, 1,000-kernel weight, and grain color),

– individual F_6 plant selections were planted at Kaghan in June, 2009, over the summer 2009 (June to October) for seed increase, and

– F_7 seed harvested at Kaghan planted at NARC in '6 row x 5 m' plots in winter 2009–10.

In general, the focus was to stringently select quality plants through modified bulks and then incorporate individual plant selection. Alternate cycles allowed for rapid seed increase and finally led to planting 2,200 '6 × 5 m' row plots of the F_7 derivatives from various bread wheat/SH combinations. Final selections were made at the end of the 2009–10 crop cycle at NARC; the number of elite selections dropped to 690 (Table 44, pp. 166-173). The best lines shall be recommended for varietal testing across Pakistan after the 2010 summer cycle at Kaghan (October, 2010).

All selected elite lines are free-threshing and have gone progressively from their tough-glumed SH parents and F_1 to free-threshing selections from the F_2 onwards. The spikes of two SH spikes of variable coloration and tough glumes, a normal free threshing bread wheat spike, and a 'bread wheat/SH' derivative that is also free-threshing are shown in Fig. 38.

Fig. 38. Spike phenotype of the utilization of the D-genome synthetic hexaploid wheats (SH) in wheat breeding: (a) two SH wheat spikes (dark and light awned) with tough glumes, (b) a free-threshing bread wheat spike, and (c) a free-threshing advanced progeny spike derived from a 'bread wheat/SH' cross.

The transfer of agronomic traits from the SHs to bread wheat developed a extensive recombination breeding effort in Pakistan around the new 'Wheat Wide Crosses and Cytogenetics' program. In general, wheats were used as the female parents for F_1 production. F_2 and F_3 advances were made around a modified bulk procedure intermixed with stress screening and selection of good agronomic plant types. From the final F_7 generation, several lines were selected. The range of variation addresses national wheat cultivation targets in both the irrigated and rainfed environments. The broad details are associated with variable heights at maturity, plant canopy intensity, early growth foliage spread, days-to-flowering, days to physiological maturity, leaf waxiness, stay-green character, spike length with terminal (apex) club shaped quality, and seed weight and appearance. Screening for resistance to stripe rust, Karnal bunt, and powdery mildew over the growing periods added information to the molecular diversity status. the national and international distribution of these advanced F_7 derivatives will permit further characterization for other factors important fornational varietal releases and deployment strategies, i.e., local and Ug99 leaf and stem rust, spot blotch, BYDV, and key quality components.

Stem rust has a unique international importance. Derivatives from this study have shown resistance to Ug99 in Kenya during the 2009 tests. Numerous crosses were made from 68 combinations that were categorized as follows:

– bread wheat/SHs resistant to rusts (27 combinations),
– bread wheat/A-genome synthetics (4 combinations),
– bead wheat/drought and rust resistant synthetics (25 combinations), and
– bread wheat/exotic synthetics, 12 combinations.

From these 68 combinations, eight were identified as resistant to Ug99 after testing in collaboration with KARI, Kenya. The entry and rust score details are 3 (5MR), 4 (15M), 9 (10M), 20 (10M), 26 (15M), 27 (20M), 50 (5MR), and 62 (10M).

Since the appearance of Ug99 in Uganda and its spread into Kenya, Ethiopia, Yemen, and Iran, Pakistan faces the danger of its entering the country. Thus, the breeding focus has shifted to test national germ plasm for Ug99 resistance and also analyze the Ug99-resistant materials within the country with a local race in lower Punjab and Sindh that has sporadic occurrence in the north. The tests against the local race were inconclusive because stem rust did not occur in the Sindh testing site during the 2009–10 cycle. The test will be repeated. Pakistan does not have race Ug99 yet, and the spores collected from minimal infections in Sindh have been sent to Australia for PCR validation to determine if Pakistan is free of Ug99 upto May 2010. The identification of resistant germ plasm moved us to have the elite synthetic-

Table 44. Phenotypic evaluation of 'bread wheat/synthetic hexaploid' F_7 derivatives produced for bread wheat improvement using exotic germ plasm during 2008–09 crop cycle (SH = synthetic hexaploid parent line, GW = 1,000-kernel weight (g), DH = day-to-heading, DM = days to maturity, PH = plant height (cm), SL = spike length (cm), and GS = grains/spike).

Line	Combination	SH	TKW	DH	DM	PH	SL	GS	Line	Combination	SH	TKW	DH	DM	PH	SL	GS
1	1	1	61.08	124	163	97	16	85	42		37	33.64	118	161	103	13	74
2		2	65.58	124	163	106	16	80	43		38	38.66	118	159	104	14	78
3		3	62.22	124	162	100	14	72	44		39	39.60	118	161	100	13	72
4	2	1	46.86	124	163	114	12	55	45		40	36.02	118	161	97	13	74
5		2	39.04	124	163	120	14	62	46		41	38.76	119	159	100	13	74
6	3	1	49.28	123	164	135	12	69	47		42	40.50	119	159	106	15	84
7		2	43.50	125	173	100	14	80	48		43	39.38	119	159	99	13	80
8		3	38.84	125	173	108	14	68	49		44	43.14	119	159	109	15	90
9		4	37.56	125	168	115	16	72	50		45	39.76	119	159	100	14	86
10		5	42.26	125	169	103	15	70	51		46	45.54	119	159	116	16	96
11		6	46.86	125	168	119	14	73	52		47	41.18	119	159	101	13	82
12		7	40.96	122	162	103	13	80	53		48	37.04	119	159	124	16	92
13		8	42.04	122	162	110	13	82	54		49	34.74	119	159	113	13	76
14		9	44.96	122	162	97	13	84	55		50	41.36	119	161	110	13	72
15		10	41.40	122	162	103	13	82	56		51	43.60	119	163	125	16	109
16		11	36.20	123	162	100	13	82	57		52	44.36	119	159	122	15	94
17		12	45.00	123	159	110	14	82	58		53	37.92	118	161	103	14	78
18		13	48.22	123	159	113	13	74	59		54	41.58	118	161	100	13	76
19		14	43.74	123	159	115	18	95	60		55	42.78	118	161	126	15	78
20		15	48.04	123	159	114	17	90	61		56	41.94	118	161	123	15	76
21		16	39.42	123	159	120	18	92	62		57	43.94	118	161	108	13	68
22		17	43.38	123	164	105	16	76	63		58	40.82	118	164	115	16	68
23		18	42.04	123	164	97	13	64	64		59	43.90	118	160	101	12	68
24		19	42.36	123	164	110	15	78	65		60	43.44	118	161	126	15	96
25		20	37.06	119	164	104	13	69	66		61	41.92	118	160	120	16	90
26		21	37.04	119	164	103	13	72	67		62	38.34	118	161	119	13	74
27		22	36.68	119	164	100	14	76	68		63	35.62	118	159	113	13	78
28		23	34.04	119	164	94	13	72	69		64	44.84	118	159	114	14	86
29		24	44.10	119	164	110	14	74	70		65	43.82	118	159	125	16	86
30		25	42.10	119	164	109	14	76	71		66	39.02	118	162	101	13	80
31		26	43.30	119	164	110	14	74	72		67	40.80	118	164	109	15	96
32		27	40.78	119	165	102	14	74	73		68	41.16	118	164	121	16	84
33		28	40.50	119	165	112	15	80	74		69	42.98	118	164	107	13	78
34		29	38.10	119	165	107	17	90	75		70	41.36	118	163	114	15	84
35		30	36.26	119	165	119	18	90	76		71	39.44	118	165	125	16	90
36		31	39.98	119	165	113	18	94	77		72	39.90	118	165	109	15	82
37		32	39.12	119	165	102	11	62	78		73	40.16	118	165	116	14	72
38		33	37.60	118	161	101	12	70	79		74	36.76	118	160	106	14	70
39		34	42.54	118	161	112	13	76	80		75	43.14	118	161	101	13	76
40		35	46.66	118	161	108	15	84	81		76	38.12	118	161	113	14	80
41		36	39.16	118	161	97	14	80	82		77	37.42	118	164	110	15	93
83		78	41.00	118	165	116	15	86	129		17	53.52	121	164	130	13	76
84		79	40.18	118	160	104	14	82	130		18	50.50	121	162	128	13	78
85		80	36.48	118	164	110	14	80	131		19	42.84	121	163	125	13	70
86		81	36.66	118	166	110	14	78	132		20	52.22	121	163	130	12	75

Table 44. Phenotypic evaluation of 'bread wheat/synthetic hexaploid' F_7 derivatives produced for bread wheat improvement using exotic germ plasm during 2008–09 crop cycle (SH = synthetic hexaploid parent line, GW = 1,000-kernel weight (g), DH = day-to-heading, DM = days to maturity, PH = plant height (cm), SL = spike length (cm), and GS = grains/spike).

Line	Combination	SH	TKW	DH	DM	PH	SL	GS	Line	Combination	SH	TKW	DH	DM	PH	SL	GS
87		82	40.16	118	166	113	14	89	133	5	1	41.74	125	171	92	15	77
88		83	37.88	118	164	104	13	83	134	6	1	40.64	123	177	122	12	40
89		84	41.21	119	166	112	18	95	135		2	32.18	123	176	117	15	49
90		85	37.08	119	165	113	16	82	136	7	1	48.76	120	173	125	16	87
91		86	39.94	119	165	120	16	87	137		2	51.96	120	173	116	16	86
92		87	41.32	119	166	115	17	92	138		3	38.34	120	173	130	16	84
93		88	41.28	119	166	117	18	96	139		4	50.10	120	173	109	15	82
94		89	38.52	119	164	121	16	94	140		5	46.92	124	173	111	16	74
95		90	39.64	119	165	125	17	90	141		6	41.54	124	174	121	16	72
96		91	46.88	119	165	103	16	92	142	8	1	45.24	125	165	113	14	76
97		92	42.60	119	164	118	17	100	143		2	45.06	125	165	118	12	72
98		93	36.24	119	162	120	17	98	144		3	48.66	125	165	106	10	56
99		94	40.32	119	161	116	15	94	145		4	46.46	125	167	110	10	51
100		95	41.92	119	164	114	16	85	146		5	42.80	125	169	114	11	60
101		96	38.22	119	165	113	16	88	147		6	45.64	125	165	109	11	54
102		97	39.60	119	165	111	15	86	148	9	1	41.12	124	163	100	10	35
103		98	40.90	119	166	109	16	82	149		2	36.86	124	164	112	11	77
104		99	40.12	118	165	120	16	90	150		3	51.82	124	163	122	12	80
105		100	38.48	118	165	110	16	82	151	10	1	46.22	123	167	108	14	86
106		101	40.32	118	166	118	17	96	152		2	47.66	123	165	120	13	78
107		102	37.36	118	166	113	17	94	153		3	50.74	120	163	115	14	86
108		103	39.04	118	164	110	17	96	154		4	36.82	122	163	111	13	80
109		104	34.84	119	166	107	16	88	155	11	1	44.54	132	172	130	11	58
110		105	40.22	119	166	120	17	90	156	12	1	41.98	129	167	107	11	36
111		106	43.10	119	166	114	16	86	157		2	58.50	126	172	128	13	65
112		107	37.98	119	162	103	16	90	158	13	1	47.08	119	165	10	15	89
113	4	1	46.54	125	165	130	13	80	159	14	1	33.54	123	162	104	11	84
114		2	52.52	125	168	125	13	76	160		2	34.92	121	162	116	13	86
115		3	53.44	125	165	126	14	71	161		3	39.18	121	162	120	13	71
116		4	55.26	125	165	131	13	78	162		4	35.06	121	162	124	14	64
117		5	52.86	125	164	130	13	80	163		5	37.90	121	162	101	12	79
118		6	54.86	125	166	114	13	70	164		6	32.14	121	162	97	13	84
119		7	52.00	125	166	120	13	66	165		7	34.16	121	162	103	13	86
120		8	52.54	125	168	127	13	70	166		8	35.22	121	158	100	12	70
121		9	56.78	125	169	128	12	73	167		9	38.78	125	160	102	12	82
122		10	48.22	122	164	133	14	70	168		10	36.65	125	160	105	12	76
123		11	55.28	122	162	131	13	69	169		11	35.16	123	159	98	13	92
124		12	55.82	122	163	137	12	66	170		12	41.12	123	159	109	12	80
125		13	52.60	122	166	132	13	86	171		13	32.18	123	158	106	13	72
126		14	49.88	123	169	133	13	74	172		14	41.28	122	160	97	14	86
127		15	53.88	123	164	128	13	66	173		15	35.20	122	160	104	13	90
128		16	52.10	123	165	130	13	70	174		16	37.28	122	163	100	13	94
175		17	30.04	122	159	101	13	94	221		20	36.74	123	156	96	13	72
176		18	30.88	122	159	96	13	92	222	19	1	50.72	124	172	123	14	47
177		19	35.12	122	162	100	13	86	223		2	52.36	126	169	116	11	56

Table 44. Phenotypic evaluation of 'bread wheat/synthetic hexaploid' F_7 derivatives produced for bread wheat improvement using exotic germ plasm during 2008–09 crop cycle (SH = synthetic hexaploid parent line, GW = 1,000-kernel weight (g), DH = day-to-heading, DM = days to maturity, PH = plant height (cm), SL = spike length (cm), and GS = grains/spike).

Line	Combination	SH	TKW	DH	DM	PH	SL	GS	Line	Combination	SH	TKW	DH	DM	PH	SL	GS
178		20	34.22	122	159	106	15	100	224	20	1	47.16	112	167	107	11	50
179		21	36.52	120	161	119	12	92	225		2	47.72	112	168	220	14	66
180		22	38.64	121	158	100	13	80	226		3	45.44	112	167	108	13	61
181		23	41.00	121	161	106	11	72	227		4	44.74	112	167	106	13	63
182		24	36.20	121	159	97	12	78	228		5	43.88	112	167	100	11	64
183		25	31.76	121	159	104	11	76	229		6	41.92	112	170	113	10	66
184		26	42.30	121	161	122	12	82	230		7	43.88	112	167	107	16	96
185		27	38.74	121	161	119	14	79	231	21	1	49.30	121	166	120	17	90
186		28	34.92	121	159	119	13	76	232		2	47.92	121	161	122	17	86
187		29	36.00	122	159	110	14	80	233	22	1	47.86	117	164	117	16	84
188		30	39.56	122	159	118	17	97	234		2	30.08	117	164	103	19	99
189		31	33.54	122	159	110	16	98	235		3	43.72	117	163	103	18	110
190	15	1	46.24	122	157	120	13	98	236		4	41.58	117	164	105	17	98
191	16	1	38.64	115	155	100	14	90	237	23	1	45.52	125	170	118	13	76
192		2	45.28	115	156	100	14	92	238		2	42.66	125	167	120	13	78
193		3	47.20	115	156	103	13	87	239		3	42.60	125	168	128	14	72
194		4	49.26	115	156	99	12	83	240		4	41.90	125	169	124	13	78
195		5	43.88	115	156	97	14	82	241		5	44.18	125	169	129	14	76
196		6	47.30	115	156	102	11	79	242		6	46.40	125	172	120	12	68
197	17	1	44.18	114	159	120	12	62	243		7	48.40	125	171	126	13	62
198		2	45.34	114	156	125	10	56	244		8	36.36	125	169	122	13	60
199		3	47.72	114	158	105	11	62	245		9	41.70	125	172	115	13	82
200		4	45.48	114	169	103	10	54	246		10	47.16	125	166	120	12	64
201		5	41.46	114	169	110	11	50	247		11	42.74	125	172	128	15	74
202	18	1	38.12	122	157	122	14	69	248		12	42.16	125	172	122	12	90
203		2	42.42	122	157	116	14	72	249		13	42.04	125	171	110	12	62
204		3	33.24	121	156	113	13	62	250		14	40.60	124	168	120	16	110
205		4	35.40	122	155	119	13	58	251		15	47.78	124	168	126	15	72
206		5	35.06	122	155	117	16	72	252		16	47.04	124	167	114	12	68
207		6	36.80	122	157	106	16	74	253		17	43.32	124	167	128	15	78
208		7	35.58	123	163	122	17	84	254		18	43.00	124	171	112	12	86
209		8	38.90	122	156	120	14	86	255		19	42.20	124	169	115	15	78
210		9	40.44	122	156	122	14	82	256		20	44.54	124	168	120	12	66
211		10	37.16	122	161	118	16	94	257		21	44.22	124	169	118	14	76
212		11	41.86	122	161	122	15	90	258		22	49.32	124	172	106	12	74
213		12	35.28	122	156	114	13	80	259		23	44.32	124	172	115	13	78
214		13	40.46	122	156	118	14	76	260		24	43.58	124	171	120	14	80
215		14	37.44	121	157	120	16	86	261		25	43.30	124	171	123	13	78
216		15	41.42	121	158	110	15	78	262		26	43.44	124	172	124	14	78
217		16	36.18	121	158	126	15	82	263		27	45.68	124	171	110	12	64
218		17	36.42	123	157	119	16	87	264		28	42.90	124	171	130	16	83
219		18	40.42	123	157	112	14	82	265		29	44.92	124	173	108	15	80
220		19	30.82	123	156	129	16	76	266		30	43.64	124	165	120	14	74
267		31	42.52	124	167	100	10	72	313		45	50.46	116	162	105	15	76
268		32	45.04	124	167	121	14	76	314		46	49.78	116	162	102	14	74

Table 44. Phenotypic evaluation of 'bread wheat/synthetic hexaploid' F_7 derivatives produced for bread wheat improvement using exotic germ plasm during 2008–09 crop cycle (SH = synthetic hexaploid parent line, GW = 1,000-kernel weight (g), DH = day-to-heading, DM = days to maturity, PH = plant height (cm), SL = spike length (cm), and GS = grains/spike).

Line	Combination	SH	TKW	DH	DM	PH	SL	GS	Line	Combination	SH	TKW	DH	DM	PH	SL	GS
269	24	1	43.58	120	157	108	14	82	315		47	48.92	116	162	103	14	76
270		2	48.88	120	164	111	15	98	316		48	45.02	125	164	100	14	82
271		3	46.82	120	164	98	15	96	317		49	48.62	125	164	99	13	83
272		4	44.30	120	164	100	13	86	318		50	49.68	125	164	110	14	90
273		5	47.40	120	164	105	14	100	319		51	45.92	125	164	98	14	86
274		6	45.52	119	164	116	15	110	320		52	45.72	119	165	110	14	80
275		7	49.44	119	164	106	15	96	321		53	45.44	119	165	110	14	84
276		8	54.92	119	164	103	15	92	322		54	46.04	119	164	99	14	80
277		9	45.56	119	164	99	12	88	323		55	49.70	119	164	103	14	82
278		10	45.20	119	164	111	12	82	324		56	43.98	119	164	102	14	84
279		11	41.96	119	164	105	13	84	325		57	48.66	119	164	100	14	82
280		12	42.30	119	164	100	13	80	326		58	50.08	119	164	105	14	86
281		13	44.56	119	164	97	13	86	327		59	43.66	120	165	100	14	82
282		14	41.76	119	164	100	13	86	328		60	44.08	120	165	105	14	82
283		15	58.58	119	164	98	13	86	329		61	48.00	120	165	103	14	80
284		16	38.66	118	164	100	13	90	330		62	49.46	120	165	103	14	78
285		17	46.70	118	164	97	13	85	331		63	47.68	120	164	115	14	86
286		18	47.66	118	164	112	12	78	332		64	46.86	120	164	115	14	80
287		19	44.10	118	164	103	13	80	333		65	47.12	120	164	104	14	84
288		20	45.76	118	164	103	12	76	334		66	41.46	120	164	100	14	82
289		21	45.28	119	164	104	11	70	335		67	43.54	120	164	103	15	84
290		22	38.66	119	164	110	12	72	336		68	43.24	120	167	110	14	86
291		23	44.90	119	164	103	13	78	337		69	47.90	120	167	110	14	80
292		24	48.64	119	164	110	14	96	338		70	41.32	120	167	108	14	89
293		25	45.60	120	164	109	13	80	339		71	48.08	117	165	120	14	88
294		26	46.48	120	164	107	12	82	340		72	48.08	117	164	103	14	79
295		27	46.12	120	164	110	13	90	341		73	45.94	117	164	115	14	68
296		28	48.86	120	167	110	15	108	342		74	48.70	117	164	100	14	68
297		29	49.34	117	164	108	13	80	343		75	48.38	117	164	104	14	72
298		30	50.62	117	164	100	13	68	344		76	45.66	117	164	125	14	76
299		31	50.60	117	164	100	13	96	345		77	41.48	117	164	106	14	78
300		32	49.52	117	164	104	12	70	346	25	1	45.78	122	160	115	14	83
301		33	49.30	116	164	102	13	80	347	26	1	45.06	122	162	92	14	82
302		34	42.88	116	164	103	12	72	348		2	55.54	125	164	93	13	84
303		35	46.60	116	164	102	12	78	349		3	55.02	125	169	90	10	70
304		36	45.68	116	164	100	10	76	350	27	1	45.20	124	157	100	13	80
305		37	36.58	125	164	104	12	72	351		2	42.16	124	157	106	14	76
306		38	54.44	125	167	103	11	70	352		3	45.20	124	157	103	15	78
307		39	43.68	125	168	105	15	88	353		4	43.48	124	156	99	15	72
308		40	40.64	125	168	103	14	78	354		5	47.46	124	157	105	15	74
309		41	51.34	116	164	100	13	60	355		6	41.50	124	157	100	14	82
310		42	47.10	116	164	99	14	74	356	28	1	46.56	119	156	95	12	62
311		43	48.24	116	164	103	14	72	357		2	42.38	123	156	96	14	100
312		44	47.64	116	164	101	14	75	358		3	41.14	126	156	98	15	60
359		4	44.18	126	156	100	12	82	405		2	48.52	129	173	120	18	106

Table 44. Phenotypic evaluation of 'bread wheat/synthetic hexaploid' F_7 derivatives produced for bread wheat improvement using exotic germ plasm during 2008–09 crop cycle (SH = synthetic hexaploid parent line, GW = 1,000-kernel weight (g), DH = day-to-heading, DM = days to maturity, PH = plant height (cm), SL = spike length (cm), and GS = grains/spike).

Line	Combination	SH	TKW	DH	DM	PH	SL	GS	Line	Combination	SH	TKW	DH	DM	PH	SL	GS
360		5	42.32	126	156	95	11	72	406	36	1	42.74	122	159	107	14	75
361		6	41.08	126	156	92	13	68	407	37	1	38.00	122	164	120	15	80
362		7	42.06	126	156	96	13	68	408		2	42.46	122	164	110	14	81
363		8	39.32	126	156	96	13	73	409		3	40.14	122	162	101	12	68
364		9	47.14	126	156	95	13	66	410		4	39.22	124	162	103	13	74
365		10	38.80	126	156	92	12	62	411		5	42.82	124	160	107	11	66
366		11	43.68	126	156	96	14	86	412		6	50.12	124	159	114	12	83
367		12	43.94	126	156	96	13	53	413		7	40.82	124	160	109	11	76
368		13	42.42	126	156	94	13	66	414		8	43.96	124	160	118	13	80
369		14	41.20	126	156	96	12	67	415		9	30.18	124	159	112	13	90
370		15	46.58	126	156	96	14	84	416		10	45.18	124	159	115	12	82
371		16	43.64	126	156	98	13	72	417		11	48.62	124	160	117	13	76
372		17	42.36	126	156	95	13	70	418		12	43.64	124	160	109	11	70
373		18	43.36	125	156	99	15	76	419		13	39.36	124	160	103	12	78
374		19	42.92	125	156	100	17	110	420		14	42.84	124	160	118	13	82
375		20	42.36	125	156	98	15	90	421		15	40.76	124	160	110	12	80
376		21	43.18	125	156	101	16	92	422		16	45.76	124	160	111	14	86
377		22	45.06	125	156	100	16	86	423	38	1	55.04	112	160	100	12	66
378		23	44.18	125	156	98	15	88	424		2	54.40	112	160	104	13	68
379	29	1	46.42	118	159	99	12	71	425	39	1	36.16	112	160	104	13	68
380	30	1	43.06	121	159	118	17	100	426	40	1	38.04	120	146	94	15	92
381		2	49.94	128	159	109	18	110	427		2	29.46	120	146	97	15	88
382		3	45.18	119	160	113	15	93	428		3	37.84	120	146	95	14	80
383	31	1	38.28	123	158	117	20	80	429		4	35.28	120	168	96	14	78
384		2	35.86	123	158	110	15	64	430		5	36.24	120	169	90	14	76
385		3	45.04	123	158	90	14	70	431	41	1	41.16	120	155	118	12	60
386		4	42.58	123	158	96	15	72	432		2	49.46	120	155	120	9	45
387		5	34.28	123	158	103	14	63	433		3	41.38	120	155	125	11	60
388		6	31.77	120	160	103	14	62	434		4	44.90	110	150	100	13	76
389		7	41.88	120	160	104	15	61	435		5	50.84	110	150	120	11	60
390		8	33.96	120	160	100	13	80	436		6	37.24	110	150	110	14	72
391		9	43.04	120	164	108	14	70	437		7	37.72	110	150	116	15	80
392		10	36.48	120	164	100	13	73	438		8	49.40	110	150	113	11	64
393		11	32.98	120	164	103	13	74	439		9	42.26	110	150	99	14	72
394		12	34.96	120	167	100	11	76	440	42	1	30.28	113	161	125	14	85
395		13	42.20	120	167	96	15	82	441		2	41.26	113	161	90	13	70
396		14	53.62	120	171	99	14	62	442	43	1	22.86	112	156	87	13	70
397		15	52.18	120	170	97	14	64	443		2	43.30	107	154	90	13	79
398	32	1	43.52	116	163	121	13	74	444	44	1	47.52	125	173	106	12	46
399		2	41.60	116	160	136	13	76	445	45	1	49.00	126	174	120	12	65
400		3	41.84	116	160	123	13	78	446	46	1	57.94	120	170	115	18	90
401	33	1	52.48	123	166	134	16	79	447		2	59.06	120	170	120	15	88
402		2	33.34	123	166	114	13	72	448	47	1	37.60	133	180	119	9	65
403	34	1	41.34	135	179	122	14	86	449		2	40.72	133	180	110	9	55
404	35	1	43.00	127	168	112	18	103	450		3	44.34	133	180	125	10	48

Table 44. Phenotypic evaluation of 'bread wheat/synthetic hexaploid' F_7 derivatives produced for bread wheat improvement using exotic germ plasm during 2008–09 crop cycle (SH = synthetic hexaploid parent line, GW = 1,000-kernel weight (g), DH = day-to-heading, DM = days to maturity, PH = plant height (cm), SL = spike length (cm), and GS = grains/spike).

Line	Combination	SH	TKW	DH	DM	PH	SL	GS	Line	Combination	SH	TKW	DH	DM	PH	SL	GS
451		4	42.72	133	180	116	9	49	497		5	28.72	125	172	130	15	66
452		5	46.54	132	180	118	9	52	498		6	37.16	125	172	122	15	74
453		6	45.56	132	177	115	10	56	499		7	37.04	125	172	128	15	76
454		7	37.60	132	178	120	9	58	500		8	30.56	125	172	123	14	70
455		8	38.87	133	180	121	9	55	501	56	1	35.46	114	146	99	13	56
456		9	44.00	133	180	127	10	58	502		2	26.20	114	146	100	13	55
457		10	44.50	133	180	106	9	53	503		3	38.56	114	146	104	12	56
458		11	40.06	133	180	103	9	50	504		4	42.06	114	146	101	11	52
459	48	1	37.42	114	151	101	13	72	505		5	38.54	114	146	99	13	58
460		2	41.00	114	151	110	16	86	506		6	44.38	119	150	100	13	50
461		3	39.16	114	151	124	15	92	507		7	45.40	119	150	104	13	56
462		4	41.38	114	151	97	16	82	508	57	1	41.70	112	166	116	14	58
463		5	36.70	114	151	119	11	76	509		2	51.02	112	163	125	14	68
464		6	38.94	114	151	105	13	60	510		3	50.40	112	166	103	13	58
465		7	43.88	111	150	98	16	82	511		4	51.06	112	165	128	14	62
466		8	42.08	111	150	113	15	92	512		5	38.68	112	163	102	14	66
467		9	45.05	111	150	120	14	74	513		6	54.48	112	163	110	13	62
468		10	40.86	111	150	107	11	68	514		7	48.18	112	163	117	13	53
469		11	50.94	111	150	101	13	72	515		8	44.60	112	163	110	14	62
470		12	33.78	111	150	110	13	68	516		9	45.48	112	163	109	13	60
471		13	45.12	111	150	96	14	78	517		10	51.48	111	162	114	17	76
472		14	45.05	111	150	115	15	78	518		11	63.18	111	162	107	13	72
473	49	1	46.90	114	165	112	15	82	519		12	50.44	111	162	103	14	76
474		2	50.98	114	156	116	14	80	520		13	52.90	111	160	108	15	80
475	50	1	51.42	123	169	130	15	72	521		14	45.36	112	160	116	15	86
476		2	53.20	123	169	108	12	50	522		15	41.54	112	160	121	15	80
477		3	49.30	123	169	103	11	50	523	58	1	45.04	120	179	129	14	81
478		4	43.98	123	169	124	14	66	524	59	1	53.98	124	179	100	11	68
479	51	1	49.92	113	153	110	14	70	525		2	53.54	124	179	111	13	72
480		2	46.98	113	153	112	16	100	526		3	44.10	124	158	115	13	77
481	52	1	40.24	114	153	99	11	60	527		4	45.62	124	158	118	13	79
482		2	33.72	114	153	106	11	57	528		5	44.96	124	158	109	13	70
483		3	39.08	114	154	95	14	82	529		6	48.72	124	158	103	13	72
484		4	35.20	114	154	97	13	72	530		7	47.18	124	173	100	12	78
485		5	41.60	114	153	95	11	62	531		8	49.00	124	173	104	13	66
486		6	38.36	114	163	105	14	100	532		9	45.46	124	173	106	13	68
487		7	36.52	114	164	110	11	45	533		10	37.52	121	173	110	13	66
488	53	1	48.44	110	154	110	13	72	534		11	42.02	120	173	103	13	68
489		2	51.50	110	154	119	16	82	535	60	1	46.08	125	162	109	11	58
490		3	42.54	110	154	108	13	70	536		2	56.56	125	162	103	11	55
491		4	45.14	110	154	125	18	86	537		3	40.36	126	162	115	13	66
492	54	1	53.66	124	171	109	16	87	538	61	1	47.24	121	164	105	11	68
493	55	1	44.36	126	173	103	20	110	539		2	38.70	121	164	105	12	72
494		2	33.72	128	171	130	13	50	540		3	37.74	121	164	105	13	88
495		3	31.90	128	171	132	15	70	541		4	37.72	121	164	110	13	66

Table 44. Phenotypic evaluation of 'bread wheat/synthetic hexaploid' F_7 derivatives produced for bread wheat improvement using exotic germ plasm during 2008–09 crop cycle (SH = synthetic hexaploid parent line, GW = 1,000-kernel weight (g), DH = day-to-heading, DM = days to maturity, PH = plant height (cm), SL = spike length (cm), and GS = grains/spike).

Line	Combination	SH	TKW	DH	DM	PH	SL	GS	Line	Combination	SH	TKW	DH	DM	PH	SL	GS
496		4	34.40	125	172	123	15	66	542		5	35.80	121	164	121	13	62
543		6	44.92	121	164	107	12	63	589	67	1	59.84	116	159	121	15	71
544	62	1	42.98	125	173	127	16	93	590		2	42.28	114	153	116	13	86
545	63	1	28.46	128	174	118	14	81	591	68	1	39.40	116	164	103	12	62
546		2	43.40	128	163	120	13	51	592		2	30.66	116	164	100	13	68
547		3	42.32	128	162	100	18	90	593		3	28.80	116	161	105	14	77
548		4	45.64	128	167	110	16	60	594		4	38.64	121	165	96	15	68
549		5	47.18	128	167	118	15	54	595		5	45.64	121	166	101	16	76
550		6	52.48	128	167	130	15	62	596		6	44.72	121	164	90	14	70
551		7	42.16	128	167	103	13	53	597		7	40.36	125	163	96	13	70
552		8	50.12	128	167	100	12	54	598		8	37.12	125	164	91	13	72
553		9	45.92	128	167	121	13	63	599		9	44.78	125	164	100	14	76
554		10	43.44	128	167	116	13	72	600		10	45.14	125	163	101	14	76
555		11	46.34	128	167	120	14	78	601		11	40.56	125	164	110	14	78
556		12	45.10	128	167	130	14	96	602		12	45.56	125	165	98	13	72
557		13	37.36	128	167	123	14	76	603		13	30.58	125	165	92	14	72
558	64	1	49.14	118	147	117	11	86	604		14	34.46	125	164	102	14	70
559		2	44.56	118	153	103	13	88	605		15	40.20	125	164	106	15	80
560	65	1	41.66	114	163	91	16	100	606		16	50.42	125	163	91	13	67
561		2	43.96	114	163	97	15	74	607		17	35.82	125	165	97	14	70
562		3	45.74	114	163	106	16	82	608		18	43.30	125	165	100	14	70
563		4	38.34	114	163	120	17	100	609		19	41.70	125	165	98	14	70
564		5	37.64	114	166	100	17	110	610		20	40.02	125	165	112	14	69
565		6	36.30	114	166	93	15	78	611		21	48.06	125	166	93	13	78
566		7	49.74	112	150	100	16	100	612		22	46.38	125	165	97	13	76
567		8	48.06	112	152	96	17	90	613		23	39.98	129	165	94	13	72
568		9	46.46	112	151	95	16	83	614		24	43.10	129	166	102	16	78
569		10	45.28	112	150	103	16	80	615		25	49.22	128	165	105	15	77
570		11	39.92	112	150	93	14	76	616		26	47.84	128	165	109	15	84
571		12	38.92	112	150	91	15	80	617		27	48.80	128	165	91	13	68
572		13	49.26	112	150	110	16	76	618		28	38.84	128	165	98	14	72
573		14	45.56	112	150	97	15	74	619		29	42.12	126	166	100	14	70
574	66	1	49.70	117	154	130	14	78	620		30	50.75	126	166	96	13	66
575		2	40.36	115	147	110	12	76	621		31	40.14	126	166	101	15	76
576		3	45.94	115	147	109	15	78	622		32	37.20	126	166	96	13	68
577		4	47.00	115	147	130	14	70	623		33	47.76	126	166	110	13	68
578		5	52.90	115	147	118	14	68	624		34	38.26	126	166	102	14	70
579		6	42.90	115	147	124	14	72	625		35	48.58	126	165	115	13	76
580		7	47.86	115	147	108	13	65	626		36	49.34	126	165	98	13	68
581		8	43.74	115	149	115	14	72	627		37	49.38	126	165	91	13	70
582		9	45.92	115	147	129	15	76	628		38	39.92	125	165	100	14	70
583		10	47.06	115	147	126	15	78	629		39	36.74	125	167	96	13	68
584		11	43.98	115	150	130	15	110	630		40	36.44	125	168	94	17	106
585		12	41.16	115	147	123	14	68	631		41	35.04	125	168	93	13	68
586		13	43.22	115	159	110	13	62	632		42	35.96	125	168	100	15	79

Table 44. Phenotypic evaluation of 'bread wheat/synthetic hexaploid' F_7 derivatives produced for bread wheat improvement using exotic germ plasm during 2008–09 crop cycle (SH = synthetic hexaploid parent line, GW = 1,000-kernel weight (g), DH = day-to-heading, DM = days to maturity, PH = plant height (cm), SL = spike length (cm), and GS = grains/spike).

Line	Combination	SH	TKW	DH	DM	PH	SL	GS	Line	Combination	SH	TKW	DH	DM	PH	SL	GS
587		14	46.60	115	158	128	13	62	633		43	34.22	125	167	112	13	70
588		15	51.02	115	159	135	14	68	634		44	41.92	112	163	100	12	40
635		45	50.74	112	157	94	12	52	676		86	38.18	118	167	102	15	54
636		46	43.88	112	163	97	13	68	677		87	44.84	118	166	97	14	76
637		47	32.52	118	165	103	13	68	678		88	49.54	118	165	103	13	72
638		48	35.92	118	165	91	12	56	679		89	47.46	118	163	106	14	74
639		49	36.72	118	166	95	13	70	680		90	45.90	114	164	98	13	68
640		50	34.20	118	165	98	13	70	681		91	49.56	114	165	110	14	74
641		51	44.38	118	165	100	15	76	682		92	45.20	114	166	97	14	73
642		52	38.04	118	165	103	13	62	683		93	43.88	114	166	95	13	68
643		53	47.76	118	165	101	13	68	684		94	40.58	114	166	103	13	70
644		54	45.58	118	163	99	13	71	685		95	36.68	114	166	99	13	72
645		55	49.38	118	165	100	14	72	686		96	42.34	114	165	106	14	69
646		56	44.98	118	163	98	13	70	687		97	44.16	114	165	108	15	78
647		57	32.82	118	165	106	15	71	688		98	42.50	114	165	103	13	68
648		58	30.32	118	165	99	13	72	689		99	38.06	114	165	104	14	70
649		59	31.40	118	165	105	16	80	690		100	42.46	114	164	108	15	78
650		60	44.84	118	165	94	13	70									
651		61	43.42	118	163	99	13	72									
652		62	40.82	118	162	93	13	69									
653		63	41.82	118	165	106	14	70									
654		64	40.84	120	165	104	13	68									
655		65	42.24	120	165	103	13	72									
656		66	44.20	120	163	97	13	72									
657		67	51.50	117	165	94	13	73									
658		68	39.32	17	165	104	13	70									
659		69	45.80	117	164	91	13	76									
660		70	40.50	119	165	103	14	80									
661		71	42.10	119	165	98	13	71									
662		72	41.40	119	165	101	14	74									
663		73	43.00	119	163	100	15	86									
664		74	50.96	119	163	96	13	72									
665		75	54.96	119	165	101	14	72									
666		76	38.88	119	162	99	13	76									
667		77	35.36	119	163	98	14	78									
668		78	30.18	117	165	105	12	50									
669		79	34.32	117	165	95	14	72									
670		80	45.96	117	165	103	15	78									
671		81	49.72	117	165	100	14	76									
672		82	45.84	118	167	94	14	71									
673		83	49.60	118	167	103	15	74									
674		84	49.64	118	167	106	17	92									
675		85	42.66	118	167	98	13	52									

based derivatives in Kenya, the hot spot site, and the eight lines identified provide stimulus to use these in breeding after we ascertain their resistance to the local race and test more elite germ plasms that are emerging. Even on the international front, synthetics from other alien resources have been incorporated in programs for addressing Ug99 resistance.

Breeding with synthetics requires some aspects to reconcile. First, the tough threshing character of the SH lines, which is a dominant trait and so inherited. Second, the presence of hybrid necrosis that causes F_1 seedlings to die prematurely due to the presence of the recessive *ne1* and *ne2* necrotic genes. Selection pressure in F_2 was for free-threshing types, whereas the necrotic combinations were automatically eliminated when F_1s were advanced; these were few.

The phenotypic attributes of the 68 combinations offer an enormous range of useful agronomic characteristics. For days-to-heading, the earliest was 110 days for combinations 41 and 53, 111 days for 48 and 57, and 112 days for 38, 65, and 68. The 1,000-kernel weight was 65 g in combination 1, 59.06 g for 46, 55.82 g for 14, 53.98 g for 59, and 53.52 g for 4; a major yield-enhancing component useful in wheat breeding. A spike length of 20 cm was in entry 55; 19 cm in 22; 18 cm for 3 and 35; 17 cm for 21, 65, and 68; and 16 cm for entry 18. Grains/spike were 110 in combinations 22, 23, 24, 28, 30, 55, 65, and 66; combinations 35 and 68 had 106; combinations 3, 14, 51, and 52 had 100; and several entries had grain numbers between 80 and 98, which also is advantageous for exploiting.

Deploying cultivars in irrigated and rain-fed locations of each of the four national provinces from the F_7 selections made is possible. For example, the eight Ug99 resistant entries (combinations 3, 4, 9, 20, 26, 27, 50, and 62) can be targeted for Baluchistan, Sindh, and lower Punjab where stem rust is prevalent and migration of Ug99 more imminent.

For the rain-fed areas of the country, 25 combinations (from 32 to 56) are ideal candidates. These have stripe rust resistance with a drought tolerant parental structure. The germ plasm is suitable for use in upper Punjab. The remaining combinations are suited for the NWFP. When choosing lines for deployment, high yield is coupled with priority constraints for the specific province; some are met here, some need to be further addressed, and the advanced are further to be studied over the next few generations.

The D-genome encoded storage protein subunits are very important because they strongly influence bread-making quality in wheat. The 690 advanced lines derived from 68 different cross combinations were subjected to SDS–PAGE to identify HMW-glutenin subunits in the D-genome. In the final analysis, sister lines derived from the same cross and having same subunit composition were not included. In the total of 68 advanced lines, 1Dx5+1Dy10 was predominant, found in 34 lines, followed by 1Dx2+1Dy12 in 22 advanced lines. Some novel HMW-glutening subunits were found. Among these novel subunits, 1Dx1.5+1Dy10 was found in six, 1Dx1.5+1Dy12 in three, 1Dx2.1+1Dy12 in four, and 1Dx2+1Dy10 and 1Dx3+1Dy12 in three advanced lines. Some rare subunits, such as 1Dx2+1DyT2, 1Dx3+1Dy10, 1Dx4+1Dy10, and 1Dx5+1Dy12, also were observed once in these advanced lines.

In this study the pre-dominant 1Dx5+1Dy10 is a superior subunit imparting better quality characteristics. Some better quality characteristics encoded by novel subunits are also very well documented. Conclusively these synthetic derivatives possess rich allelic diversity for HMW-GS which the conventional germplasm lacks due to the presence of only two subunits (1Dx5+1Dy10 and 1Dx2+1Dy12). So, more options become available to the wheat breeders if utilizing synthetic hexaploids in recombination breeding.

Phenotypic and molecular characterization of candidate wheat cultivars and their evaluation to key biotic stresses.

Alvina Gul Kazi, Awais Rasheed, Farrukh Bashir, and Abdul Mujeeb-Kazi.

Six candidate wheat genotypes derived from synthetic hexaploids are the subject for varietal release. Morphological observations (Table 45, p. 175) and their resistance to key biotic stresses, including leaf, stem, and stripe rust (Table 46, p. 176), were made.

The grain color of all these lines was amber, red, or their combination. Kazi-1 showed the maximum 1,000-kernel weight (55.70 g) followed by Kazi-2 (46.23 g). An important yield determinant, wheat grains having a 1,000-kernel weight greater than 55 g are categorized as extra large. Kazi-6 took the minimum number of days to mature (154), fol-

Table 45. Pedigree and phenotypic data of six candidate lines for varietal release (TKW = 1,000-kernel weight and DPM = days to physiological maturity).

Line	Pedigree	Grain color	TKW (g)	DPM	Plant height (cm)	Spike length (cm)	Awn color
Kazi-1	Not available	Amber	55.70	163	118	14.66	Light Brown
Kazi-2	QT8343/PASTOR*2/OPATA	Amber	46.23	170	102.3	12.66	Light Brown
Kazi-3	TURACO/5/CHIR3/4/SIREN//ALTAR84/ *Ae. tauschii* (205)/3/3*BUC/6/CNO	Red	44.11	171	108.6	13.66	Light Brown
Kazi-4	TURACO/5/CHIR3/4/SIREN//ALTAR84/ *Ae. tauschii* (205)/3/3*BUC/6/FCT	Amber-red	43.45	170	102.3	12.66	Light Brown
Kazi-5	MAYOOR//TKSN1081/*Ae. tauschii* (222)/3/CNO	Amber-red	41.45	160	123	13.3	Light Brown
Kazi-6	TURACO/5/CHIR3/4/SIREN//ALTAR84/ *Ae. tauschii* (205)/3/3*BUC/6/BCN	Red	40.80	154	119.6	10.6	Light Brown

lowed by Kazi-5 (160 days). Kazi-2 and Kazi-4 have the same minimum plant height (102.3 cm). Kazi-1 exhibited the maximum spike length (14.66 cm) followed by Kazi-3 (13.66 cm). All lines have light brown awns.

All genotypes were resistant to stripe rust at the adult-plant stage. Kazi-1–Kazi-5 were immune to stripe rust whereas Kazi-6 showed a trace stripe rust and Kazi-6 showed a terminal response of 5R. Similarly, stem rust resistance in these genotypes was evaluated at seedling stage under glass house conditions. The inoculum used to screen these genotypes were collected from two different locations (Sindh and Bahawalpur). Both inoculum were used separately for seedling tests (Table 46). All the lines tested with Sindh inoculum were resistant except Kazi-3, which showed an intermediate response and also gave the same response when tested with the inoculum from Bahawalpur. Kazi-5 exhibited an intermediate response to stem rust with Sindh inoculum but was immune when tested with inoculum from Bahawalpur. Heterogeneity in stem rust response also was observed in Kazi-1 for both inoculum types. We concluded that the stem rust inoculum from both location is diverse and has different virulence pattern. All genotypes had intermediate resistance to leaf rust at the seedling stage except for Kazi-4 and Kazi-6, which were immune. Kazi-1, Kazi-2, and Kazi-3 are

Table 46. Disease resistance data of six candidate lines for varietal release.

Line	Stripe rust (adult)	Stem rust (seedling) Matli, Sindh	Stem rust (seedling) Bahawalpur	Leaf rust (seedling)	Powdery mildew (seedling)	Spot blotch (seedling)
Kazi-1	0	1	4	4	R	4
Kazi-2	0	1	;	4	R	2
Kazi-3	0	12	23	34	R	2
Kazi-4	0	0	;	0	S	5
Kazi-5	TR	3	;	4	S	4
Kazi-6	5R	0	0	0	S	4

resistant powdery mildew, but the others are susceptible. However, powder mildew is not a big problem in most areas and susceptible cultivars can be deployed to Punjab and Sindh. For spot blotch, Kazi-2 and Kazi-4 were found to be resistant and the others showed a moderate reaction.

These six cultivars, along with eight Pakistani commercial wheat cultivars, also were evaluated for their molecular diversity using 264 SSR markers (Roder et al. 1998). The other elite Pakistani cultivars included Inqilab-91, Pak-81, Watan, Rohtas-90, Pasban-90, TJ-83, Bakhtawer-92, and Pirsabak-85. Kazi-6 grouped in a separate subcluster with Pak-81 and Inqilab-91 (Fig. 39). On the

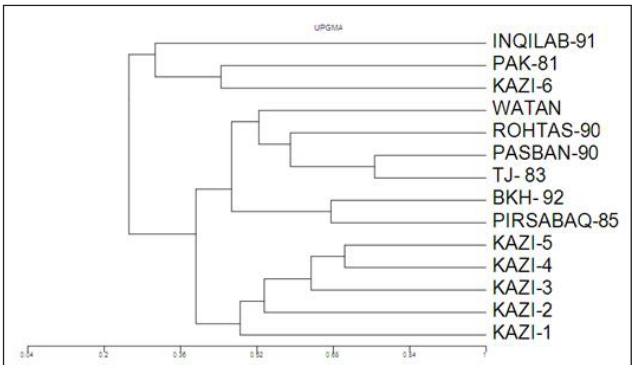


Fig. 39. Genetic diversity evaluation of the six candidate lines along with eight commercial cultivars of Pakistan using SSR markers.

other hand, Kazi 1–Kazi-5 were in a separate subcluster in which no current Pakistani cultivar was grouped; showinbg the unique genetic composition of Kazi-1–Kazi-5. Kazi-1 and Kazi-2 showed the maximum genetic diversity among all six candidate lines. Conclusively deploying these cultivars due to their molecular diversity from the existing commercial cultivars will be beneficial and enhance field diversity that will be difficult for the dynamic biotic stresses to cope with.

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Genetic discrimination for some quality attributes in genotypes with T1BL·1RS.

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High yield potential, better adaptability, and resistance to several biotic components are the advantages of the T1BL·1RS translocation in wheat. Cultivars carrying the T1BL·1RS translocation, such as Seri-82, Kavkaz, Neuzucht, and Lorvin10 and their derivatives, were highly involved in crossing programs from early 1970s to 1990s. Earlier reports suggested a negative effect of T1BL·1RS on dough and bread-making properties (Pena et al. 1990). Later, Mujeeb-Kazi et al. (1995) suggested that the adverse bread-making quality is not an exclusive function of T1BL·1RS. So, identifying genotypes with promising quality characteristics and carrying T1BL·1RS is of breeder's interest. In this study, 36 genotypes carrying the T1BL·1RS translocation were studied for some physio-chemical characteristics contributing to end-use quality.

The maximum variability was found for the gluten index (46.75%) followed by falling number (25.06%). The most consistent character was moisture content, which had a co-efficient of variability of 3.69%. Falling number ranged from 330.5 sec to 941.50 sec with an average of 466.30 sec. Eighteen of the 36 genotypes were found significantly different from mean at a CD 0.05. The standard deviation forfalling number was very high, and it also exhibited a higher co-efficient of variation (25.06%). Falling number is an indirect measure of α-amylase activity, and grains with a value greater than 400 sec have very low or no α-amylase activity. Protein content ranged from 9.03–14.97% with an average of 11.93%. Eighteen cultivars performed significantly different from the mean, and 15 had protein percentage greater than the overall mean. The co-efficient of variation for protein content was 12.53%. T1B·1R-510 showed the maximum protein content, whereas T1B·1R-500 had the minimum water content. Grain texture is the most important trait that determines hardness or softness of wheat. Hardness scores ranged from 27.33–59.67 with an average of 45.35. Replications were nonsignificant, whereas the genotypes were statistically significant. For co-efficient of variation for this trait, four genotypes were significantly different from mean and the others were not significant at a $CD_{0.05}$. Grain hardness is a key determinant for the classification of wheat and end-product quality (Campbell et al. 1999). Grain hardness primarily influences rheological properties of dough. The most important physical difference between the endosperm of hard and soft wheats lies in the adhesion between the starch granules and the surrounding protein matrix (Simmonds et al. 1973). All 36 wheat genotypes fell into the soft wheat category according to the NIR hardness scale. Moisture content ranged from 9.23–10.48% with an average of 9.78%. The co-efficient of variation for this trait was 3.69%, indicating lower variability among genotypes for this trait compared to others. Moisture content is greatly influenced by variation in the processing of grain and the method of grinding as well as variation in climatic conditions and temperature during harvest.

Thousand-kernel weight ranged from 22.40–50.00 g with an average of 35.69 g. The co-efficient of variability (6.02%) was lower for this trait (Table 47, p. 177). Fourteen genotypes were significantly different from the mean at $CD_{0.05}$. 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). Most of the genotypes are in the medium graincategory according to this scale. The ash content of these genotypes ranged from 1.45–1.85% with an average of 1.65%. 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. Zahoor (2003) reported an ash content of 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 endosperm flour.

Wet gluten was observed maximum in T1B·1R-488 (36.38%) and minimum in T1B·1R-492 (18.67%). The average wet gluten was 24.53%. Five genotypes performed significantly different from mean at CD0.05. Wet gluten has a strong effect on dough rheology and baking performance. Wet forms are more quickly incorporated into low-protein

Table 47. Some quality attributes in genotypes with T1BL·1RS. Means significantly different from mean at a CD = 0.05.

Genotype	Falling number	Protein (%)	Hardness score	Moisture (%)	1,000-kernel weight	Ash (%)	Wet gluten (%)	Gluten index
T1B·1R-469	330.50	10.30	34.00	9.60	39.40	1.65	21.44	58.70
T1B·1R-470	343.67	10.97	36.00	9.73	41.20	1.78	20.97	40.51
T1B·1R-471	427.33	11.35	49.50	9.65	33.80	1.74	23.12	29.17
T1B·1R-472	461.00	11.53	51.00	10.10	30.40	1.60	22.09	11.05
T1B·1R-474	538.50	14.13	46.00	9.83	31.40	1.83	35.71	36.54
T1B·1R-475	510.00	12.93	46.67	9.60	29.83	1.66	27.02	82.13
T1B·1R-476	427.50	11.93	41.33	9.47	29.68	1.66	22.00	64.00
T1B·1R-478	336.00	12.80	43.67	9.57	33.00	1.80	25.20	58.45
T1B·1R-479	434.00	12.97	45.00	9.43	27.40	1.70	26.34	67.00
T1B·1R-480	419.50	12.40	27.33	9.83	33.00	1.52	23.45	92.75
T1B·1R-481	422.00	13.90	42.33	9.50	22.40	1.69	27.33	83.00
T1B·1R-482	462.00	12.23	42.33	9.67	32.50	1.68	26.97	22.52
T1B·1R-483	464.50	12.93	55.00	9.40	30.80	1.65	25.61	8.48
T1B·1R-485	455.00	12.27	53.00	10.13	31.20	1.56	26.84	12.44
T1B·1R-486	501.00	11.43	49.67	10.47	33.20	1.51	26.65	12.70
T1B·1R-487	477.50	12.33	52.50	9.63	36.80	1.60	28.93	16.27
T1B·1R-488	450.00	13.73	53.00	9.63	37.40	1.69	36.38	27.20
T1B·1R-489	405.50	12.50	48.00	9.43	30.00	1.83	26.33	59.81
T1B·1R-490	377.33	11.37	48.00	9.41	33.40	1.60	23.79	46.10
T1B·1R-491	397.50	11.43	42.67	9.23	29.80	1.66	19.75	61.00
T1B·1R-492	441.50	10.27	38.67	10.17	31.80	1.63	18.67	73.00
T1B·1R-493	323.33	11.37	48.00	9.38	34.80	1.54	21.29	66.00
T1B·1R-494	421.00	10.00	47.33	9.59	32.50	1.54	21.85	81.18
T1B·1R-496	501.50	11.17	47.33	9.54	36.60	1.61	23.02	45.44
T1B·1R-497	449.50	10.03	47.33	9.53	36.00	1.62	19.47	85.00
T1B·1R-498	437.00	10.87	48.00	10.10	38.80	1.61	20.45	83.21
T1B·1R-500	538.00	9.03	45.67	10.18	33.80	1.51	21.40	82.00
T1B·1R-501	403.50	9.57	26.00	9.70	33.20	1.68	22.34	88.22
T1B·1R-502	398.00	10.43	31.67	9.47	35.00	1.45	20.13	36.88
T1B·1R-503	403.50	10.20	46.00	9.50	39.40	1.55	20.30	83.05
T1B·1R-508	458.50	11.70	55.67	10.48	48.60	1.61	28.56	86.00
T1B·1R-509	691.00	13.93	57.67	10.33	43.00	1.57	27.31	78.50
T1B·1R-510	556.50	14.97	59.00	10.14	48.20	1.78	23.45	54.76
T1B·1R-511	941.50	13.77	56.00	10.07	46.60	1.77	22.78	65.34
T1B·1R-512	724.00	14.87	59.67	10.05	50.00	1.85	27.76	43.23
T1B·1R-514	458.00	11.90	46.67	10.61	49.60	1.64	28.54	45.00
Mean	466.30	11.93	46.32	9.78	35.68	1.65	24.53	55.18
Standard deviation	116.87	1.50	8.10	0.36	6.58	0.10	4.10	25.80
CV(%)	25.06	12.53	17.48	3.69	18.44	6.02	16.69	46.75

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).

Conclusively, T1B·1R-512 showed over all better quality characteristics with a falling number of 724 sec, indicating no α-amylase activity. Protein content is 14.87%, 1,000-kernel weight is 50 g, and wet gluten is 27.76%, which make it distinct in this group of genotypes. The reported negative effects of T1BL·1RS translocations on wheat quality also can be avoided by the introgression of superior HMW-glutenin subunits at *Glu-B1* (7+8) and *Glu-D1* (5+10).

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Pasting properties of wheat flours of eight hard white spring wheat cultivars.

Saqib Arif, Qurrat-ul-ain Afzal, Tahira Mohsin Ali, Awais Ahmed, Atif Warsi, Mubarik Ahmed, Abid Hasnain, Awais Rasheed, Alvina Gul Kazi, and Abdul Mujeeb Kazi.

Starch is the main fraction of wheat. One of the main functions of starch is to set the pasting properties of wheat flour. Other factors, including protein, particle size distribution, α-amylase, starch damage, sugar, and salt, have a minor to significant impact on pasting properties of wheat flour (Nagao 1995; Wei 2002; Mousia et al. 2004). The pasting properties of wheat flour from eight hard white spring wheat (HWSW) cultivars grown in two locations in Pakistan were studied (Table 48, p. 179). The pasting profile of wheat cultivars was evaluated in terms of pasting temperature, peak viscosity, time to reach peak viscosity, hot-paste viscosity, breakdown viscosity, setback viscosity, and cold-paste viscosity using a Brabender Microviscoamylography (Model 803201, Brabender, Germany).

The pasting temperature of wheat cultivars ranged from 56.8–62.8°C and 56.5–58.4°C at Nawabshah and Tandojam, respectively. Imdad, Mehran, Abadgar, Moomal, and SKD-1 showed significant variation in pasting temperature with location. Thus, providing credence to the notion that a change in growing conditions can significantly affect the architecture of wheat kernel, which subsequently modifies the physico-chemical properties of wheat flour from the corresponding wheat cultivars.

Peak viscosity is a very important parameter that governs the thickening property of wheat flour. Viscosity is highest when there is a maximum number of swollen intact starch granules present in the wheat flour slurry (Thomas and Atwell 1999). Peak viscosity was found to vary significantly with location and ranged between 1,029–1,296 BU and 875–1,230 BU for HWSW cultivars at Nawabshah and Tandojam, respectively. The extent of variation with location ranged between 18–174 BU. The lowest and highest variation were in Anmol and Mehran, respectively. Sowing conditions could affect peak viscosity; researchers have found increased peak viscosity under late-sown conditions (Singh et al. 2010).

The time to reach peak viscosity is basically the measure of gelatinization of starch granules. Variation in location significantly effected time to reach peak viscosity of all HWSW cultivars except Anmol. The time to reach peak viscosity ranged between 14:30–16:30 min at Nawabshah and 13:10–16:20 min at Tandojam. SKD-1 at Tandojam took the least time to reach peak viscosity whereas Anmol took the longest.

Breakdown viscosity also measures the extent of fragility of starch granules (Thayumanavan and Kumari 1998). Higher breakdown values reflect less ability of starch granules to withstand high shear conditions often encountered in food processes such as blending, homogenization, and extrusion. Lower breakdown values indicate a high shear

resistance of starch granules. Breakdown viscosity of the flours ranged between 395–695 BU, with the lowest and highest values in Imdad at Tandojam and TJ-83 at Nawabshah, respectively. Change in location significantly effected the breakdown viscosity of the wheat cultivars; the minimum in Mehran and the maximum variations in Abadgar. Breakdown is low in flours with a high protein content (Singh et al. 2010) and higher amylose content (Miuria et al. 2002; Blazek and Copeland 2008). Singh et al, (2010) also found an increase in breakdown under rain-fed conditions.

Table 48. Pasting properties of wheat flours of eight hard white spring wheat cultivars at two locations in Pakistan, Nawabshah (NS) and Tandojam (TJ). Different superscript letters within the same column are significantly different at a $p < 0.05$.

Location	TD-1	Imdad	Mehran	Abadgar	Moomal	Anmol	SKD-1	TJ-83
Pasting temperature								
NS	57.3^{a}	58.3^{a}	56.8^{a}	62.8^{a}	57.0^{a}	57.2^{a}	58.5^{a}	58.6^{a}
TJ	57.1^{a}	56.6^{b}	58.3^{b}	57.0^{b}	56.5^{b}	57.4^{a}	60.4^{b}	58.4^{a}
Peak wiscosity								
NS	1,108^{a}	1,174^{a}	1,049^{a}	1,065^{a}	1,029^{a}	1,212^{a}	1,145^{a}	1,296^{a}
TJ	999^{b}	1,033^{b}	875^{b}	1,202^{b}	992^{b}	1,230^{b}	1,074^{b}	1,208^{b}
Time to reach peak wiscosity								
NS	16:30^{a}	15:40^{a}	16:00^{a}	14:55^{a}	15:15^{a}	16:20^{a}	14:30^{a}	15:00^{a}
TJ	14:00^{b}	16:10^{b}	14:35^{b}	15:10^{b}	16:00^{b}	16:35^{a}	13:10^{b}	16:20^{b}
Breadown								
NS	479^{a}	578^{a}	623^{a}	403^{a}	606^{a}	542^{a}	597^{a}	695^{a}
TJ	528^{b}	395^{b}	597^{b}	640^{b}	396^{b}	569^{b}	523^{b}	592^{b}
Hot-paste viscosity								
NS	629^{a}	594^{a}	424^{a}	662^{a}	423^{a}	682^{a}	548^{a}	601^{a}
TJ	471^{b}	638^{b}	277^{b}	561^{b}	597^{b}	660^{a}	550^{a}	606^{a}
Setback								
NS	378^{a}	613^{a}	516^{a}	585^{a}	563^{a}	561^{a}	523^{a}	501^{a}
TJ	540^{b}	669^{b}	467^{b}	557^{b}	579^{a}	566^{a}	472^{b}	515^{a}
Cold-paste viscosity								
NS	1,146^{a}	1,166^{a}	934^{a}	1,214^{a}	981^{a}	1,221^{a}	998^{a}	1,048^{a}
TJ	962^{b}	1,213^{b}	796^{b}	1,089^{b}	1,115^{b}	1,153^{b}	853^{b}	1,056^{a}

The hot-paste viscosity of TD-1, Imdad, Mehran, Abadgar, and Moomal varied significantly with location. The cultivars at Nawabshah ranged between 423–682 BU and those at Tandojam 277–660 BU. The variation in hot-paste viscosity in the cultivars ranged between 2–174 BU.

Cold-paste viscosity measured at 50°C varied with the change of location and the extent of variation ranged between 8–184 BU. Mehran at Tandojam and Abadgar at Nawabshah showed lowest and highest CPV values, respectively. Setback viscosity is the measure of retrogradation tendency of starch granules (Karim et al. 2000). When a gelatinized starch slurry is cooled, the leached out amylose chains reassociate with each other. Therefore, higher setback values reflect increased retrogradation tendency of starch granules. TD-1, Imdad, Mehran, Abadgar, and SKD-1 showed significant differences in setback viscosity with location. Compared to other HWSW cultivars, TD-1 at Nawabshah showed a very low setback value of 378 BU, whereas Imdad at Tandojam showed highest setback value of 669. Peak viscosity for the cultivars was found to be positively correlated with cold-paste viscosity with a Pearson correlation coefficient of 0.660 at $p < 0.01$. Cold-paste viscosity was found to be positively correlated with hot-paste viscosity (0.857) and time to reach peak viscosity (0.626) with Pearson coefficients $p < 0.01$.

The pasting properties of wheat flour were related to cultivar as well as growing location. Furthermore, other cultivars, including approved and those in the pipeline, shall be exploited for their pasting profiles.

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Arabinoxylan levels in hard wehat of various origin.

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A total of 39 hard wheat samples representing seven origins (Australia, Argentina, Brazil, Canada, France, Pakistan, and the Russian Federation) and two classes (white and red) were analyzed for their total arabinoxylan (AXt) and water-extractable arabinoxylan (WeAX) content.

The mean, minimum, and maximum values for AXt and WeAX of wheat of various origin are given in Table 49. AXt and WeAX were 45.6–84.2 mg/g and 5.2–12.4 mg/g in meal and 11.1–24.1 mg/g and 2.7–8.7 mg/g in flour, respectively. The highest and lowest values were found in Pakistani wheat. However, the highest mean values for AXt and WeAX in meal and flour were found in the wheats from Argentina. These values were significantly different from those of Pakistani wheats. On the other hand, Pakistani wheat was not statistically different from Australian wheat in their AX levels. For WeAX, the lowest meal value was in Australian and French wheats. The flours of Pakistani and Australian wheats contained the lowest amount of WeAX. The variation observed in the AX content of these wheats is due to their different genetic background and market classes. Hong et al. 1989; Anderson et al. 1992; Saulnier et al. 1995; Lempereur et al. 1997 all reported that AX content varies with genotype and environment. Furthermore, we analyzed the relationship of AX content with other quality parameters and found no clear relationship. Li et al. (2009) also found no significant relationship of AX with test weight, protein, or hardness. However, some obvious relationships were found between the AXt and WeAX. AXt was positively related with WeAX in meal (r = 0.919**) and flour (r = 0.949**). In flour, AXt and WeAX was directly related with their amount in meal. Highly significant relationships were found between AXt in meal and flour (r = 0.892**), and WeAX in meal was significantly related with that in flour (r = 0.872**).

Table 49. Minimum, maximum and mean[1] values for total arabinoxylan (AXt) and water-extractable arabinoxylan (WeAX) content of wheat of different origin.

Origin	Arabinoxylan contents (mg/g)			
	Meal		Flour	
	AXt	WeAX	AXt	WeAX
Australia				
Min	51	6.3	12.2	3.6
Max	63	8.2	17.2	6.5
Mean	55[a]	7.2[ad]	15.1[a]	5.3[a]
Argentina				
Min	67.2	8.7	18.0	7.2
Max	77.3	10.4	21.1	8.0
Mean	72.4[bc]	9.5[ab]	19.6[b]	7.6[b]
Brazil				
Min	65.4	8.5	17.7	6.7
Max	76	10.0	20.1	7.7
Mean	70.7[ab]	9.2[bc]	18.9[bc]	7.2[bc]
Canada				
Min	55	6.9	15.0	5.5
Max	63	8.1	17.4	6.5
Mean	58.5[ab]	7.4[bc]	16.4[ac]	6.0[ab]
France				
Min	51	6.1	12.5	3.7
Max	66	8.7	17.7	6.6
Mean	59.5[ab]	7.2[c]	15.7[a]	5.6[ac]
Pakistan				
Min	45.6	5.2	11.1	2.7
Max	84.2	12.4	24.1	8.7
Mean	59.9[a]	7.9[d]	15.2[ac]	5.3[a]
Russia				
Min	67	8.9	17.9	6.8
Max	75	10.0	20.7	7.8
Mean	70.7[c]	9.3[ab]	18.8[b]	7.3[b]

Saulnier et al. (1995) evaluated the usefulness of wheat as poultry feed with a major emphasis on AX content in 22 wheat cultivars grown at different locations in France. They found that cultivars with a high natural variation (CV 8%) in total AX content ranging from 5.53–7.79% with the mean value of 6.63%. In our study, the AX content of French wheat ranged from 51–66 mg/g (5.1-6.6%) with the mean value of 59.5 mg/g (5.95%). These varied results were obtained due to different samples. In another study by Lempereur et al. (1997), the AX content ranged from 4.07–6.02% in durum wheat cultivars grown in France under different agronomic conditions. The value of AX in durum wheat is similar to that of bread wheat (Medcalf & Gilles 1968). All wheats except those from the Russian Federation were not found statistically different from the French wheat in their AXt level (meal). Furthermore, the AXt and WeAX content of French wheat (meal and flour) were found to be significantly different from that of Russian wheat but not significantly different from Canadian wheat. Canadian wheat samples ranged from 55–63 mg/g (5.5–6.3%) with the mean value of 58.2 mg/g (5.82%). Wang et al. (2006) reported almost the same range (5.45–7.32%) for AX content in six commercially grown samples of common hard spring wheats. In addition to French wheat, Canadian wheat was found to be not significantly different from Brazilian wheat in AX level. Specifically, the WeAX content in the flour of Canadian wheat was not statistically different from that of all wheat samples in the study.

In this study, a total of 39 hard spring wheats were analyzed; 24 were white and 15 were red. The AX content of the white wheats varied widely (Table 50). However, the mean AX content of the red wheats was greater than that of the white wheats. We have not found consistent differences in AX content of white wheats and red wheats (Table 50).

Table 50. Levels of arabinoxylan in red and white wheats of different origin.

	Meal				Flour			
	Total arabinoxylan content		Water-extractable arabinoxylan content		Total arabinoxylan content		Water-extractable arabinoxylan content	
Type	Red	White	Red	White	Red	White	Red	White
Minimum	51.0	45.6	6.1	5.2	12.5	11.1	3.7	2.7
Maximum	77.3	84.2	10.4	12.4	21.1	24.1	8.0	8.7
Mean	68.0	59.2	8.8	7.8	18.1	15.3	6.9	5.3

The development of high quality wheat depends on a thorough understanding of the influential factors and parameters. No doubt, AXs have a significant impact on the quality of the end-product. Because they are mainly concentrated in outer layer, AX was greater in the meal than in the flour. AX varied with the growing location irrespective of wheat class (red or white). Wheat of different origin with a known AX content can be better utilized for its desired end-product. Information about AX level will be useful for a country like Pakistan that imports wheat from diversified origins.

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High-molecular-weight glutenin subunit variation in B-genome amphiploids (2n=6x=AABBBB).

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Sixteen B-genome amphiploids (2n=6x=42; AABBBB) were analyzed for their HMW -glutenin subunit diversity using SDS-PAGE. Three durum and 13 *Ae. speltoides* accessions were utilized to develop these amphiploids. All the durum parents had null subunit encoded by the allele *Glu-A1c* at the *Glu-A1* locus. Three different subunit pairs, 7+8, 17+18, and 13+16, were found at the *Glu-B1* locus in the amphiploids (Table 51). The locus contributed by the Ae. *speltoides* was designated as *Glu-B^{s}1*. The allelic classification at this locus followed international recommendations (McIntosh et al. 1998, 2007), although the genes were designated with Roman numerals to avoid possible ambiguities for inclusion in Wheat Gene Catalogue. The name of each allele was sequentially based on frequency among accessions.

Table 51. Allelic variation at the *Glu-1* loci in B-genome amphiploids (2n=6x=42; AAAABB) derived from *Aegilops speltoides*.

Line	Pedigree	*Glu-A1*	*Glu-B1*	*Glu-B^{s}1*
B1	CETA/*Ae. speltoides* (127)	Null	13+16	*Glu-B^{s}1-IV*
B2	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. speltoides* (133)	Null	7+8	*Glu-B^{s}1-V*
B3	ARLIN_1/*Ae. speltoides* (134)	Null	17+18	*Glu-B^{s}1-I*
B4	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. speltoides* (135)	Null	7+8	*Glu-B^{s}1-VI*
B5	CETA/*Ae. speltoides* (139)	Null	13+16	*Glu-B^{s}1-VII*
B6	ARLIN_1/*Ae. speltoides* (126)	Null	17+18	*Glu-B^{s}1-VIII*
B7	ARLIN_1/*Ae. speltoides* (128)	Null	17+18	*Glu-B^{s}1-IX*
B8	ARLIN_1/*Ae. speltoides* (130)	Null	17+18	*Glu-B^{s}1-X*
B9	ARLIN_1/*Ae. speltoides* (131)	Null	17+18	*Glu-B^{s}1-XI*
B10	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. speltoides* (143)	Null	7+8	*Glu-B^{s}1-XII*
B11	ARLIN_1/*Ae. speltoides* (144)	Null	17+18	*Glu-B^{s}1-XIII*
B12	ARLIN_1/*Ae. speltoides* (147)	Null	17+18	*Glu-B^{s}1-II*
B13	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. speltoides* (147)	Null	7+8	*Glu-B^{s}1-II*
B14	ARLIN_1/*Ae. speltoides* (156)	Null	17+18	*Glu-B^{s}1-III*
B15	CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. speltoides* (156)	Null	7+8	*Glu-B^{s}1-III*
B16	ARLIN_1/*Ae. speltoides* (131)	Null	17+18	*Glu-B^{s}1-XIV*

A very rich diversity was contributed by *Ae. speltoides*. All accessions used to develope these amphiploids had a unique allele at *Glu-B^{s}1* except for *Ae. speltoides* lines 134 and 139. Lines B13 and B15 encoded only x-type subunits, whereas all the remaining accessions encoded both x- and y-type subunits at the *Glu-B^{s}1* locus. A total of 14 different subunits were observed in these 16 amphiploids. Lines B14 and B15 and B12 and B13 had same durum and *Ae. speltoides* parents, therefore they did not show any heterogeneity between one another. Previously, Fernández-Calvín (1990) identified HMW-glutenin subunits in *Ae. searsii* and *Ae. speltoides* and concluded that these subunits moved within the range of B-genome in hexaploid wheat. However, *Ae. speltoides* is the only species that could explain the variability for HMW-subunits previously described for the B genome of wheat, and therefore, cannot be excluded as a possible donor of this genome. These genetic resources can be a good source for using the allelic variation in *Ae. speltoides* in common hexaploid wheat through standard breeding methodologies.

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Bread quality of hard white spring wheat cultivars grown at two locations.

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The bread-making performance of flours from eight Pakistani hard white spring wheat (HWSW) cultivars grown at two locations were evaluated for their quality attributes including loaf height (LH), loaf volume (LV), specific loaf volume (SLV), and sensory and textural properties (Table 52).

Table 52. Loaf height, loaf volume, specific loaf volume, and crumb firmness of breads prepared from hard white spring wheat cultivars grown at Nawabshah (NS) and Tandojam (TJ), in Pakistan.

Cultivar	Loaf height (cm)		Loaf volume (cm^3)		Specific loaf volume (cm^3/g)		Crumb firmness (g)	
	NS	TJ	NS	TJ	NS	TJ	NS	TJ
TD-1	8.4	7.0	709.8	591.5	5.1	4.2	829.90	946.83
Imdad	7.6	8.0	642.2	676.0	4.6	4.8	865.64	850.10
Mehran	8.1	9.5	684.5	802.8	4.9	5.7	837.27	802.70
Abadgar	7.1	10.0	600.0	845.0	4.3	6.0	909.30	750.40
Moomal	6.1	7.8	515.5	659.1	3.7	4.7	964.91	858.40
Anmol	7.1	7.8	600.0	659.1	4.3	4.7	925.60	869.13
SKD-1	5.8	7.9	490.1	667.6	3.5	4.8	990.69	852.30
TJ-83	6.3	8.4	532.4	709.8	3.8	5.1	940.41	835.80

Breads varied in their LH, LV, and SLV with the change of genotype and location. Many workers have found the significant effects of genotype and environment on bread-making quality in wheat (Johansson et al. 1999; Anjum and Walker 2000; Mladenov et al. 2001; Yong et al. 2004; Finlay et al. 2007). The cultivars ranged from 5.8–8.4 cm (LH), 490.1–709.8 cm^3 (LV), and 3.5–5.1 cm^3/g (SLV) when grown at Nawabshah, and, they ranged from 7.0–10.0 cm (LH), 591.5 to 845.0 cm^3 (LV), and 4.2 to 6.0 cm^3/g (SLV) when grown at Tandojam. All the cultivars, except TD-1, performed better at Tandojam than at Nswabshah for LH, LV, and SLV. The highest and lowest values for LH, LV, and SLV were by Abadgar (at Tandojam) and SKD-1 (at Nawabshah), respectively.

Quality assessment of bread by sensory evaluation is a subjective evaluation based on personal judgment. The results are, therefore, not absolute and reflect consumer preference. Several workers suggest that baking scores be made in addition to loaf volume (Bhatt and Derera 1975; Mladenov et al. 2001). Similar to physical properties, the sensory characteristics of breads varied with cultivar and location. The results were inline with previous studies (Mladenov et al. 2001). All the cultivars had scores greater than 70 out of 100 at both locations (Table 53). Bread from TD-1 (at Nawabshah) was superior with respect to sensory attributes. Marginal differences were observed between the dough scores of different cultivars. One reason would be the optimum addition of water as assessed by Farinograph. The dough scores of cultivars grown at Nawabshah and Tandojam ranged from 23 to 25 and 24 to 25, respectively.

Table 53. Dough and bread quality scores of Pakistani wheat cultivars grown at Nawabshah (NS) and Tandojam (TJ), in Pakistan.

Cultivar	Dough score (27)		External score (18)		Internal score (55)		Total score (100)	
	NS	TJ	NS	TJ	NS	TJ	NS	TJ
TD-1	25	25	18	11	48	43	91	79
Imdad	23	25	17	16	46	45	86	86
Mehran	24	24	18	18	48	48	90	90
Abadgar	25	24	15	18	44	45	84	87
Moomal	25	24	9	11	40	43	74	78
Anmol	24	25	15	17	35	46	74	88
SKD-1	24	24	11	15	39	41	74	80
TJ-83	25	25	14	18	38	46	76	89

Breads were analyzed for external quality including external symmetry, break and shred, crust character, and color. All cultivars had external scores in between 15 and 18 out of 18 except Moomal, SKD-1, and TD-1. Moomal had the lowest external score (9) when grown at Nawabshah and was nearly similar at Tandojam with a score of 11. Cultivars SKD-1 and TD-1 scored an 11 at both locations, however, they performed better with the change of location as SKD-1 scored a 15 at Nawabshah and TD-1 scored an 18 at Tandojam.

Internal scores were given on the basis of internal grain, texture, crumb body, crumb color, taste/aroma, and mouth feel. At Nawabshah, the cultivars ranged from 35 to 48, and the lowest internal score was given to the bread from Anmol due to its inferior internal grain, texture, and crumb body. This cultivar's internal quality was found in a narrow range (41–48) at Tandojam. Mehran had the highest score (48) at both locations. All the cultivars had nearly same taste, aroma, and mouth feel.

The crumb firmness varied with cultivar and growing location (Table 52, p. 183). Crumb firmness of breads was 829.90–990.69 g at Nawabshah and 750.40–946.83g at Tandojam. All cultivars except TD-1 produced firmer breads when grown at Nawabshah. Cultivars having the highest and lowest SLV, e.g., Abadgar (at Tandojam) and SKD-1 (at Nawabshah), respectively, also have the lowest and highest crumb firmness, which agrees with previous studies by Axford et al. (1968), Maleki et al. (1980), and Courtin et al. (2001).

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Marker-assisted selection for stripe rust resistance genes and allelic variation at the Glu-1 locus in synthetic-derived, advanced lines.

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SDS-PAGE analysis was utilized to study HMW-GS composition in 95 advanced synthetic derivatives, five local cultivars (Inqilab-91, Seher, Bhakkar, TD-1, and Fareed), a durum wheat, and SH-854 (Table 54, pp. 185-186). These advanced synthetic derivatives were partitioned into 27 different HMW-GS combinations (Table 55, p. 186-187). At the *Glu-A1* locus, the composition of alleles was only contributed by x-type subunits, 1Ax1, 1Ax2*, and null, which are controlled by alleles *Glu-A1a*, *Glu-A1b*, and *Glu-A1c*, respectively. Of the advanced synthetic derivatives, the null allele was the most frequent (63, 61.76%), followed by 1Ax1 (20, 19.60%), and 1Ax2* (19, 18.62%). The predominant null allele at this locus was reported previously by several workers. A higher proportion of the null allele has been reported in synthetic hexaploids (Pena et al. 1995) and in the world collection of wheat cultivars (Payne and Lawrence 1983) and justifies the predominance of null in these advanced synthetic derivatives. The subunit composition 'null 17 + 18 and 5 + 10' was the most frequent, found in 15 advanced lines (Kazi-09, Kazi-24, Kazi-32, Kazi-33, Kazi-53, Kazi-55, Kazi-58, Kazi-60, Kazi-61, Kazi-63, Kazi-65, Kazi-69, Kazi-71, Kazi-102, and Kazi-108). Another frequent subunit composition was 'null 7 + 8 and 5 + 10' was found in nine entries (Seher, Bhakkar, Kazi-17, Kazi-43, Kazi-49, Kazi-50, Kazi-51, Kazi-52, and Kazi-92). Apart from the null allele, 39 (38.23%) of the 102 advanced lines had either 1Ax1 or 1Ax2*, which impart better quality to wheat flour and are associated with higher extensibility and better dough strength (Branlard and Dardevet 1985). Several other subunits with different unique compositions also were found in these advanced synthetic derivatives. The subunits null and 2* also were found in large number of entries. The majority

Table 54. Pedigrees of advanced lines used to assay stripe rust resistance genes and allelic variation at the *Glu-1* locus.

Entry #	Name	Pedigree
1	INQILAB	WL 711/CROW`S'
2	SEHER	CHILL/2* STAR/4/BOW//BUC/PVN/3/2* VEE#10
3	BHAKKAR	P20102/PIMA/SKA/3/TTR`S'/BOW`S'
4	TD-1	MAI'S' X NORTENO65 X H68
5	FAREED	PT'S'/3/TOB/LFN//BB/4/BB/HD-832-5//ON/5/G-V/ALD'S'//HPO
6	SH	D67.2/P66.270//AE.SQUARROSA (634)
7	DURUM	ROK/KML
8	KAZI-9	SERI.1B*2/3/KAUZ*2/BOW/KAUZ/4/PBW343*2/KUKUNA
9	KAZI-10	WHEAR/TUKURU//WHEAR
10	KAZI-11	WHEAR/KUKUNA/3/CBO.1/3*BATAVIA//2*WBLLI
11	KAZI-12	TURACO/5/CHIR3/4/SI-REN/ALTAR84/*Ae. tauschii* (205)/3/3*BUC/6/CNO
12	KAZI-13	CHAPIO/INQALAB 91 x RABI//GS/CRA
13	KAZI-14	CHAPIO/INQALAB 91 x RABI//GS/CRA
14	KAZI-15	TURACO/5/CHIR3/4/SI-REN/ALTAR84/*Ae. tauschii* (205)/3/3*BUC/6/BCN
15	KAZI-16	TURACO/5/CHIR3/4/SI-REN/ALTAR84/*Ae. tauschii* (205)/3/3*BUC/6/CNO
16	KAZI-17	TURACO/5/CHIR3/4/SI-REN/ALTAR84/*Ae. tauschii* (205)/3/3*BUC/6/FCT
17	KAZI-18	TURACO/5/CHIR3/4/SI-REN/ALTAR84/*Ae. tauschii* (205)/3/3*BUC/6/FCT
18	KAZI-19	SABUF/3/BCN/CETA/*Ae. tauschii*/4/BCN
19	KAZI-20	SABUF/3/BCN/CETA/*Ae. tauschii*/4/CNO
20	KAZI-21	SABUF/3/BCN/CETA/*Ae. tauschii*/4/FCT
21	KAZI-22	MAYOOR//TKSN1081/*Ae. tauschii*/3/OPA
22	KAZI-23	MAYOOR//TKSN1081/*Ae. tauschii*/3/CNO
23	KAZI-24	MAYOOR//TKSN1081/*Ae. tauschii*/3/FCT
24	KAZI-25	MAYOOR//TKSN1081/*Ae. tauschii*/3/FCT
25	KAZI-26	YS/PASTOR
26	KAZI-27	12*2
27	KAZI-28	KARIEGA/SAAR
28	KAZI-29	KARIEGA/SAAR

Table 54. Pedigrees of advanced lines used to assay stripe rust resistance genes and allelic variation at the *Glu-1* locus.

Entry #	Name	Pedigree
29	KAZI-30	KARIEGA/SAAR
30	KAZI-31	KARIEGA/SAAR
31	KAZI-32	KARIEGA/SAAR
32	KAZI-33	KARIEGA/SAAR
33	KAZI-34	KARIEGA/SAAR
34	KAZI-35	KARIEGA/SAAR
35	KAZI-36	KARIEGA/SAAR
36	KAZI-37	KARIEGA/SAAR
37	KAZI-38	KARIEGA/SAAR
38	KAZI-39	KARIEGA/SAAR
39	KAZI-40	KARIEGA/SAAR
40	KAZI-41	KARIEGA/SAAR
41	KAZI-42	KARIEGA/SAAR
42	KAZI-43	KARIEGA/SAAR
43	KAZI-44	KARIEGA/SAAR
44	KAZI-45	KARIEGA/SAAR
45	KAZI-46	KARIEGA/SAAR
46	KAZI-47	KARIEGA/SAAR
47	KAZI-48	KARIEGA/SAAR
48	KAZI-49	KARIEGA/SAAR
49	KAZI-50	KARIEGA/SAAR
50	KAZI-51	KARIEGA/SAAR
51	KAZI-52	KARIEGA/SAAR
52	KAZI-53	KARIEGA/SAAR
53	KAZI-54	KARIEGA/SAAR
54	KAZI-55	KARIEGA/SAAR
55	KAZI-56	KARIEGA/SAAR
56	KAZI-57	KARIEGA/SAAR
57	KAZI-58	KARIEGA/SAAR
58	KAZI-59	KARIEGA/SAAR
59	KAZI-60	KARIEGA/SAAR
60	KAZI-61	KARIEGA/SAAR
61	KAZI-62	KARIEGA/SAAR
62	KAZI-63	KARIEGA/SAAR
63	KAZI-64	KARIEGA/SAAR
64	KAZI-65	KARIEGA/SAAR
65	KAZI-66	KARIEGA/SAAR
66	KAZI-67	KARIEGA/SAAR
67	KAZI-68	KARIEGA/SAAR
68	KAZI-69	KARIEGA/SAAR
69	KAZI-70	KARIEGA/SAAR
70	KAZI-71	KARIEGA/SAAR
71	KAZI-72	KARIEGA/SAAR
72	KAZI-73	KARIEGA/SAAR
73	KAZI-74	FILIN/KARIEGA
74	KAZI-75	FILIN/KARIEGA
75	KAZI-76	FILIN/KARIEGA
76	KAZI-77	FILIN/KARIEGA
77	KAZI-78	FILIN/KARIEGA
78	KAZI-79	FILIN/KARIEGA

Table 54. Pedigrees of advanced lines used to assay stripe rust resistance genes and allelic variation at the *Glu-1* locus.

Entry #	Name	Pedigree
79	KAZI-80	FILIN/KARIEGA
80	KAZI-81	FILIN/KARIEGA
81	KAZI-82	FILIN/KARIEGA
82	KAZI-83	FILIN/SAAR
83	KAZI-84	FILIN/SAAR
84	KAZI-85	FILIN/SAAR
85	KAZI-86	FILIN/SAAR
86	KAZI-87	FILIN/SAAR
87	KAZI-88	FILIN/SAAR
88	KAZI-89	PFAU/WEAVER*2/3/WEAVER/ESDA//BORL95
89	KAZI-90	BL 1496/MILAN/3/CROC_1/*Ae. tauschii* (205)//…
90	KAZI-91	MILAN/S87230//BABAX
91	KAZI-92	PSN/BOW//SERI/3/MILAN/4/AT-TILA
92	KAZI-93	CROC_1/*Ae. tauschii* (205)//KAUZ/3/BJY/COC//PRL/BOW
93	KAZI-94	KRICHAUFF/2*PASTOR
94	KAZI-95	OASIS/SKUAZ//4*BCN/3/WBLL1
95	KAZI-96	BABAX/3/PRL/SARA//TSI/VEE#5/4/WBLL1
96	KAZI-97	JNRB.5/PIFED
97	KAZI-98	CROC_1/*Ae. tauschii* (205)//BORL95/3/KENNEDY
98	KAZI-99	D67.2/P66.270//*Ae. tauschii* (320)/3/…
99	KAZI-100	QT6581/4/PASTOR//SITE/MO/3/CHEN/…
100	KAZI-101	BL 1496/MILAN/3/CROC_1/ *Ae. tauschii* (205)//…
101	KAZI-102	KAUZ//ALTAR 84/AOS/3/MILAN/KAUZ/4/HUITES
102	KAZI-103	CAR//KAL/BB/NAC/4/VEE/PJN//2*TUI/5/MILAN
103	KAZI-104	INQALAB91*2/TUKURU
104	KAZI-105	FRET2/TUKURU//FRET2
105	KAZI-106	TUKURU//BAV92/RAYON
106	KAZI-107	SANSU/CHIBA
107	KAZI-108	YACO//ALTAR 84/*Ae. tauschii* (191)/3/…
108	KAZI-109	SCA/A*Ae. tauschii* (409)//PAS-TOR/3/PASTOR
109	KAZI-110	KS940935.7.1.2/2*PASTOR
110	KAZI-111	LOCAL CHECK **CHECK**
111	KAZI-112	CROC_1/*Ae. tauschii* (205)//BORL95/3/FILIN/4/…
112	KAZI-113	SLVS/3/CROC_1/*Ae. tauschii* (224)//OPATA

Table 55. High-molecular-weight glutenin subunit composition and quality score of 102 synthetic-derived, advanced lines.

Subunit combination	Number of entries	Quality score	Entry name
1, 6+8, 5+10	1	10	SH-854
1, 17+18, 2+12	1	8	Kazi-45
1, 7+8, 5+10	2	10	Kazi-54, Kazi-109
1, 17+18, 5+10	3	10	Kazi-86, Kazi-88, Kazi-91
1, 13+16, 5+10	8	10	Kazi-72, Kazi-73, Kazi-74, Kazi-75, Kazi-76, Kazi-77, Kazi-78, Kazi-79
1, 7, 5+10	4	9	Kazi-94, Kazi-98, Kazi-100, Kazi-103
1, 7+9, 2+12	1	8	Kazi-97
2*, 6+9, 5+10	1	10	Kazi-80
2*, 6+8, 5+10	1	10	Kazi-70
2*, 13+16, 2+10	1	8	Kazi-62
2*, 13+16, 5+10	1	10	Kazi-44
2*, 7+8, 5+10	2	10	TD-1, Kazi-23
2*, 17+18, 5+10	8	10	Inqilab-91, Kazi-31, Kazi-37, Kazi-39, Kazi-40, Kazi-67, Kazi-68, Kazi-56
2*, 7+8, 2+12	5	8	Kazi-34, Kazi-36, Kazi-38, Kazi-41, Kazi-110
Null, 7+8, 5+10	9	8	Seher, Bhakkar, Kazi-17, Kazi-43, Kazi-49, Kazi-50, Kazi-51, Kazi-52, Kazi-92
Null, 13+16, 5+10	6	8	Kazi-16, Kazi-18, Kazi-19, Kazi-27, Kazi-59, Kazi-96
Null, 6+8, 2+12	1	6	Kazi-22
Null, 13+16, 2+12	2	6	Kazi-20, Kazi-81

Table 55. High-molecular-weight glutenin subunit composition and quality score of 102 synthetic-derived, advanced lines.

Subunit combination	Number of entries	Quality score	Entry name
Null, 17+18, 5+10	15	8	Kazi-09, Kazi-24, Kazi-32, Kazi-33, Kazi-53, Kazi-55, Kazi-58, Kazi-60, Kazi-61, Kazi-63, Kazi-65, Kazi-69, Kazi-71, Kazi-102, Kazi-108
Null, 7+8, 2+12	6	6	Kazi-21, Kazi-25, Kazi-26, Kazi-83, Kazi-107, Kazi-112
Null, 13+16	1	4	Durum
Null, 17+18, 2+12	8	6	Kazi-29, Kazi-30, Kazi-42, Kazi-46, Kazi-47, Kazi-105, Kazi-106, Kazi-111
Null, 7+9, 5+10	2	8	Kazi-15, Kazi-82
Null, 6+8, 5+10	4	8	Kazi-28, Kazi-57, Kazi-64, Kazi-99
Null, 13+16, 2+10	1	6	Kazi-48
Null, 7, 5+10	5	7	Kazi-66, Kazi-84, Kazi-85, Kazi-95, Kazi-104
Null, 7+9, 2+12	3	6	Fareed-06, Kazi-93, Kazi-101

of entries possessed either subunit 17+18 or 5+10. Subunit 17+18 was found in 35 (34.31%) of the 102 entries, subunit 7+8 in 24 (22.54%), 13+16 in 20 (19.60), 7 in nine (8.82%), 6+8 in seven (6.86%), and 6+9 in one (0.98%). All these subunits are controlled by allele *Glu-D1*. Subunit 5+10 was found in 73 (71.56%) of the 102 entries, 2+12 in 27 (26.47%), and 2+10 in two (1.96%), all of these are controlled by allele *Glu-B1*.

The variation in the HMW-glutenin subunits of wheat are known to be correlated with bread making quality. Payne et al. (1987) were able to determine the overall quality of a cultivar in terms of HMW-glutenin subunits by adding together the score of individual subunits. For most entries, the quality score was 8 or greater. Twenty-five entries (Inqilab-91, Kazi-31, Kazi-37, Kazi-39, Kazi-40, Kazi-67, Kazi-68, Kazi-56, SH-854, Kazi-54, Kazi-109, Kazi-86, Kazi-88, Kazi-91, Kazi-72, Kazi-73, Kazi-74, Kazi-75, Kazi-76, Kazi-77, Kazi-78, Kazi-79, Kazi-80, TD-1, Kazi-23, Kazi-44, and Kazi-70) scored 10, four entries (Kazi-94, Kazi-98, Kazi-100, Kazi-103) scored 9, and the remaining 44 scored 8 according to Payne et al. (1987).

Marker-assisted selection for effective stripe rust resistance genes. Marker assisted selection for stripe rust resistance genes *Yr15*, *YrSp*, and *YrTp-1* were carried out on these advanced lines. The SSR marker GWM155-1A, linked to stripe rust resistance gene *YrSp* (Yan-Ling et al. 2003), was used to detect a 147-bp fragment in the 102 entries. Only two entries, Kazi-84 and Kazi-85, had *YrSp*.

The SSR marker GWM413-1B, which is linked to stripe rust resistance gene *Yr15* (Peng et al. 2000), was used to detect a 96-bp fragment in the germ plasm. Five of the entries, Kazi-44, Kazi-77, Kazi-78, Kazi-84, and Kazi-85, had *Yr15*.

The SSR marker WMC477-2B, which is linked to stripe rust resistance gene *YrTp-1* (Yin et al. 2006) was used to detect a 167-bp fragment in the 102 entries. Six entries had gene *YrTp-1*; Kazi-09, Kazi-17, Kazi-28, Kazi-44, Kazi-84, and Kazi-85. Some of 102 entries possessed more than one gene of stripe rust resistance (Table 56). Kazi-84 and Kazi-85 have all of three stripe rust resistance genes, *Yr15*, *YrSp*, and *YrTp-1*, and Kazi-44 has *Yr15* and *YrTp-1*.

Table 56. Marker-assisted selection for effective yellow rust resistance genes *Yr15*, *YrSp*, and *YrTp-1* (+ = present and – = absent).

Entry	Marker		
	GWM155-3A$_{147bp}$ (*YrSp*)	GWM413-1B$_{96bp}$ (*Yr15*)	WMC477-2B$_{167bp}$ (*YrTp-1*)
Kazi-9	–	–	+
Kazi-17	–	–	+
Kazi-28	–	–	+
Kazi-44	–	+	+
Kazi-77	–	+	–
Kazi-78	–	+	–
Kazi-84	+	+	+
Kazi-85	+	+	+

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Grain quality characteristics of synthetic derived genotypes advanced by doubled haploidy.

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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. To capture the diversity in synthetic hexaploids for grain quality parameters, 35 synthetic-derived, advanced lines were screened for essential quality characteristics. A significant genotype effect was shown for all the characters studied (Table 57, p. 189).

The falling number of these genotypes ranged from 266.67–788.00 sec with an average of 416.97. Replications were not significant but genotypes were significant at $P_{0.05}$. Five genotypes were found significant at $CD_{0.05.}$ Falling number is an important determinant of α-amylase activity and also an indicator of sprout damage and flour set up ability. Mailhott and Patton (1988) reported that all types of bread flour should have falling number values of 200–300 sec. Wheat flour with a falling number value higher than 400 sec has very low or no α-amylase activity. In our study, 16 genotypes had a falling number greater than 400 sec. DH-4 showed the lowest value, 266.67 sec, and DH-13 the maximum.

Protein content was the maximum in DH-12 (16.03%) and the minimum in DH-34 (9.70%). All genotypes were significantly different at $CD_{0.05}$ except DH-3, DH-13, DH-17, DH-18, DH-25, and DH-32. Mean protein content was 12.78% with a CV = 10.05%, indicating better variability among genotypes for this character. Broad-sense heritability was 0.71, which indicated a lesser role of the environment in the transfer of this trait. Protein content in Pakistani wheat cultivars was 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 breeding for both traits difficult. However, identifying lines with high yield and high protein is of prime importance, and advanced lines with promising grain yield can be an important sources of higher protein content.

Grain texture is the most important trait that determines the hardness or softness of wheat. Hardness scores were 30.00–53.67, with an average of 45.10. Replications were not significant but genotypes were statistically significant. Twenty-nine genotypes were significantly different from mean, whereas the others were not significant at $CD_{0.05.}$ Grain hardness is the key determinant for the classification of wheat and end product quality (Campbell et al. 1999). Grain hardness primarily influences the rheological properties of dough. 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 35 genotypes fall into the soft wheat category according to the NIR hardness scale. Some authors also reported that kernel size exerts an effect on grain hardness, however they differ in their opinion about the extent of the effect. Williams et al. (1987) emphasized that kernel size exerts a small effect, whereas Pomeranz et al. (1988) reported a direct effect of kernel size on grain hardness.

Moisture content was 9.22–11.33% with an average of 10.25%. The co-efficient of variation for this trait was 6.20%, indicating lower variability among genotypes compared to other traits. Moisture content is greatly influenced by variation in the processing of the grains and the method of grinding as well as variation in the climatic conditions and temperature during harvest.

Table 57. Quality characteristics of 35 synthetic-derived, advanced lines (lines with an * are significantly different from mean at CD 0.05).

Genotype	Falling number	Protein (%)	Hardness score	Moisture (%)	1,000-kernel weight (g)	Ash (%)	Wet gluten (%)	Gluten index
DH-1	427.00	14.67*	46.67	9.50	29.80*	1.78	33.62	49.80
DH-2	316.50	13.53*	47.00	9.37*	39.80	1.63	26.74	76.10
DH-3	490.00	12.77	39.00*	9.53	37.60	1.55	29.36	48.50
DH-4	266.67*	12.80	49.00*	9.42*	43.00*	1.72	26.10	61.00
DH-5	393.00	14.73*	45.33	9.41*	38.20*	1.83	25.60	55.00
DH-6	387.00	12.23*	34.00*	9.57	32.00*	2.00*	28.10	36.00
DH-7	467.50	13.40*	41.33*	9.57	36.80	1.74	34.26	64.92
DH-8	528.00	15.40*	51.67*	9.65	35.40*	1.78	27.70	44.50*
DH-9	339.00	12.47*	51.00*	9.22*	42.20*	1.69	27.05	10.89*
DH-10	296.00	11.67*	48.67*	9.78	22.90*	1.90	30.19	37.55
DH-11	343.67	12.00*	42.33*	9.57	44.20	1.70	28.20	13.96*
DH-12	389.50	16.03*	48.00*	9.70	38.60	1.72	37.75*	46.50
DH-13	788.00*	12.70	47.33*	9.40*	33.40	1.65	28.03	40.50
DH-14	375.00	12.37*	42.33*	9.83	27.20*	1.78	26.89	37.00
DH-15	368.50	12.00*	50.33*	10.73	40.80	1.88	25.49	37.00
DH-16	465.00	13.27*	47.33*	10.96	37.80	1.59	25.38	92.50*
DH-17	456.50	12.60	37.00*	10.82	32.40	1.77	17.05*	91.00*
DH-18	526.50	12.67	30.00*	10.53	31.60*	1.60*	31.85	62.00
DH-19	477.00	12.50*	37.33*	10.80	32.20	1.81	28.76	37.35
DH-20	531.00	12.07*	34.33*	10.62	32.60	1.88	28.97	33.2*
DH-21	537.50	12.43*	47.33*	11.33*	28.80*	1.71	24.06	80.50
DH-22	442.50	12.03*	35.33*	10.79	32.80	1.70	22.83	32.00*
DH-23	636.50*	13.33*	48.67*	11.20*	26.40*	1.82	25.68	47.00
DH-24	388.50	14.00*	52.67*	10.62	48.00*	1.59	32.67	46.50
DH-25	241.00*	12.87	53.67*	10.63	40.00	1.70	27.08	29.50*
DH-26	262.00*	14.17*	51.33*	10.62	27.80*	1.90	26.72	66.00
DH-27	478.50	11.70*	46.33	10.74	33.80	1.75	28.45	83.00*
DH-28	495.00	11.17*	47.33*	10.47	38.00	1.70	19.61	96.00*
DH-29	365.00	12.57*	36.00*	10.62	42.80*	1.89	26.93	71.50
DH-30	299.50	10.80*	48.33*	10.92	38.20	1.79	18.94	93.00
DH-31	283.00	11.67*	47.00	10.63	49.00*	1.94	20.38	70.50
DH-32	328.00	12.77	47.00	10.70	34.80	1.70	22.24	90.00*
DH-33	464.50	11.93*	50.67*	10.39	40.00	1.61	22.09	83.00
DH-34	396.00	9.70*	48.33*	11.03	48.40*	1.52*	19.27	52.00
DH-35	345.00	14.47*	48.33*	10.12	34.80	1.66	30.57	90.50
Mean	416.97	12.78	45.10	10.25	36.07	1.75	26.65	56.99
Standard deviation	113.09	1.28	6.09	0.64	6.45	0.11	4.77	24.29
CV(%)	27.12	10.05	13.49	6.20	17.88	6.29	17.90	42.62
Heritability	0.53	0.71	0.62	0.85	0.92	0.65	0.77	0.63

Thousand-kernel weight ranged from 22.90 (DH-10) to 48.40 g (DH-34) with an average of 36.07 g. The co-efficient of variability (17.88%) was sufficient for this trait among genotypes. Sixteen of the advanced lines were significantly different from the mean at $CD_{0.05.}$ Both 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 > 55 g (very large). According to this scale, most of the genotypes are medium grained. Zanetti et al. (2001) reported a 1,000-kernel weight of 42.4–48.7 g in 128 wheat cultivars and Anjum et al. (2002) reported grain weight of 31.43–37.28 g in Pakistani wheat cultivars.

The ash content of these genotypes ranged from 1.52% (DH-34) to 2.0% (DH-6) with an average of 1.75%. All the genotypes were significantly different from the mean at $CD_{0.05}$. Ash content is the inorganic material left after flour is burned, is an important determinant of the extraction rate, and influences flour color and quality. Zahoor (2003) reported the ash contents of 0.30–0.53% in Pakistani wheat cultivars. The higher ash content found in these genotypes indicates the presence of a higher bran proportion than of endosperm flour.

Maximum wet gluten was observed DH-12 (37.75%) and the minimum in DH-17 (17.05%); the average was 26.65%. Both replication and genotype 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 effects dough strength, gas retention and controlled expansion, structural enhancement, water absorption and retention, and natural flavor (Grausgruber et al. 2000).

Previously, these advanced lines were found promising for better yield and drought tolerance in Pakistan (unpublished data). Most of these have good characteristics for useful quality traits and offer variability for these traits. A detailed analysis of advanced lines carrying higher protein content and higher thousand gain weight is required in order to exploit these genotypes further via a a recombination-breeding program.

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Unique high-molecular-weight glutenin subunits in synthetic hexaploids to bridge the gap at the Glu-D1 locus.

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Break-making quality in wheat is largely controlled by high-molecular-weight glutenin subunits (HMW-GS). The D-genome encoded subunits have the most influence but have limited variability. New subunits found in the D-genome synthetic hexaploids can be deployed for break-making quality improvement but require proper identification and characterization for different quality parameters. We identified the HMW-GS in 33 synthetic hexaploids using SDS-PAGE (Tables 58 and 59).

At the *Glu-A1* locus, the x-type subunits 1, 2*, and null, which encode *Glu-A1a, Glu-A1b*, and *Glu-A1c* respectively, were observed. The subunits null and 1 were not very frequent. The null subunit, which does not code for any protein, was the most frequent and was found in 23 (79.31%) accessions. The remaining accessions were found to possess subunit 1, found in four (13.79%), and 2* in two (6.90%) genotypes at the *Glu-A1* locus.

Five different co-dominant alleles are at the *Glu-B1* locus, *Glu-B1b, Glu-B1c, Glu-B1d, Glu-B1f, and Glu-B1i* controlling the subunits 7+8, 7+9, 6+8, 13+16, and 17+18, respectively. In these subunits, four are x- and four are y-type. The *Glu-B1d* allele controlling the subunit 6+8 was less frequent (6.90%) among all the subunits at this locus. and the most frequent was 17+18 at *Glu-B1*, which appeared in 13 (44.83%) accessions. Other subunits were 7+8, present in six (20.69%), 13+16 in five (17.24%), and 7+9 in three (10.34%) genotypes.

Valuable genetic variability was found at the *Glu-D1* locus in these synthetics. The the allelic variation of HMW-GS strongly influence the variability in bread-making quality and the D-genome strongly influences bread-making quality

Table 58. Allelic frequencies of HMW-GS at *Glu-1* loci in 33 synthetic hexaploids accessions.

Locus	Subunit	Allele	Number of accessions	Proportion	Frequency
Glu-A1	1	a	4	0.1379	13.79
	2*	b	2	0.0690	6.90
	null	c	23	0.7931	79.31
Glu-B1	6+8	d	2	0.0690	6.90
	7+8	b	6	0.2069	20.69
	7+9	c	3	0.1034	10.34
	17+18	i	13	0.4483	44.83
	13+16	f	5	0.1724	17.24
Glu-D1	5+10	d	10	0.3103	31.03
	1.5+10	ah	2	0.0690	6.90
	1.5+12	aj	2	0.0690	6.90
	2+12	a	8	0.2759	27.59
	2.1+12	n	3	0.1034	10.34
	1.5+ T2	ag	1	0.0345	3.45
	2+ T2	x	3	0.1050	6.90

Table 59. Allelic composition and frequency in 33 synthetic hexaploid accessions.

Subunit composition	Allelic combination	Number of accessions	Accession
Null, 17+18, 2+T2	c, i, x	1	E-1
Null, 7+8, 2+12	c, b, a	2	E-2, E-24
Null, 7+8, 2.1+12	c, b, n	3	E-3, E-27, E-28
Null, 7+8, 1.5+T2	c, b, ag	1	E-4
Null, 6+8, 1.5+12	c, d, aj	2	E-5, E-9
1, 17+18, 5+10	a, i, d	2	E-7, E-17
Null, 17+18, 1.5+10.5	c, i, ah	2	E-8, E-22
1, 13+16, 5+10	a, f, d	1	E-10
Null, 17+18, 2+12	c, i, a	2	E-11, E-29
Null, 17+18, 5+10	c, i, d	4	E-12, E-14, E-23, E-16, E-30
2*, 17+18, 5+10	b, i, d	1	E-15
1, 17+18, 2+12	a, i, a	1	E-17
2*, 13+16, 5+10	b, f, d	1	E-19
Null, 7+9, 2+12	c, c, a	3	E-20, E-21,E-30
1, 13+16, 2+12	a, f, a	1	E-25
Null, 13+16, 2+T2	c, f, x	1	E-26, E-13

(William et al. 1993; Pfluger et al. 2001). At *Glu-D1,* nine different co-dominant alleles, *Glu-D1a, Glu-D1ah, Glu-D1ag, Glu-D1aj ,Glu-D1d, Glu-D1f*, *Glu-D1i*, *Glu-D1n,* and *Glu-D1x* controlling subunits 5+10, 2+12, 2.1+12, 1.5+10, 1.5+12, 1.5+T2, and 2+T2, were found. The *Glu-D1d* allele controlling subunit 5+10 is the most important and superior bread-making quality subunit and was found most frequently (31.03%). Li et al. (2009) reported the superiority of this allele among all the other alleles at the *Glu-1* locus. Luo et al. (2001) reported the association of subunit 5+10 with sedimentation volume and longer pelshenke time. They also reported that the presence of 5+10 subunit in a genotype results in greater whole-meal flour protein. Payne et al. (1981) established that subunit 5+10 has a superior quality effect over 2+12 and all other alleles at *Glu-D1*. Eight genotypes (27.59%) had 2+12, encoded by *Glu-D1a*. The unique subunits 1.5+T2 and 2+ T2 also were observed at this locus. Other important subunits at *Glu-D1* were 1.5+12, found in two (6.90%), and 2.1+12 in three (10.34%) accessions. Subunit 1.5+10 was present in two of the 33 synthetics, and this subunit has better overall quality characteristics than genotypes having other subunits (Pena et al. 1995).

Eighteen different HMW-GS compositions were observed in the synthetic hexaploid wheats (Table 59, p. 191). Four (13.79%) genotypes had the combination null, 17+18, 5+10. Other frequent subunit compositions were null, 7+9, 2+12 and null, 7+8, 2.1+12 recorded in 3 (10.34%) accessions. Several other sub-units with different unique combinations like T2 were also found in this group of accessions. Four synthetics showed the presence of rare allele T2 at *Glu-D1* locus with either of subunit 1.5 or 2. The quality effects of genotypes with T2 subunit were not determined because these are rare subunits and their quality effects are yet to be determined. Superior bread making quality characteristics in a genotypes are accredited by presence of either of subunit 1 or 2* at *Glu-A*1 along with subunits 7+8, 17+18 or 13+16 at *Glu-B1*. Seven of these synthetics had one of these combinations. From these results, it is concluded that synthetics have good potential towards the bread making quality and their utilization in breeding programmes can become a first choice to the breeder emphasizing on wheat breeding for high grain quality.

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QTL-based phenotyping of a molecular mapping population for salinity tolerance in wheat.

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In developed countries, around 90% of the wheat area is rain-fed. In developing countries, wheat is mainly cultivated in irrigated areas, especially by large producers such as India and China. Irrigated land is believed to be most productive but, unfortunately, it faces a great production constraint in salinity. Currently, about 20% of the 275 x 10^6 ha of irrigated land globally is salt affected. The most important and the productive part of Pakistan, the Indus Plain, is effected by salinity of varying degrees. In Pakistan, about 5.30 x 10^6 ha of land are salt-affected. Therefore, nationally and internationally, salinity represents a great threat to our food production and limits it in many farming systems including the arid and semi-arid regions.

To be able to effectively utilize saline land and water resources, we can either improve the soil, breed for salt tolerance to enable them to grow under harsher conditions, or develop new crops that have high productivity in a highly

saline environment. Knowing the complex physiology and genetics of salt tolerance for crop development poses a difficult task for plant breeders. So far, little progress has been made. This slow progress can be accredited to different factors, such as the genetic, physiological, and biochemical basis for salt tolerance, which are not well understood due to polygenic inheritance in plants such as wheat; an incomplete knowledge of the effects of salinity on plants; and unproductive selection methods. The success of a breeding program can be enhanced by knowledge of these areas. Some basics for improving salt tolerance can be the availability of suitable genetic variability in the cultivated species or their wild relatives, methods of selection and screening large numbers of genotypes for salt tolerance, and a suitable breeding methodology.

Salinity affects the most important and productive land of Pakistan as well as of the world. Salt-tolerant cultivars will play a great role in achieving future production goals. To develop salt tolerance in existing or new crops, the diversity in germ plasm becomes important. Genetic variation for salt tolerance has been reported in many crop species, including wheat, and the potential exists for improvements using conventional and novel breeding techniques. Our objectives were to exploit genetic diversity for salt tolerance in 100 F_1-based, doubled haploids developed from the parental lines Prinia and 2407, develop plant population in hydroponic culture at 75 mMol NaCl and perform phenotyping for salinity tolerance using different physiological tests and assessing agronomical data, and identify good, agronomic, salt-tolerant lines on the basis of this phenotyping that could be used in wheat breeding and improvement programs.

The germ plasm consisted of 75 doubled-haploid (DH) lines and three check cultivars Kharchia and Shorawaki (salt tolerant), and PBW-343 (sensitive). This plant material was screened for salt tolerance under hydroponic conditions using K^+:Na^+ analytical parameters complimented by other test indicators such as relative growth rate, chlorophyll content, K^+/Na^+ ratio, and ion flux (Table 60, pp. 193-195; Table 61, p. 195-196, and Table 62, p. 197-198).

Table 60. Mean values of shoot/root length,and relative growth rates of 93 doubled haploid lines and check cultivars Kharchia, Shorawaki, and PBW-343 (The relative growth rate is gram per gram of dry weight of the sample per day).

Line	Shoot length (cm)	Root length (cm)	Shoot dry weight		Natural log shoot dry weight 1st and 2nd harvests	Relative growth rate (g/g/d)
			1st harvest (g)	2nd harvest (g)		
DH1	21.3	3.0	0.0240	0.249	0.223	0.022
DH4	25.4	4.9	0.0290	0.283	0.216	0.022
DH6	16.9	4.8	0.0117	0.108	0.431	0.043
DH7	18.3	4.2	0.0155	0.170	0.056	0.006
DH9	26.2	8.0	0.0153	0.207	0.217	0.022
DH10	24.4	8.0	0.0133	0.146	0.127	0.013
DH11	16.0	2.9	0.0160	0.189	0.172	0.017
DH12	21.9	3.3	0.0289	0.167	0.190	0.019
DH13	22.6	5.8	0.0250	0.175	0.005	0.001
DH14	13.5	4.8	0.0160	0.229	0.177	0.018
DH15	18.7	5.0	0.0170	0.185	0.378	0.038
DH16	17.7	3.3	0.0160	0.159	0.118	0.012
DH17	17.6	3.6	0.0204	0.296	0.163	0.016
DH18	27.6	3.6	0.0262	0.347	0.168	0.017
DH19	31.6	9.0	0.0246	0.301	0.165	0.016
DH20	32.0	6.4	0.0267	0.285	0.079	0.008
DH21	36.7	12.2	0.0250	0.378	0.247	0.025
DH22	21.3	4.5	0.0301	0.385	0.159	0.016
DH23	25.4	3.7	0.0208	0.212	0.360	0.036
DH24	24.5	4.5	0.0312	0.216	0.114	0.011
DH25	22.3	6.9	0.0190	0.253	0.010	0.001
DH26	27.3	4.9	0.0206	0.246	0.075	0.007
DH27	25.7	5.3	0.0221	0.291	0.302	0.030

Table 60. Mean values of shoot/root length,and relative growth rates of 93 doubled haploid lines and check cultivars Kharchia, Shorawaki, and PBW-343 (The relative growth rate is gram per gram of dry weight of the sample per day).

Line	Shoot length (cm)	Root length (cm)	Shoot dry weight		Natural log shoot dry weight 1st and 2nd harvests	Relative growth rate (g/g/d)
			1st harvest (g)	2nd harvest (g)		
DH28	27.8	6.6	0.0202	0.202	0.359	0.036
DH29	34.2	4.6	0.0340	0.317	0.137	0.014
DH30	27.0	5.5	0.0385	0.318	0.172	0.017
DH32	27.5	3.8	0.0317	0.249	0.119	0.012
DH33	20.1	4.9	0.0180	0.164	0.269	0.027
DH34	20.9	4.5	0.0324	0.277	0.133	0.013
DH35	30.3	6.9	0.0360	0.200	0.079	0.008
DH36	24.0	4.8	0.0200	0.394	0.668	0.067
DH39	24.3	3.4	0.019	0.030	0.470	0.047
DH40	22.9	4.3	0.018	0.028	0.442	0.044
DH41	22.7	6.8	0.028	0.036	0.243	0.024
DH42	30.5	4.8	0.024	0.034	0.357	0.036
DH43	25.2	4.9	0.016	0.032	0.681	0.068
DH44	20.9	4.5	0.019	0.022	0.126	0.013
DH45	26.0	7.8	0.018	0.028	0.474	0.047
DH46	25.1	4.4	0.020	0.024	0.167	0.017
DH47	28.0	4.7	0.016	0.023	0.386	0.039
DH48	24.4	3.8	0.020	0.032	0.470	0.047
DH49	16.8	3.5	0.014	0.021	0.442	0.044
DH51	26.2	4.6	0.058	0.074	0.250	0.025
DH52	17.8	5.3	0.027	0.042	0.420	0.042
DH53	21.5	5.6	0.045	0.060	0.288	0.029
DH57	16.4	5.7	0.016	0.017	0.036	0.004
DH59	17.2	5.7	0.013	0.019	0.411	0.041
DH60	25.4	3.4	0.018	0.029	0.471	0.047
DH61	29.8	5.8	0.015	0.024	0.470	0.047
DH62	27.5	4.8	0.013	0.018	0.349	0.035
DH63	23.4	6.3	0.018	0.020	0.110	0.011
DH64	11.7	3.5	0.016	0.030	0.624	0.062
DH65	24.3	4.7	0.015	0.028	0.619	0.062
DH66	23.4	4.1	0.019	0.027	0.362	0.036
DH67	23.3	6.3	0.013	0.026	0.660	0.066
DH68	17.0	5.8	0.011	0.020	0.625	0.062
DH69	16.9	7.2	0.018	0.024	0.278	0.028
DH70	31.1	4.8	0.024	0.037	0.423	0.042
DH72	27.3	5.6	0.022	0.040	0.590	0.059
DH73	26.6	7.3	0.013	0.024	0.653	0.065
DH76	20.1	5.4	0.008	0.010	0.281	0.028
DH77	25.8	10.3	0.022	0.040	0.593	0.059
DH78	22.9	4.0	0.030	0.043	0.334	0.033
DH79	24.7	5.7	0.030	0.038	0.102	0.010
DH81	22.3	5.2	0.030	0.032	0.028	0.003
DH82	26.7	7.5	0.040	0.052	0.215	0.021
DH83	19.9	4.9	0.020	0.026	0.198	0.020
DH84	25.0	4.4	0.020	0.027	0.271	0.027
DH85	22.6	4.7	0.030	0.041	0.381	0.038

Table 60. Mean values of shoot/root length,and relative growth rates of 93 doubled haploid lines and check cultivars Kharchia, Shorawaki, and PBW-343 (The relative growth rate is gram per gram of dry weight of the sample per day).

Line	Shoot length (cm)	Root length (cm)	Shoot dry weight		Natural log shoot dry weight 1st and 2nd harvests	Relative growth rate (g/g/d)
			1st harvest (g)	2nd harvest (g)		
DH87	22.90	3.20	0.030	0.040	0.165	0.016
DH89	21.10	3.80	0.030	0.039	0.253	0.025
DH90	14.50	4.90	0.010	0.022	0.394	0.039
DH91	21.60	6.20	0.030	0.033	0.121	0.012
DH92	16.80	7.30	0.020	0.024	0.121	0.012
DH93	23.50	5.10	0.030	0.034	0.148	0.015
Kharchia	21.00	3.00	0.030	0.035	0.230	0.023
PBW-343	14.90	5.20	0.010	0.016	0.099	0.010
Shorawaki	18.50	5.60	0.020	0.034	0.506	0.051

Table 61. Mean values of leaf fresh/dry weight and chlorophyll content of 93 doubled haploid lines and the check cultivars Kharchia, Shorawaki and PBW-343.

Line	Leaf fresh weight (g)	Leaf dry weight		Chlorophyll absorbance (at 666 nm)	Chlorophyll content (mg/mg)
		g	mg		
DH1	0.0750	0.0054	5.4000	1.1940	0.0118
DH4	0.0633	0.0051	5.1000	1.0180	0.0107
DH6	0.0640	0.0056	5.6000	1.0160	0.0097
DH7	0.0410	0.0041	4.1000	0.4747	0.0061
DH9	0.0793	0.0005	0.5100	0.9300	0.0974
DH10	0.0118	0.0020	2.0000	0.5040	0.0133
DH11	0.0357	0.0028	2.8000	0.3623	0.0068
DH12	0.0911	0.0052	5.1583	0.0500	0.0004
DH13	0.3332	0.0189	18.8588	0.5220	0.0015
DH14	0.0773	0.0054	5.4000	1.1370	0.0113
DH15	0.0540	0.0043	4.3000	0.6680	0.0083
DH16	0.0410	0.0035	3.5000	0.6820	0.0104
DH17	0.1010	0.0082	8.2000	1.8760	0.0123
DH18	0.0443	0.0023	2.3000	0.5280	0.0122
DH19	0.0363	0.0016	1.6000	0.3610	0.0118
DH20	0.0490	0.0028	2.8000	0.5200	0.0098
DH21	0.0413	0.0052	5.2000	0.5240	0.0053
DH22	0.1127	0.0064	6.3775	1,4298	0.0120
DH23	0.1126	0.0044	4.4000	1.0650	0.0129
DH24	0.0879	0.0050	4.9764	0.4870	0.0052
DH25	0.0763	0.0062	6.2000	0.5440	0.0046
DH26	0.0840	0.0045	4.5000	1.0860	0.0129
DH27	0.0890	0.0050	5.0378	1.1295	0.0120
DH28	0.0091	0.0005	0.5151	0.8500	0.0880
DH29	0.1196	0.0068	6.7722	1.5183	0.0120
DH30	0.1154	0.0065	6.5311	1.4643	0.0120
DH32	0.1258	0.0071	7.1214	1.5966	0.0120
DH33	0.1630	0.0092	9.2237	2.0680	0.0120
DH34	0.0790	0.0045	4.4719	1.0026	0.0120
DH35	0.2089	0.0118	11.8248	2.6511	0.0121
DH36	0.0444	0.0049	4.9000	0.6020	0.0065
DH39	0.0926	0.0041	4.1000	0.9370	0.0122

Table 61. Mean values of leaf fresh/dry weight and chlorophyll content of 93 doubled haploid lines and the check cultivars Kharchia, Shorawaki and PBW-343.

Line	Leaf fresh weight (g)	Leaf dry weight		Chlorophyll absorbance (at 666 nm)	Chlorophyll content (mg/mg)
		g	mg		
DH40	0.0690	0.0033	3.3000	0.6970	0.0112
DH41	0.0540	0.0031	3.1000	0.7920	0.0136
DH42	0.0483	0.0050	5.000	0.5070	0.0054
DH43	0.0636	0.0038	3.8000	0.7780	0.0109
DH44	0.1093	0.0040	4.0000	0.9030	0.0120
DH45	0.0166	0.0021	2.1000	0.6420	0.0162
DH46	0.0383	0.0023	2.3000	0.3760	0.0086
DH47	0.1140	0.0062	6.2000	1.0350	0.0089
DH48	0.0396	0.0012	1.2000	0.5030	0.0222
DH49	0.0061	0.0003	0.3453	0.0774	0.0105
DH51	0.1941	0.0110	10.9852	2.4629	0.0121
DH52	0.0076	0.0004	0.4302	0.0964	0.0108
DH53	0.0090	0.0005	0.5094	0.1142	0.0110
DH57	0.0041	0.0002	0.2321	0.0520	0.0098
DH59	0.0075	0.0004	0.4245	0.0952	0.0108
DH60	0.0055	0.0003	0.3113	0.0698	0.0104
DH61	0.0048	0.0003	0.2717	0.6000	0.1172
DH62	0.0093	0.0005	0.5264	0.8201	0.0831
DH63	0.0065	0.0004	0.3679	0.1450	0.0198
DH64	0.0713	0.0040	4.0368	0.0850	0.0010
DH65	0.0068	0.0004	0.3849	0.8300	0.1150
DH66	0.0041	0.0002	0.2321	0.2110	0.0467
DH67	0.0065	0.0004	0.3679	0.0730	0.0092
DH68	0.0052	0.0003	0.2943	0.0810	0.0130
DH69	0.0040	0.0002	0.2264	0.1270	0.0279
DH70	0.0109	0.0006	0.6169	0.6870	0.0592
DH72	0.0098	0.0006	0.5547	0.6030	0.0577
DH73	0.0081	0.0005	0.4585	0.5330	0.0616
DH76	0.3091	0.0175	17.4974	3.9229	0.0121
DH77	0.0221	0.0013	1.2535	0.9110	0.0388
DH78	0.0875	0.0050	4.9543	1.1108	0.0120
DH79	0.0763	0.0043	4.3158	0.9676	0.0120
DH81	0.1870	0.0106	10.5825	0.7000	0.0035
DH82	0.0175	0.0010	0.9890	0.2217	0.0116
DH83	0.1996	0.0113	11.2949	2.5323	0.0121
DH84	0.1924	0.0109	10.8878	0.5001	0.0024
DH85	0.0329	0.0019	1.8614	0.4173	0.0118
DH87	0.0340	0.0019	1.9238	0.4313	0.0118
DH89	0.0891	0.0050	5.0456	1.1312	0.0120
DH90	0.2130	0.0121	12.0543	2.7026	0.0121
DH91	0.0566	0.0032	3.2036	0.7183	0.0119
DH92	0.1034	0.0059	5.8551	1.3127	0.0120
DH93	0.2430	0.0138	13.7513	0.7390	0.0029
Kharchia	0.0577	0.0027	2.7000	3.0900	0.0616
PBW-343	0.0321	0.0028	2.8000	0.8910	0.0170
Shorawaki	0.0532	0.0026	2.6000	3.0100	0.0623

Table 62. Mean values for sodium ion flux, potassium ion flux, and potassium:sodium ratios after 5 and 15 days of salinization in doubled haploid lines and the check cultivars Kharchia and Shorawaki. (J_s = net ion transport rate from roots to shoots in moles/gram/day).

Line	K^+:Na^+		Sodium (J_s) (mol/g/d)	Potassium (J_s) (mol/g/d)
	5 days	15 days		
DH1	1.35	1.70	18.57	63.64
DH4	0.97	0.89	39.35	28.57
DH6	2.77	2.75	9.31	24.51
DH7	0.73	0.69	9.58	0.53
DH9	1.69	1.84	1.36	25.50
DH10	1.87	1.87	1.23	4.53
DH11	1.20	1.22	29.26	36.49
DH12	1.44	1.46	16.28	27.18
DH13	1.76	1.81	53.55	104.54
DH14	1.43	1.55	8.69	31.21
DH15	1.55	1.63	3.31	13.33
DH16	1.72	1.72	9.03	15.98
DH17	0.65	0.50	19.47	0.91
DH18	1.44	1.33	73.22	81.92
DH19	0.94	0.92	29.21	22.45
DH20	0.78	0.72	50.28	9.94
DH21	0.96	0.86	68.18	31.05
DH22	1.66	1.67	37.79	69.42
DH23	0.59	0.60	76.95	47.03
DH24	0.51	0.56	25.51	21.14
DH25	0.99	0.59	274.32	40.46
DH26	0.92	0.84	120.70	51.95
DH27	1.67	1.69	10.13	21.46
DH28	0.89	0.87	38.20	29.09
DH29	0.82	0.83	23.97	21.08
DH30	1.45	1.58	3.29	15.76
DH32	1.81	1.72	9.45	11.88
DH33	0.79	0.64	140.41	42.78
DH34	0.51	0.55	19.73	16.13
DH35	0.85	0.83	43.70	35.04
DH36	2.66	2.77	1.48	10.25
DH39	0.91	0.91	44.08	40.49
DH40	1.00	0.87	71.10	16.02
DH41	1.67	1.75	6.64	15.62
DH42	1.64	1.80	5.98	36.23
DH43	0.97	0.91	86.13	59.58
DH44	1.39	1.40	74.09	107.09
DH45	0.88	0.85	114.05	77.54
DH46	0.67	0.61	47.71	7.00
DH47	0.72	0.69	137.85	85.14
DH48	1.79	1.80	4.13	11.56
DH49	0.86	0.79	19.02	2.70
DH51	1.65	1.70	9.46	17.56
DH52	1.84	2.02	4.43	13.77
DH53	1.71	1.88	4.70	15.79

The relative growth rate (gram per gram of dry weight of the sample per day = g/g/d) of the shoots were calculated according to the following formula where WS_1 and WS_2 are shoot weights at harvest time t_1 and t_2 and In is the natural log:

RGR (g/g/d) = In WS_2 – $InWS_1$ / $t_2 – t_1$

Chlorophyll content was calculated according to the following formula:
Chlorophyll (mg/mg dry weight of sample) = (chlorophyll absorbance – 0.01) x 5 / 92.6474 x dry weight of sample

The ion fluxes for sodium and potassium were calculated according to the following formula:

$J_s = OS_2–OS_1/t_2-t_1 x InWR_2–InWR_1/WR_2–WR_1$

Relative growth rate (g/g/d). The relative growth rate (RGR) reflects the growth potential under the stress conditions imposed over plants and the overall growth and development and total biomass production by the plants during the period of stress (Table 60, pp. 194-196). The tolerant check lines showed RGR values of 0.0230 g/g/d (Kharchia) and 0.0506 g/g/d (Shorawaki). The sensitive line PBW-343 had an RGR value of 0.00099 g/g/d. The RGR values for the DHs ranged from minimum of 0.0005 g/g/d (DH-13) to the maximum of 0.0681 g/g/d (DH-43). A few lines showed RGR values more than the tolerant checks, DH-72 (0.0590 g/g/d), DH-77 (0.0593 g/g/d), DH-65 (0.0619 g/g/d), DH-64 (0.0624 g/g/d), DH-68 (0.0625 g/g/d), DH-73 (0.0653 g/g/d), DH-67 (0.0660 g/g/d), and DH-36 (0.0668 g/g/d). These lines possess good potential to grow and develop under salt stress.

The mean data for shoot length also shows a good positive correlation for the relative growth rates of plants. Thirty-ong DH lines had an RGR value between those of both tolerant check cultivars ranging from 0.0470 g/g/d (DH-45) to 0.0243 g/g/d (DH-41), which is promising growth potential under stress. The remaining 35 DH lines had RGR values less than both the tolerant checks, from 0.0223 g/g/d (DH-1) to 0.0005 g/g/d (DH-13), indicating poor growth under salt stress.

Relative growth rates were calculated on the shoot dry weight basis, because it also effects the ion accumulation in the shoot and, hence, the K^+:Na^+ ratio. We observed

Table 62. Mean values for sodium ion flux, potassium ion flux, and potassium:sodium ratios after 5 and 15 days of salinization in doubled haploid lines and the check cultivars Kharchia and Shorawaki. (J_s = net ion transport rate from roots to shoots in moles/gram/day).

Line	K^+:Na^+		Sodium (J_s) (mol/g/d)	Potassium (J_s) (mol/g/d)
	5 days	15 days		
DH57	0.66	0.52	102.89	28.71
DH59	0.89	0.66	100.91	36.50
DH60	1.90	2.06	11.46	50.30
DH61	0.73	0.71	21.46	9.13
DH62	0.72	0.70	12.72	0.94
DH63	0.35	0.38	70.54	30.22
DH64	1.27	1.33	4.28	11.65
DH65	2.52	2.77	1.76	21.11
DH66	0.78	0.78	47.92	35.80
DH67	1.57	1.54	47.01	68.87
DH68	0.81	0.81	66.41	54.00
DH69	0.89	0.81	18.80	6.59
DH70	2.26	2.54	7.11	31.84
DH72	2.63	2.56	14.91	34.02
DH73	3.32	3.47	5.21	28.26
DH76	1.97	1.99	39.60	84.08
DH77	0.77	0.70	14.79	3.04
DH78	1.74	1.65	15.008	16.22
DH79	0.77	0.70	21.236	10.75
DH81	0.92	0.83	32.759	21.43
DH82	0.55	0.54	8.221	2.90
DH83	1.50	1.56	16.945	30.40
DH84	1.74	1.86	14.504	39.31
DH85	1.53	1.58	0.902	5.68
DH87	0.82	0.71	24.920	6.05
DH89	0.97	0.76	49.326	15.86
DH90	1.97	2.12	15.849	57.62
DH91	0.50	0.51	13.522	7.82
DH92	1.65	1.68	10.586	20.15
DH93	1.80	1.89	16.269	38.24
Kharchia	3.29	3.82	1.757	17.65
PBW-343	0.67	0.60	156.593	73.74

that the relative growth rate showed a positive correlation (Pearson's correlation coefficient, r = 0.3588) with the K^+:Na^+ ratio taken 15 days after salinization. The DH lines having high RGR values also showed higher K^+:Na^+ ratio and vice versa, for instance, DH-36, with a higher RGR value of 0.0668 g/g/d, also had a higher K^+:Na^+ ratio (2.768) and DH-25, with a lower RGR value of 0.0010 g/g/d, also showed a lower K^+:Na^+ ratio (0.587).

Chlorophyll content (mg/mg). Chlorophyll carries out photosynthesis in plants and so directly effects growth and development. A decrease in chlorophyll content results in a reduction of photosynthesis under salt stress. We observed that lines that could not survive under salt stress also had a lower chlorophyll content, in contrast with salt-tolerant lines, which showed a greater chlorophyll content in their leaves. A decrease in chlorophyll content means a decrease in photosynthesis, which will affect the overall growth and survival of plants. Therefore, chlorophyll content can be used as an indicator of salt tolerance. The check cultivars, Kharchia, Shorawaki, and PBW-343, had a chlorophyll content of 0.0616 (mg/mg), 0.0623 (mg/mg), and 0.0170 (mg/mg), respectively. Five lines had a chlorophyll content greater than the tolerant checks. The maximum chlorophyll content was in DH-61, 0.1172 mg/mg. The chlorophyll content showed a positive correlation (r = 0.3409) with the relative growth rate, which suggests that a greater chlorophyll content contributes to more biomass production and the overall growth and development of the plant makes them tolerant to salt stress. However, the correlation between the chlorophyll content and Na^+ flux was negative (r = –0.189); favoring the fact that more uptake and accumulation of Na^+ in the shoot results in more chlorophyll damage and reduction.

K^+:Na^+ descrimination. Sodium ion exclusion and a greater accumulation of K^+ is one of the adaptations to salt stress that results in enhanced performance under stress conditions. Increased Na^+ exclusion improves salt tolerance and grain yield in wheat. Several sources of enhanced Na^+ exclusion and higher salt tolerance have been identified within the Triticeae. Bread wheat is, in general, a better Na^+ excluder and shows higher K^+:Na^+ ratio. The K^+:Na^+ ratios for all DH lines and checks were calculated after 5 and 15 days of salinization (Table 62, pp. 197-198). The maximum K^+:Na^+ ratio after 15 days of salinization was in Kharchia (3.823), followed by Shorawaki (3.560). The K^+:Na^+ ratio for PBW-343 (sensitive check) was 0.600. The K^+/Na^+ ratios for the DH lines ranged from 3.469 to 0.380. The maximum K^+:Na^+ ratio was in DH-73 (3.469).

The correlation between relative growth rate and K^+:Na^+ ratio also was positive. A positive correlation means that plants having good potential to grow under salt stress have better ability to exclude Na^+, which ultimately results in development of tolerance against salt stress. The D genome contributes to lower rates of Na^+ accumulation and a higher

K^+:Na^+ ratio in the leaves, in both diploid *Ae. tauschii* (DD) and hexaploid (AABBDD) wheat. This trait of high K^+:Na^+ ratio could be used to increase salt tolerance of current wheat culitvars, which is supported by this study.

Ion fluxes (J_s, Mol/g/d). The ability of plants to uptake sodium and potassium ion also affects their tolerance to salt. The net ion uptake (ion flux) for potassium is higher in salt-tolerant cultivars, whereas ion flux for sodium is higher in salt-sensitive cultivars. Th e ion flux for potassium in the DH lines ranged from a high of 107.091 mol/g/d in DH-44, which was higher than the tolerant check Shorawaki (95.872 mol/g/d), to a low of 0.532 mol/g/d in DH-7.

The correlation between K^+ flux and K^+:Na^+ ratio also was found to be positive (r = 0.147), suggesting that the lines having a greater ability to discriminate potassium and sodium ions also have a greater ability to uptake and accumulate potassium and exclude sodium ions. This potassium/sodium ion discrimination is the most important character used to determine the salt tolerance of genotypes.

Generally, the tolerant genotypes showed fluxes of K^+ and less of Na^+. Variation in the uptake of K^+ and Na^+ has already been reported in wheat genotypes. Enhanced leaf K^+:Na^+ ratios and a greater flux of K^+ and less of Na^+ can be used to develop an effective screening procedure that can be more fruitful while breeding for salt tolerance.

Conclusion. The relative growth rate values for DN lines ranged from 0.0005 g/g/d to 0.0681 g/g/d/. Chlorophyll content was between 0.0004 mg/mg and 0.1172 mg/mg, whereas the K^+:Na^+ ratios observed were from a minimum of 0.380 to a maximum of 3.469. Ion flux for K^+ in the DH lines ranged from a high of 107.091 mol/g/d to a low of 0.532 mol/g/d. The DH lines that showed good performance for both agronomic and physiological parameters were DH-36, DH-65, DH-72, DH-73, and DH-76.

These results will become more authenticated after genotyping this germ plasm. Furthermore, the entries of agronomic promise serve as a useful source of salinity tolerance for different wheat breeding programs aimed at developing salt-tolerant wheat cultivars.

Cytological and molecular characterization of elite Pakistani wheat germ plasm for the T1BL·1RS chromosome translocation.

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Food security is an important issue prevailing throughout the world; which is lacking in one-third of the population. According to production estimates for the year 2025, a world population increase to 8.5 x 10^9 will require a doubling in food supplies. Food security could be achieved by developing and applying improved agricultural technologies. Our objectives were to cytologically characterize the elite Pakistani wheat cultivars and germ plasm of the National Uniform Wheat Yield Trials for the T1BL·1RS translocation using conventional somatic cytology and C-banding technique, screen the germ plasm for T1BL·1RS through molecular diagnostics using SSR markers, and validate the characterized germ plasm through biochemical analysis using glucose phosphate isomerase (GPI) and low-molecular-weight glutenin subunits (LMW-GS).

Germ plasm. The experimental material comprised of two sets of germ plasm. The first group comprised of the 40 elite Pakistani wheat cultivars (Table 63, pp. 200-201). The second group comprised of all entries of the National Uniform Wheat Yield Trials (NUWYT) of two crop cycles, 2008–09 and 2009–10, for both rain-fed and irrigated categories. The total number of entries of NUWYT (rainfed and irrigated) was 62 as first crop cycle comprised of 30 entries (Table 64, pp. 201-203) and second crop cycle comprised of 32 entries (Table 65, pp. 204). These wheat lines/entries were analyzed for the presence of the T1BL·1RS translocation. This study was conducted in the research laboratory of the Wheat Wide Crosses and Cytogenetics Programme in National Agriculture Research Centre, Islamabad, during 2009–10.

Results and discussion. Elite Pakistani wheat germ plasm was characterized for the T1BL·1RS translocation using the cytological, molecular, and biochemical techniques. The 40 selected and approved wheat cultivars and the National Uniform Wheat Yield Trial entries for 2008–09 and 2009–10 crop cycles, i.e., were analyzed using a conventional somatic cytological technique, C-banding, and SSR markers, and then validated by using a biochemical marker, GPI.

Table 63. A list of Pakistani wheat cultivars characterized for the T1BL·1RS translocation by cytology (the presence of two satellites (6B,6B) shows the presence of translocation and four satelllites (6B,6B,1B,1B) shows its absence), C–banding, SSR marker, and glucose phosphate isomerase (GPI) analyses (1RS +ve indicates presence of heterochromatin bands of rye, 1RS –ve shows absence).

Cultivar	Parentage	Cytological validation (number of satellites)	C–banding diagonistics	Molecular diagonistics	GPI validation
Lasani-2008	LUAN/Kohistan-97	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Punjnad-88	K4500.2/BJY	6B,6B	1RS +ve	1RS +ve	1RS +ve
Miraj-2008	Sparrow/Inia//V.7394/ WL711/13/BAU'S'	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Kiran-95		6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Farid-2006	PT'S'/3/TOB/LFN/BB/4/ BB/HD-832-5//ON/5/G-V/ ALD'S'//HPO	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Bhittai	VEE/TRAP//Soghat-90	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
AS-2002	KHP/D31708// CM74A370/3/Ciano79/4/ RL6043/*4NAC	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Faislabad-2008	PBW65/2*Pastor	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Khirman		6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Pirsabak-05	Munia/CHTO//Amsel	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Momal-2002	BUC'S'/4/TZPP/IRN46	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Zarghoon-79	CC/Inia/3/TOB/CTFN// BB/4/7C	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Saleem-2000	Cham-6//Kite/PGO	6B,6B	1RS +ve	1RS +ve	1RS +ve
Marvi-2000	CMH-77A917/PKV 1600//RL6010/6*SKA	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Shafaq-2006	V 81094(LU 26/HD 21790/ 2* Inqalab 91)	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Chakwal-50	Attila/3/HUI/CARC// CHEN/CHTO/4/Attila	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Sehar-2006	CHILL/2* Star/4/BOW// BUC/PVN/3/2*VEE#10	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
TD-1	MAI'S' X NORTENO65 X H68	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Imdad-2005		6B,6B	1RS +ve	1RS +ve	1RS +ve
Pak-81	KVZ//BUHO//KAL/BB	6B,6B	1RS +ve	1RS +ve	1RS +ve
Shalimar-88	PB81/HD2182/PB81	6B,6B	1RS +ve	1RS +ve	1RS +ve
Pavon	VCM//CNO/7C/3/KAL/ BB	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Auqab-2000	Crow's'/NAC//BOW'S'	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Tatara	JUP/ALD'S'//KLT'S	6B,6B	1RS +ve	1RS +ve	1RS +ve
Sarsabz	M20/79	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Chakwal-97	BUC'S'/FCT'S'	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Margalla-99	Opata/BOW'S'	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Inqalab-91	WL 711/Crow's'	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Wafaq-2001	Opata/Rayon//Kauz	6B,6B	1RS +ve	1RS +ve	1RS +ve
Blue Silver	1154-388/AN/3/YT54/ N10B//LR64	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Tandojam-83	TZPP/PL/7C	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
GA-2002	DWL 5023/S N B// SNB	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Pirsabak-85	KVZ/BUHO/KAL/BB	6B,6B	1RS +ve	1RS +ve	1RS +ve

Table 63. A list of Pakistani wheat cultivars characterized for the T1BL·1RS translocation by cytology (the presence of two satellites (6B,6B) shows the presence of translocation and four satelllites (6B,6B,1B,1B) shows its absence), C–banding, SSR marker, and glucose phosphate isomerase (GPI) analyses (1RS +ve indicates presence of heterochromatin bands of rye, 1RS –ve shows absence).

Cultivar	Parentage	Cytological validation (number of satellites)	C–banding diagonistics	Molecular diagonistics	GPI validation
Chakwal-86	Fln/ACS/ANA	6B,6B	1RS +ve	1RS +ve	1RS +ve
LU-26	Blue Silver/Khushal	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Pasban-90	Inia F 66/A.Distchum// Inia66/3/GEN	6B,6B	1RS +ve	1RS +ve	1RS +ve
Bhakkar-2002	P20102/PIMA/ SKA/3/TTR'S'/ BOW'S',Pb.23826-D-1a-1a-1t-1t-0t	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
Rohtas-90	Inia F 66/A.Distchum// Inia66/3/GEN	6B,6B	1RS +ve	1RS +ve	1RS +ve
Fakhr-e-Sarhad	PFAU'S'/Seri//BOW'S'	6B,6B	1RS +ve	1RS +ve	1RS +ve
Zardana	CNO S/8156 TOB 66 CNO6-PVN	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve

Table 64. Wheat genotypes in National Uniform Wheat Yield Trails (Rainfed, 1-11, and Irrigated, 12-30), 2008–09, characterized for the T1BL·1RS translocation by cytology (the presence of two satellites (6B,6B) shows the presence of translocation and four satelllites (6B,6B,1B,1B) shows its absence), C–banding, SSR marker, and glucose phosphate isomerase (GPI) analyses (1RS +ve indicates presence of heterochromatin bands of rye, 1RS –ve shows absence).

Cultivar	Parentage	Cytological validation (number of satellites)	C–banding diagonistics	Molecular diagonistics	GPI validation
NR-358	PFAU/Weaver*2//Kiritati CGSS01B00076T-099Y-099M-099B-75Y-0B-01D	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
PR-98	CMH84.3379/ CMH78.578//Milan CMSS93Y006285-7Y-010Y-010M-010Y-10M-0Y-3KBY-0KBY	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NR-360	PFAU/Seri.1B// AMAD/3/Waxwing CGSS02Y00153S-099M-099Y-099M-46Y-0B-01D	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
SN-151	Kambara-1 CGSS9500016F-099Y-099B-099Y-099B-15Y-0B-0SY	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
04FJS35	PASTOR// HXL7573/2*BAU CMSS97M00306S-0P-95Y-90M-010Y	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
AZRC-2008-1	Tracha's//CMH76-252/ Pvn's ICW93-0065-6AP-0L-3AP-0L-1AP-0AP	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve

Table 64. Wheat genotypes in National Uniform Wheat Yield Trails (Rainfed, 1-11, and Irrigated, 12-30), 2008–09, characterized for the T1BL·1RS translocation by cytology (the presence of two satellites (6B,6B) shows the presence of translocation and four satelllites (6B,6B,1B,1B) shows its absence), C–banding, SSR marker, and glucose phosphate isomerase (GPI) analyses (1RS +ve indicates presence of heterochromatin bands of rye, 1RS –ve shows absence).

Cultivar	Parentage	Cytological validation (number of satellites)	C–banding diagonistics	Molecular diagonistics	GPI validation
PR-99	Hamam-4/Star"S"/Liz 0F-0K-2F-0K	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
KT-4	Altar84/ *Ae.squarrosa*219//SER CMBW91Y00892S-8Y-11KBY-2KBY-010M-9Y-3M-0Y-0SY	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
V-05003	Karvan2/4/Burgus/ Sort12-13//Kal/BB/3/ Pak81	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NRL-0320	FRET 2 CGSS96Y00146T-099B-099Y-099B-16Y-0B-0SY	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
5C011	Skauz/BAV92 CMSS96M03611S-1M-010SY-010M-010SY-8M-0Y	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
DN-62	SW89.5181/Kauz CMSS93B00824S-24Y-010M-010Y-010M-9Y-0M-0HTY	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
V-04178	Shalimar88/90A-204// MH97	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
SM-07018	Shalimar-88/Atilla// MH97	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
22-03	Snb(s')//Kea(s')/Snb(s')	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
B-07/Bkhtwr	LFN/1158.57//Prl/3/ Hahn/4/Kauz CMBW 89Y1044-0t0PM-8Y-010M-020B-0NPL-0T0Y	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
V-05082	Chenab2000/ Inqal-ab-91	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
PR-90	CNDO/R143//Ente/ Mexi-213/ CMSS93Bo1824M-040Y-73Y-010M-010Y-010M-10Y-0M	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
ZAS70	Inqalab 91*2/Tukuru CGSS99B00015F-099Y-099M-099Y-099M-31Y-0B	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
33010	KT/Bage//Fnu/3/Chak-wal-86 BR.4457-1B-5B-3B-0B	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve

Table 64. Wheat genotypes in National Uniform Wheat Yield Trails (Rainfed, 1-11, and Irrigated, 12-30), 2008–09, characterized for the T1BL·1RS translocation by cytology (the presence of two satellites (6B,6B) shows the presence of translocation and four satelllites (6B,6B,1B,1B) shows its absence), C–banding, SSR marker, and glucose phosphate isomerase (GPI) analyses (1RS +ve indicates presence of heterochromatin bands of rye, 1RS –ve shows absence).

Cultivar	Parentage	Cytological validation (number of satellites)	C–banding diagonistics	Molecular diagonistics	GPI validation
AUP-4008	Gen*2//Buc/ Flk/3/Buchin CMSS96M03098S-12M-010SY-010M-010SY-3M-0Y	6B,6B	1RS +ve	1RS +ve	1RS +ve
NIA-8/7	SHA4/Weaver// Skauz*2/SRMA	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
V-05066	Amsel/Attila// Inqal-ab-91/Pew'S'	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
CT-03457	Attila*2/Yaco CGSS96B00134F-099B-028Y-099M-4Y-0B	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
66284	Inqalab-91/CB-271	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
SD-4085/3	Sarsabz/Sunco*2	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NR-356	Oasis/ Skauz//4*BC/3/2*Pastor CMSS00Y01881T-050M-030Y-030M-030WGY-33M-0Y-01D	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
9268	7012/PBW-222	6B,6B	1RS +ve	1RS +ve	1RS +ve
V-05BT006	Maya/Mon'S//Hork/ Fsd85 Iotech-0R4-1R1-2R7-3RK-0R	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
V-04022	Inqalab-91/3/Crow/ Nac//Bow'S'	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve

Conventional somatic cytology. Wheat cultivars show secondary constrictions on chromosomes 1B, 6B, and 5D when analyzed by conventional somatic cytological techniques. The 5D chromosome is resolved rather inconsistently. Generally, the secondary constriction of 1RS does not get resolved in T1BL·1RS wheat cultivars. The detection of only two satellited chromosomes in the somatic cells gives a quick, initial indication of the presence of 1RS in T1BL·1RS. The satellites on the short arm of chromosomes 1B and 6B were frequently observed, however, the chromosome 5D satellite appeared infrequently. The satellites of chromosome 5D were visible only in the good preparations. The translocated lines showed only two satellites on the short arm of 6B chromosome, whereas lines lackiing T1BL·1RS had six satellites, a pair of satellites on short arm of each of 1B, 6B, and 5D chromosomes (Fig. 40).

The difference observed between chromosomes 1B and 6B was that the short arm of the chromosome 1B was shorter than the short arm of chromosome 6B. There was a significant difference between the length of the long and the short arm of chromosome 1B whereas chromosome 6B had nearly equal short and long arms.

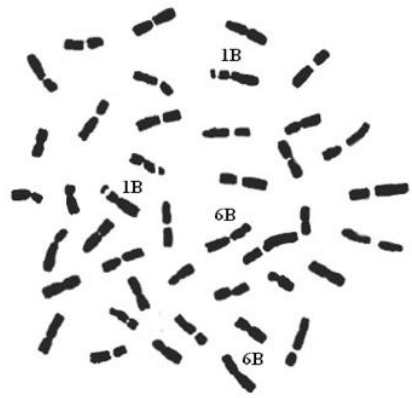

Fig. 40. Representative cell of wheat containing 42 chromosomes showing satellites on 1B and 6B chromosomes.

Table 65. Wheat genotypes in National Uniform Wheat Yield Trails (rain-fed, 1-12 and irrigated, 13-32), 2009–10, characterized for the T1BL·1RS translocation by cytology (the presence of two satellites (6B,6B) shows the presence of translocation and four satelllites (6B,6B,1B,1B) shows its absence), C–banding, SSR marker, and glucose phosphate isomerase (GPI) analyses (1RS +ve indicates presence of heterochromatin bands of rye, 1RS –ve shows absence).

Line/cultivar	Cytological validation (number of satellites)	C–banding diagonistics	Molecular diagonis-tics	GPI validation
NUWYT-1	6B,6B	1RS +ve	1RS +ve	1RS +ve
NUWYT-2	6B,6B	1RS +ve	1RS +ve	1RS +ve
NUWYT-3	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-4	6B,6B	1RS +ve	1RS +ve	1RS +ve
NUWYT-5	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-6	6B,6B	1RS +ve	1RS +ve	1RS +ve
NUWYT-7	6B,6B	1RS +ve	1RS +ve	1RS +ve
NUWYT-8	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-9	6B,6B	1RS +ve	1RS +ve	1RS +ve
NUWYT-10	6B,6B	1RS +ve	1RS +ve	1RS +ve
NUWYT-11	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-12	6B,6B	1RS +ve	1RS +ve	1RS +ve
NUWYT-13	6B,6B	1RS +ve	1RS +ve	1RS +ve
NUWYT-14	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-15	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-16	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-17	6B,6B	1RS +ve	1RS +ve	1RS +ve
NUWYT-18	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-19	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-20	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-21	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-22	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-23	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-24	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-25	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-26	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-27	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-28	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-29	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-30	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-31	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve
NUWYT-32	6B,6B,1B,1B	1RS –ve	1RS –ve	1RS –ve

Somatic cells of the germ plasm lines were analyzed, chromosome were counted, and satellites observed at metaphase. Each germ plasm entry had a normal euploid status with 42 chromosomes. Twelve of the 40 wheat cultivars had only 6B satellites and the remaining 28 had four satellites, chromosomes 1B and 6B, and in rare cases 5D also was observed. The cultivars with translocation 6B satellites included Punjnad-88, Chakwal-86, Saleem-2000, Imdad-2005, Pak-81, Shalimar-88, Tatara, Wafaq-2001, Pirsabak-85, Pasban-90, Rohtas-90, and Fakhr-e-Sarhad.

Two of the 30 NUWYT entries for the 2008–09 crop cycle had only 6B satellites, and the remaining 28 entries had four satellites for chromosomes 1B and 6B. Only 6.66% of the NUWYT entries for this crop cycle possessed the T1BL·1RS translocation. This trial had no translocated entry present in the rain-fed category, both were in the irrigated group. For the 2009–10 crop cycle, ten of the 32 NUWYT entries exhibited only 6B satellites, and the remaining 22 entries had four satellites for chromosomes 1B and 6B. For the 2009–10 crop cycle, 31.25% of the NUWYT entries had the T1BL·1RS translocation, an increase.

C-banding technique. Unlike wheat, heterochromatin is present at the terminal ends of rye chromosomes making it possible to identify 1RS Robertsonian translocations, 1R substitutions, or 1R additions in wheat. The C-banding for a particular chromosome is believed to be the constant for that chromosome. The wheat and rye karyotypes were produced by this technique, and these karyotypes are helpful in the identification of rye chromosomes in the wheat background. The B genome of wheat is the most heterochromatic, followed by A genome, which is slightly more heterochromatic than the D genome. The D genome is the least heterochromatic in terms of the total heterochromatin per genome in wheat. Chromosomes 4A, 1B, 2B, 5B, and 6B are the most heterochromatic chromosomes. When analyzed by C-banding, the T1BL·1RS chromosome contained prominent banding sites on the long arm terminal end, at the centromeric region, and terminal and subterminal sites on the short arm, with a fainter interstitial band on the long arm. Banding patterns observed were very clear in good preparations.

Prominent bands on the terminal end of the long arm, and centromeric, terminal, and subterminal bands on the short arm were shown in 12 of the 40 wheat cultivars, and these cultivars were classified as translocated. Quite clear banding patterns were observed in the good preparations. Cultivars with the translocation included Punjnad-88, Chakwal-86, Saleem-2000, Imdad-2005, Pak-81, Shalimar-88, Tatara, Wafaq-2001, Pirsabak-85, Pasban-90, Rohtas-90, and Fakhr-e-Sarhad.

Two of the 30 NUWYT entries of the 2008–09 crop cycle showed prominent bands on terminal end of long arm, and centromeric, terminal, and subterminal bands on the short arm, and remaining 28 entries showed bands specific for arm 1BS. Only 6.66% of the NUWYT entries for this crop cycle possessed T1BL·1RS. This trial had no T1BL·1RS entry present in the rain-fed category, both translocation lines were present in the irrigated set. For the 2009–10 crop cycle, ten of 32 NUWYT entries showed prominent bands on terminal end of long arm, the centromeric, terminal, and the subterminal band on the short arm, and remaining 22 entries showed bands specific for arm 1BS, indicating that 31.25% of the NUWYT entries for this crop cycle possessed the translocation.

Cytological techniques provide a powerful tool for the identification of the T1BL·1RS translocation in germ plasm. The conventional somatic cytological technique used proved to be an efficient means for characterizing the germ plasm with T1BL·1RS through the identification of the satellites present on short arm of the chromosome 6B. Secondary constrictions present on the chromosomes 1B, 6B, and 5D are very helpful for the characterization of germ plasm carrying translocations. Rye chromatin also can be characterized using C-banding. First established as a technique applicable to animal chromosomes, chromosome banding is now an established procedure for the characterization of rye chromatin in plants. To apply chromosome banding and in situ hybridization, the base essentials include ideal chromosome contractions and a high number of mitotic metaphase spreads.

Molecular characterization. Many scientists use molecular diagnostics to identify the T1BL·1RS translocation. Molecular markers, including SSRs or microsatellites, have been used efficiently for the identification of this translocation. When rye-specific SSR primers are used to identify T1BL·1RS, they generate diagnostic bands that help identify translocation genotypes. The 1RS-specific RYE-NOR marker was used for the molecular evaluation of the Pakistani wheat cultivars and the entries of the two NUWYT trials.

Amplification and polymorphism generated by RYE-NOR. The RYE-NOR is specific to the short arm of rye and amplifies bands of 300 bp, 400 bp, 700 bp, and 800 bp in the T1BL·1RS genotypes; it generated no amplification in the nontranslocation genotypes. Wheat cultivars that were amplified by the marker included Punjnad-88, Chakwal-86, Saleem-2000, Imdad-2005, Pak-81, Shalimar-88, Tatara, Wafaq-2001, Pirsabak-85, Pasban-90, Rohtas-90, and Fakhr-e-Sarhad. The maximum number of bands observed was three and the minimum was two.

Of the 30 NUWYT entries observed for the 2008–09 crop cycle, only two were amplified by the marker The remaining 28 entries were not amplified by this marker, thus, only 6.66% of the NUWYT entries for this crop cycle possessed T1BL·1RS. This trial had no entry with the translocation present in the rain-fed category, both translocation lines were present in the irrigated set. For the 2009–10 crop cycle, ten of the 32 NUWYT entries observed were amplified by the marker, the remaining 22 entries were not, thus, 31.25% of the NUWYT entries for this crop cycle possessed T1BL·1RS.

Biochemical validation with glucose phosphate isomerase analysis. Biochemical diagnostics using GPI allelic variation and LMW-GS allelic variation were used for further validate results obtained by using cytological and molecular techniques. The rye proteins specific to 1R had unique characteristics compared to each another and to wheat proteins, which makes it possible to characterize 1RS in wheat-rye translocation lines. The short arms of chromosome 1R, 1A,

1B, and 1D have GPI loci and at least two enzyme subunits are controlled by each GPI locus. The appearance of unique, rye GPI bands makes possible the identification of rye in a wheat background.

Twelve of the 40 wheat cultivars observed showed the unique 1RS GPI bands, indicating the T1BL·1RS translocation. These bands were distinguishable from those of 1BS of the normal wheat. Cultivars with the T1BL·1RS translocation included Punjnad-88, Chakwal-86, Saleem-2000, Imdad-2005, Wafaq-01, Shalimar-88, Tatara, Pak-81, Pirsabak-85, Pasban-90, Rohtas-90, and Fakhr-e-Sarhad.

Of the 30 NUWYT entries for the 2008–09 crop cycle, only two had unique 1RS GPI bands and the remaining 28 entries showed diagnostic bands for arm 1BS; only 6.66% of the NUWYT entries for this crop cycle possessed T1BL·1RS. The genotypes specified for the rain-fed category had no translocation and only two genotypes for irrigated areas had translocation. For the 2009–10 crop cycle, ten of the 32 NUWYT entries had unique 1RS GPI bands; the remaining 22 entries showed bands specific for 1BS, 31.25% of the NUWYT entries.

Low-molecular-weight glutenin subunit analysis. The LMW-GS comprise of an important class of glutenins. Approximately 40% of the total wheat gluten fraction is represented by LMW-GS. The LMW-GS are encoded by the *Glu-A3, Glu-B*3, and *Glu-D3* loci on the short arm of chromosomes 1D, 1B, and 1D, respectively. Thus, analyzing the LMW-GS is an efficient means to identify the T1BL·1RS translocation. *Glu-B3j*, a low-molecular-weight subunit encoded by rye chromatin is the indicator of the T1BL·1RS translocation.

Sixteen wheat cultivars were validated using LMW-GS analysis. Among these 16, seven had the T1BL·1RS translocation and the remaining nine lacked it (Table 66). Cultivars with the T1BL·1RS translocation expressed subunit *Glu-B3j*. Cultivars found to have T1BL·1RS included Punjnad-88, Pak-81, Shalimar-88, Tatara, Pirsabak-85, Pasban-90, Rohtas-90, and Fakhr-e-Sarhad; Blue Silver, Sarsabz, Bhakkar-2002, Margalla-99, Auqab-2000, Inqalab-91, Lasani-2008, Shafaq-2006, and Miraj-2008 lacked *Glu-B3j*.

Table 66. Low-molecular-weight glutenin subunit (LMW-GS) validation for the T1BR·1RS translocation in Pakistani wheat cultivars (1RS +ve indicates presence of heterochromatin bands of rye, 1RS –ve shows absence).

Cultivar	LMW–GS validation
Punjnad-88	1RS +ve
Pak-81	1RS +ve
Shalimar-88	1RS +ve
Lasani-2008	1RS –ve
Shafaq-2006	1RS –ve
Inqalab-91	1RS –ve
Fakhr-e-Sarhad	1RS+ve
Blue Silver	1RS –ve
Pasban-90	1RS +ve
Sarsabz	1RS –ve
Bhakkar-2002	1RS –ve
Pirsabak-85	1RS +ve
Rohtas-90	1RS +ve
Miraj-2008	1RS –ve
Margalla-99	1RS –ve
Auqab-2000	1RS –ve

This study generated valuable information about Pakistani wheat cultivars and the entries of NUWYT for two crop cycles. These results showed that the percent of entries with the T1BL·1RS translocation increased from 6.66% in the 2008–09 trial to 31.25% in 2009–10. This increase is desirable because many advantages are associated with this translocation. The T1BL·1RS lines tend to have greater above-ground biomass, 1,000-kernel weight, test weight, higher grain yield, and spike fertility.

Along with high yield, stability, and adaptability, the T1BL·1RS lines also may possess resistance to *Septoria tritici* blotch and moderate aluminium toxicity tolerance. In addition, resistance to several important pathogens, such as leaf, strip, and stem rusts and downy mildew has been associated with chromosome arm 1RS. Grains of T1BL·1RS lines are rich in trace minerals. A positive effect of T1BL·1RS on the concentration of iron and zinc in the grain of the CIMMYT wheat germ plasm also has been reported. The T1BL·1RS translocation lines have a high harvest index and water-use efficiency under drought conditions than their non-T1BL·1RS counterparts. The T1BL·1RS wheat–rye translocation also is useful for improving the adaptability of wheat to zinc deficient and acidic soils. However, some quality concerns exist about T1BL·1RS germ plasm. The advantages are making the T1BL·1RS lines desirable to be used in the breeding programs worldwide, and future strategies to exploit these sources to get more benefit from this sort of novel germ plasm should be encouraged.

QTL analysis of a wheat double haploid mapping population for salinity tolerance.

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Through the combined use of DNA marker technology and advanced statistical methods, chromosome regions that contain the genes that determine quantitative traits (QTL) can be identified. Gene cloning technologies that originally targeted major genes are increasingly including those genes responsible for quantitative, multigenic traits. Identifying genes behind these QTL is a big challenge. However, now that a few plant QTL have been cloned and accurately tagged, they might be accurately positioned within 2 cM or less on the genome. We see circumstances when map-based cloning using only original mapping data would be a realistic option that avoids time-consuming and expensive fine mapping. Keeping this in view, our study was designed to find DNA markers linked to salinity tolerance traits in common wheat using the QTL mapping technique to map QTL for salt tolerance traits in a wheat mapping population, use in vitro screening for salt tolerance monitoring physiological parameters through hydroponic tests, and study the phenotype to identify entries with good morphological characters.

Materials and methods. The plant material consisted of 61 double haploids (DH) derived from the cross of 'Croc/Stylet'. Croc is salinity resistant and Stylet is salinity susceptible. Seeds of these lines were collected from the Wheat Wide Crosses, Islamabad. A hydroponic experiment was conducted at two salinity levels, at 0 mM NaCl, which served as a control experiment under nonsaline conditions, and at 75 mM NaCl, under saline conditions to evaluate the performance of the mapping population and parents.

Screening of parents for physiological characteristics. The mean values for both parents revealed a large amount of variation for these traits at 0 mM. The K^+:Na^+ ratio was higher in Croc (2.5) than Stylet (0.1). Chlorophyll a content was higher in Croc than Stylet. Croc showed a significantly higher value in different physiological traits than Stylet. A significant difference in total chlorophyll was noted between Croc (5.3 mg/g) and Stylet (52 mg/g). The same pattern was observed for sugar content, which was higher in Croc (3.01 mg/g) than Stylet (0.12 mg/g). Thus, Croc showed better results than Stylet (Table 67). There was a significant difference in both parents at 75 mM. The K^+:Na^+ ratio was higher in Croc (1.25) than Stylet (0.07). Chlorophyll content was higher in Croc (Table 67). A significant increase in sugar content under saline conditions was observed in Croc (4.15 mg/g) compared to Stylet (1.25 mg/g). The data revealed that Croc showed better performance under 75 mM salt stress than Stylet.

Table 67. Physiological characterization of a 'Croc/Stylet' mapping population and the parents at two salinity levels.

Parameter	Salt concentration (mM)	Mean (±SD)		Mapping population: Minimum value	Maximum value	Skewness	Kuertosis	h^2
K^+:Na^+	0	2.50	0.10	0.11	2.32	–0.24	–1.17	0.82
	75	1.25	0.07	0.07	1.23	0.14	–0.44	0.54
Chlorophyll a	0	4.02	0.43	0.43	3.87	0.11	–0.25	0.44
	75	2.80	0.10	0.10	2.72	0.12	–1.10	0.72
Chlorophyll b	0	2.20	0.07	0.08	2.00	0.75	0.26	0.56
	75	1.50	0.06	0.06	1.42	0.87	0.25	0.73
Total chlorophyll	0	5.30	0.52	0.52	5.16	0.19	–0.56	0.48
	75	4.23	0.18	0.19	4.05	0.26	–0.95	0.73
Sugar	0	3.01	0.12	0.13	2.92	–0.14	-1.08	0.80
	75	4.15	1.25	1.27	4.15	–0.01	-0.11	0.84

Screening of the parent lines for morphological characteristics. Significant variability was observed in both parents at control (0 mM) conditions. Both parents differed significantly from each other for all the parameters studied (Table 68, p. 208). The shoot length of Croc was 37.3 cm, whereas that of Stylet was 18 cm. Root length was greater in Croc (21.50 cm) than Stylet (5.44 cm). The shoot dry weight of Croc was 0.7 g and Stylet was 0.16 g. Root dry weight of Croc was 0.060 g, whereas of Stylet was 0.006 g.

At saline conditions (75 mM NaCl), Croc performed better than Stylet (Table 68). The means of the different morphological parameters showed significant differences in both parents. The shoot length of Croc was 33cm and that of Stylet 14 cm. The difference in root length also was large; Croc was 21.2 cm and Stylet was 3.00 cm. Fresh shoot weight was significantly different in both parents; Croc had a weight of 0.52 g and Stylet had 0.07 g. Shoot dry weight also was significantly higher in Croc (0.06 g) than in Stylet (0.01 g). The root dry weight of Croc was 0.280 g, whereas that of Stylet was 0.004 g.

Table 68. Morphological characterization of a 'Croc/Stylet' mapping population and the parents at two salinity levels.

Parameter	Salt concentration (mM)	Croc_1	Stylet	Mapping population				h^2
		Mean±SD		Minimum value	Maximum value	Skewness	Kuertosis	
Shoot length (cm)	0	37.4	18.0	19.0	36.3	–0.38	1.45	0.68
	75	33.0	14.0	14.0	32.0	–0.81	2.51	0.67
Root length (cm)	0	21.5	5.440	5.44	20.6	0.39	–0.31	0.59
	75	21.2	3.000	3.02	20.5	1.35	2.74	0.76
Shoot fresh weight (g)	0	0.70	0.16	0.16	0.60	–0.11	-0.73	0.58
	75	0.52	0.07	0.08	0.51	0.18	-0.74	0.75
Shoot dry weight (g)	0	0.12	0.03	0.03	0.12	1.85	5.05	0.14
	75	0.06	0.01	0.01	0.06	0.48	–0.26	–0.12
Root dry weight (g)	0	0.06	0.006	0.006	0.054	2.44	9.05	0.42
	75	0.28	0.004	0.004	0.023	0.44	1.09	0.46

Screening of the mapping population for physiological characteristics. The mean, maximum, and minimum values revealed that variation exists among the members of mapping population (Table 68). No transgressive segregation at the lower and upper limits of any trait was observed. The graphical representation of these traits also revealed that, in most of the cases, the data distribution is normal and there is no need to standardize the data set.

K^+:Na^+ ratio. The K+:Na+ ratio ranged from 0.11 to 2.32 in the population at the control conditions. The skewness of the potassium sodium ratio was –0.24 and kurtosis was –1.17. The negative value for skewness indicated that most of the lines had a value less than the average. At 75 mM salt, the K^+:Na^+ ratio was 0.07–1.23, which is less than the control. The skewness of K^+:Na^+ ratio at saline conditions was positive (0.14) being greater than its mean value. The heritability under control condition (0 mM) was 0.82 and at salt stress conditions (75 mM) was 0.54, which is less than the control condition and, thus, could not be made a criteria of selection for salinity tolerance.

Chlorophyll a. Chlorophyll a was higher in 0 mM salt (3.87 mg/g) and reduced in saline conditions (2.72 mg/g). Under the control conditions chlorophyll was 0.43–3.87 mg/g and 0.10–2.72 mg/g at saline conditions (75 mM NaCl). The skewness of chlorophyll a in the population is 0.12 in saline (75 mM NaCl) and 0.11 in nonsaline (0 mM) conditions. In both cases, the positive value for skewness showed that the performance of most of the lines was equal or greater than the mean value. Kuertosis in the population in saline conditions was –1.1 and in nonsaline conditions was –0.25. The estimate pf heritability of chlorophyll a under control conditions was 0.44 and 0.72 under salt stress.

Chlorophyll b. Chlorophyll b content was higher under control conditions (0.08–2.00 mg/g) and reduced in saline conditions (0.06–1.42 mg/g). The skewness of chlorophyll b in the population was 0.87 in saline and 0.75 in nonsaline conditions. Skewness was positive in both saline and nonsaline conditions. Kurtosis in the population under saline conditions was 0.26 and 0.25 in nonsaline. Heritability for chlorophyll b under control conditions was 0.56 and 0.73 under salt stress.

Total chlorophyll. The total chlorophyll also decreased due to salinity stress, ranging from 0.52 mg/g to 5.16 mg/g under the control conditions and 0.19 mg/g to 4.05 mg/g uder saline conditions. The skewness in the population was 0.26 in saline and 0.19 in control conditions. The population showed a higher skewness value for total chlorophyll at saline conditions (0.26) than at control conditions (0.19). A positive value for skewness showed that they were equal or greater than the mean value. Kurtosis in the population under saline conditions was –0.95 and in nonsaline conditions was –0.56. Heritability for total chlorophyll was 0.48 and 0.73 under control and salt stress, respectively.

Sugar content. Sugar content increased in stressed conditions; the range of sugar was 0.13–2.92 mg/g at the control conditions and 1.27–4.15 mg/g in saline. A significant increase in sugar content was observed in saline conditions; increasing from 2.92 mg/g in he control and 4.15 mg/g in saline. Skewness in population was –0.01 in saline and –0.14 in nonsaline conditions. Sugar showed a negative value for skewness indicating less than the mean value. Skewness is much less in saline conditions than in the control conditions. Kurtosis in the population under saline conditions was –0.11 and –1.08 in nonsaline conditions was. Heritability for sugar contents in the control was 0.80 and 0.84 under salt stress conditions.

Screening of the mapping population for morphological characters. Sufficient variability was observed in the 61 lines for the morphological characters studied.

Shoot length. The shoot length ranged from 14.0–32.0 cm in the population under saline conditions, which is within the limits of the parental means (14.0–33.0 cm). Under control conditions, shoot length ranged from 19.0–36.3 cm; a decrease under salinity. The skewness of shoot length was –0.81 under saline and –0.38 under control conditions, a negative move from the mean values. Kuertosis was –2.51 at saline and 1.45 at control conditions. Heritability for shoot length was 0.68 under control and 0.67 under salt stress condition.

Root length. Root length was higher in the control (5.44–20.50 cm), which was within the limit of the parental mean (5.44–21.50 cm) and reduced under saline conditions (3.02–20.50 cm). Under saline conditions the parental mean for root length was 3.00–21.20 cm. The skewness of root length in the population is 1.35 in saline and 0.39 in nonsaline conditions. A positive value for the skewness showed that these values were equal or greater than the mean value. Kurtosis in saline conditions was 2.74 and –0.31 in nonsaline conditions. The negative value showed that these values were less than the mean value. Under control conditions, heritability for root length was 0.59 and 0.76 under salt stress.

Shoot fresh weight. Fresh weight of the shoots was higher in control conditions (0.16–0.60 g) and reduced in saline (0.08–0.51 g). The parental mean values for shoot fresh weight was 0.70 g under control conditions and 0.52 g under saline. The skewness of shoot fresh weight in the population was 0.18 under saline and –0.11 under nonsaline conditions. The skewness for shoot fresh weight under saline condition was higher with a positive value and smaller in controlled conditions with value negative from the mean. The kurtosis in saline conditions was –0.74 and in nonsaline conditions was –0.73. Heritability for shoot fresh weight under control conditions was 0.58 and under salt stress was 0.76.

Shoot dry weight. The dry weight of the shoot also decreased due to salinity stress ranging from 0.03–0.12g under control conditions, within the limit of their parental mean values (0.03–0.12g). On the other hand, under saline condition, it ranged from 0.01–0.06 g, exactly within the parental mean value (0.01–0.06 g). Skewness in the population was 0.48 under saline and 1.85 under control conditions, showing a positive shift from the mean value. Kurtosis under saline conditions was 5.05 and under nonsaline conditions was –0.26. Heritibility for shoot dry weight was 0.14 under control and –0.12 under salt stress conditions.

Root dry weight. The root dry weight decreased under stress conditions. A significant decrease under saline condition was observed. At control conditions root dry weight was 0.006–0.054g with the parent mean 0.006–0.060 g. The root dry weight ranged from 0.004 to 0.023 g under salt stress, which is within the parental mean values (0.004–0.028 g). Skewness in the population was 0.44 under saline and 2.44 under nonsaline conditions and higher under control conditions (2.44) than under saline condition (0.44). Kurtosis under saline conditions was 9.05 and 1.09 under nonsaline. Heritability for root dry weight was 0.42 under control and 0.46 under salt stress conditions.

Correlation between different physiological parameters at 0 mM. Correlation ranged from 0.98 to –0.42 among the different physiological and morphological parameters. The maximum correlation was observed between chlorophyll a and total chlorophyll, and the minimum correlation was observed between the sugars and K^+:Na^+. The correlation matrix showed positive correlation between most of the physiological parameters at a 0 mM salinity level. There are fewer negative correlations, between chlorophyll a and sugar (–0.26), chlorophyll b and sugar (–0.36), total chlorophyll and sugar (–0.31), sugar and K^+:Na^+ (–0.42), sugar and shoot length (–0.12), sugar and root length (–0.12), sugar and shoot fresh weight (–0.23), chlorophyll a and shoot dry weight (–0.088), chlorophyll b and shoot dry weight (–0.14), total chlorophyll and shoot dry weight (–0.11), and K^+:Na^+ and shoot dry weight (–0.1). Positive correlations were observed among rest of the characteristics.

Correlation between pifferent physiological parameters at 75 mM. The correlation among the different physiological and morphological parameters ranged from 0.98 to –0.31. The maximum correlation was observed between chlorophyll a and total chlorophyll, and the minimum correlation was observed between the sugar and root length. Correlation matrix showed a positive correlation between most of the physiological parameters at 75 mM salinity. Fewer negative correlations were observed between different parameters; chlorophyll a and sugar (–0.21), chlorophyll b and sugar (–0.26), total chlorophyll and sugar (–0.23), sugar and shoot length (–0.13), sugar and root length (–0.31), K^+:Na^+ and root length (–0.14), sugar and shoot fresh weight (–0.29), chlorophyll a and shoot dry weight (–0.18), chlorophyll b and shoot dry weight (–0.28), total chlorophyll and shoot dry weight (–0.22), sugar and shoot dry weight (–0.14), chlorophyll a and root dry weight (–0.35), chlorophyll b and root dry weight (–0.37), total chlorophyll and shoot dry weight (–0.37), and sugar and shoot dry weight (–0.02).

Genotyping. ***Formation of linkage groups.*** The data were subjected to Mapmaker/EXP version 3.0 to construct genetic linkage maps with a LOD threshold of 2.0 and maximum distance of 50 cM between adjacent markers. Centimorgan (cM) values were calculated from recombination frequencies based on the Kosambi mapping function. The LOD score is defined as the base-10 logarithm of the ratio of the maximum likelihood values assuming linkage versus no linkage. Nine linkage groups were formed for the chromosomes 1A, 6A, 7B, 1D, 2D, 4D, 5D, 6D, and 7D (Fig. 41). The 2D chromosome was divided into two linkage groups 2D.1 and 2D.2 due to the greater recombination fraction among the adjacent markers.

QTL analysis. QTL mapping analyses for the traits tested were performed using the composite interval mapping method implemented by the software package Q Gene V. 4.1. A log- likelihood (LOD) score threshold of 2.0 was used to identify regions containing putative loci associated with traits under study. Three data pools for each trait were prepared and subjected to QTL analysis. A third data pool for the difference at both salinity levels was used to identify any additional QTL controlling the difference of these traits. The total LOD score and variance explained (R^2) in each trait were determined in a multiple-QTL model that included all of the significant QTL. Results for the different traits follow.

Chlorophyll a content. Total of two QTL for Chlorophyll a were identified on chromosomes 6A and 7D (Table 69). A QTL on 6A was identified to control chlorophyll a under 0 mM conditions and 7A controls the trait at 75 mM salt-stress conditions. These QTL on 6A and 7D were linked with their respective markers and explained 17.6% (6A) and 17.0% (7D) of the variation (R^2) chlorophyll a. The additive effect of 6A and 7D were 0.407 and –0.329, respectively (Table 69).

Table 69. QTL for various traits using composite interval mapping in Richard's Salinity Mapping Population.

Trait		QTL location	Closest marker	LOD score	R^2	Additive effect
Chlorophyll a	0 mM	7D	BARC 57	2.560	17.6%	0.407
	75 mM	6A	BARC 169	2.470	17.0%	–0.329
Chlorophyll b	0 mM	6A	BARC 169	2.290	15.9%	–0.188
	0 mM	7D	BARC 53	2.160	15.0%	0.205
	75 mM	6A	BARC 169	2.670	18.3%	–0.168
Total chlorophyll	0 mM	7D	BARC 53	2.170	15.2%	0.550
	75 mM	6A	BARC 169	2.710	18.5%	–0.528
Sugar	0 mM	1D	*Xgdm19*	2.060	14.4%	1.252
	75 mM	1D	*Xgdm19*	2.840	19.3%	1.361
	75 mM	5D	*X292.5D*	2.820	19.2%	–30.03
K^+:Na^+ ratio	75 mM	2D2	BARC159	2.210	15.4%	–1.021
Shoot length	75 mM	1A	BARC 62	4.675	29.7%	2.094
	75 mM	2D2	*X320.D*	4.041	26.3%	13.154
Shoot fresh weight	75 mM	6D	*Xgdm14*	2.490	17.2%	–0.529
	Difference	4D	*Xgdm61*	2.590	17.8%	–0.070
Shoot dry weight	Difference	1A	BARC 62	2.890	19.6%	-0.01

Chlorophyll b content. Three QTL for Chlorophyll b were identified on chromosomes 6A and 7D under 0 mM conditions and one on chromosome 6A under salt stress condition (Table 69, p. 210). These QTL are exactly linked with their respective markers and explained 15.9% (6A), 15.0% (7D), and 18.3% (6A, 75 mM) of the variation for chlorophyll b. The additive effects of 6A were –0.188 (6A), 0.205 (7B), and –0.168 (6A, 75 mM).

Total chlorophyll content. Two QTL for total chlorophyll were identified on chromosomes 7D and 6A (Table 69, p. 210). These QTL are exactly linked with respective markers and explained 15.2% (7A) and 18.5% (6A) of the variation for total chlorophyll. The additive effects of 6A and 7D were 0.550 and –0.528, respectively.

Sugar content. Three QTL for sugar were identified on chromosome 1D under 0 mM conditions and two were identified on chromosomes 1D and 5D under salt-stress conditions (Table 69, p. 210). The QTL on chromosome 1D at both salinity levels were exactly linked with their respective marker and explained 14.4% and 19.3% of the variation. The additive effects of the 1D QTL were 1.252 and 1.361. A third QTL is found on 5D chromosome. The position of the QTL was 226.0 cM on the genetic map of chromosome 5D. This QTL explained 19.2% of the variation for sugar and the additive effect of 5D was –30.03.

K^+:Na^+ ratio. One QTL is found on chromosome 2D2 for K^+:Na^+ ratio (Table 69, p. 210). The position of the QTL was 422.0 cM on genetic map of chromosome 2D2. This QTL explained 15.4% of the variation for the K^+:Na^+ ratio and the additive effect of 2D was –1.021.

Shoot length. One QTL is found on 1D chromosome for shoot length (Table 69, p. 210). The position of the QTL was 34.0 cM on the genetic map of chromosome 1D. This QTL explained 13.9 % of the variation for shoot length. The additive effect of 1D was –4.029.

Shoot fresh weight. Two QTL for shoot fresh weights were identified on chromosomes 6D and 4D (Table 69, p. 210). The position of the QTL was 332.0 cM on genetic map of chromosome 6D and 2.0 cM on the genetic map of chromosome 4D. These QTL explained 17.2% (6D) and 17.8% (4D) of the variation for shoot fresh weight. The additive effect of 6D and 4D were –0.529 and –0.07, respectively.

Shoot dry weight. One QTL is found on 1A chromosome for shoot dry weight (Table 69, p. 210). The position of QTL was 152.0 cM on genetic map of chromosome 1A. This QTL explained 19.6% of the variation shoot dry weight. The additive effect of 1A was –0.01.

Characterization of the mapping population for morphological and physiological traits. A wheat DH population subjected to salt stress of 75mM NaCl indicated highly significant differences among the population for all the morpho-physiological traits studied. However, the differences in the parents for the physiological parameters studied were more prominent than morphological traits. Croc has a 25 times higher K^+:Na^+ ratio than Stylet under control conditions, whereas it has 18 times greater K^+:Na^+ ratio than Stylet at 75 mM salt stress. These results revealed that Croc is more tolerant than Stylet. Under salt-stress conditions, there is a decrease in the K^+:Na^+ ratio. The salinity tolerance in wheat is associated with the accumulation of K^+ and exclusion of Na^+ under saline conditions. These results indicated that parents slightly decrease K^+ accumulation under salinity stress. We observed a similar percent decrease in the K^+:Na^+ ratio. In Richard's population, genotype HW775*C22 showed the highest K^+:Na^+ ratio and HW775*A13 the lowest.

Sodium competes with K^+ for uptake through a common transport system and does this effectively because the Na^+ concentration in saline environments is usually considerably greater than that of K^+. The sensitivity of some crops to salinity is due to the inability to keep Na^+ and Cl^- out of transpiration streams. Plants limiting the uptake of toxic ions or maintaining normal nutrient ion contents could show greater tolerance, which also was the case with our study. Uptake mechanisms that discriminate similar ions, such as Na^+ and K^+, can provide a useful selection criteria for salt tolerance in wheat and breeding for efficient nutrient uptake. All the wheat genotypes studied showed a decreasing trend in K^+ content due to salinity stress. The decrease in K^+ was due to the presence of excessive Na^+ in the growth medium because high external Na^+ content is known to have an antagonistic effect on K^+ uptake in plants. Salt tolerance is reported to be associated with K^+ content because of its involvement in osmotic regulation and competition with Na^+. Regulation of K^+ uptake, prevention of Na^+ entry, and efflux of Na^+ from cell are commonly used by plants to maintain desirable K^+:Na^+ ratio in the cytosol.

The chlorophyll content of leaves generally decreases under salt stress. The oldest leaves start to develop chlorosis and fall with prolonged period of salt stress. In this study, the chlorophyll a content of the leaves decreased due to salt stress. In both parents, the percent reduction varied from 76.6 % in Stylet and to 30.3 %Croc. Stylet was more sensitive towards salt stress shown by a greater decrease in chlorophyll a content in the leaves under salt stress. Croc had less of a decrease in chlorophyll a content of the leaves (30.3%) than that of Stylet, showing tolerant behavior under salt stress. The mapping population also had a decreased chlorophyll a content within the range of the parental reduction. Genotype HW775*C16 showed high chlorophyll a content and genotype HW775*A51 showed a low content in the mapping population. The chlorophyll b content in leaves of Croc decreased about 32% and in Stylet about 14.2%. The population also had a decreased chlorophyll b content. In the population, genotype HW775*C29 showed a higher content of chlorophyll b and genotype HW775*C16 had a lower content.

Similarly, total chlorophyll also decreased in the parents as well as in the population. In Croc, the total chlorophyll reduction was 20.2 % and the reduction was 65.3% in Stylet. These results revealed that Stylet was more sensitive to salt stress because of a more pronounced decrease in chlorophyll. In the population, genotype HW775*C27 showed a higher content of chlorophyll b and genotype HW775*B7 had a lower content. The reduction in chlorophyll content is to be expected under stress; being membrane bound, its stability is dependent on membrane stability, which under saline condition seldom remains intact.

During salt stress, an increase in sugar concentration has been reported in many species. In this study, the sugar content in the leaves of Croc increased 27.5% under salt stress whereas Stylet increased 90.4% to overcome salinity stress. Similar behavior was observed in the population with with genotype HW775*B19 under salt stress showing less of an increase in sugar content, exhibiting tolerance, and genotype HW775*C35 showing an increase in sugar content under salt stress.

Shoot length also decreased due to salt stress. The percent reduction in shoot length in Croc was 11.7% and 22.2% in Stylet, twice the reduction in shoot length. Stylet was sensitive for salt tolerance and the population also showed a reduction in shoot length under salt stress conditions. Genotype HW775*C36 had the highest shoot length and genotype HW775*A35 the smallest.

Root length decreased in Croc by 1.4% and in Stylet by 44.8%. A similar range of reduction was observed in the population. Genotype HW775*C35 in the population had the longest root length and with HW775*C32 the shortest under salt stress. Shoot fresh weight decreased 25.7% in Croc and 57% in Stylet. These results indicate that Croc, the tolerant parent, produced a smaller reduction in shoot fresh weight than Stylet. The population also showed a similar range of reduction in shoot fresh weight where HW775*C35 showed less reduction in shoot fresh weight and genotype HW775*C34 showed greater reduction under salt stress.

Shoot dry weight and root dry weight also decreased under salinity stress. Shoot dry weight decreased 50.0% and 53.0% of root dry weight in Croc, whereas the reductions in Stylet were 66.6% of shoot dry weight and 33.3 % of root dry weight under salt stress conditions. Genotype HW775*B6 had less of a reduction in shoot dry weight and genotype HW775*B17 in root dry weight showing tolerant behavior in these traits. Because salinity stress results in a clear stunting of plants, it also results in a considerable decrease in fresh and dry weights of leaves, stems, and roots. The frequency distributions for a majority of the traits were normal and variation for most of the morphological traits studied was more prominent in the population than among the parents.

Linkage map construction. The genetic map depicted that the D genome had three to eight times the number of markers compared to the A and B genomes (Fig. 41, p. 213). The chromosome length of the A and B genomes also was less than that of the D genome.

Salt tolerance is located on D genome of bread wheat. Experiments on hexaploid bread wheat and its ancestors confirmed that *Ae. tauschii* and *T. aestivum* had high K^+:Na^+ ratios, whereas *T. turdigum* subsp. *dicoccoides* and *Ae. speltoides* had low ratios. These studies demonstrated that the D genome contains a trait for enhanced K^+:Na^+ discrimination, which is located on chromosome 4D. In our study, QTL located for K^+:Na^+ ratio were found on chromosome 2D, possibly because our map is not highly dense and the QTL is a false positive. As far as the total map length is concerned, Richards map spanned 2,195.1 cM with a average distance of 2 cM between two markers.

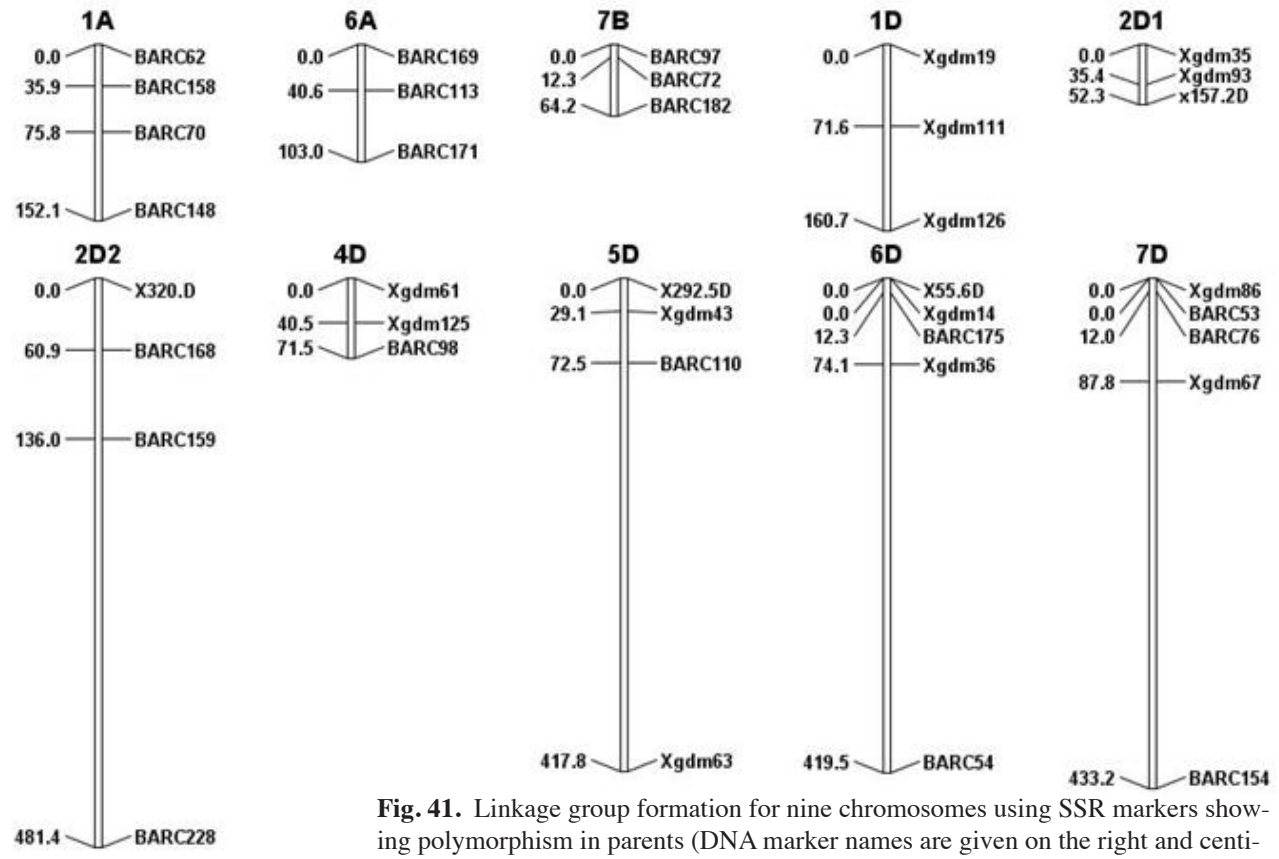

Fig. 41. Linkage group formation for nine chromosomes using SSR markers showing polymorphism in parents (DNA marker names are given on the right and centimorgan distances are on the left).

QTL mapping. The advent of molecular markers has revolutionized the genetic analysis of complex traits, and reports on the location of QTL for yield in many cereal crops are commonplace. However, QTL analysis in bread wheat has been hampered in part by its large genome size, estimated to be around 14,500 Mbp/1C, with the large majority of this DNA being repetitive sequences. Thus, many markers are required to cover the whole genome adequately. In addition, because of the relatively recent origin of the species, hexaploid wheat also suffers from relatively low levels of polymorphism. In consequence, detailed genetic maps of the whole genome are much more difficult to achieve than for most other crop species.

We observed QTL for shoot dry weight and shoot length located on chromosome 1A. Regions of QTL for total chlorophyll and chlorophyll b are located on 6A. QTL influencing chlorophyll content have been well identified in rice but none were found on chromosome B. QTL for sugar were detected on two chromosomes, one on chromosome 1D and a second on chromosome 5D. Chromosome 2D contains a region for K^+:Na^+ ratio and shoot length. The K^+:Na^+ ratio and shoot length decrease with salt stress. Shoot fresh weight has QTL on chromosomes 4D and 6D. A total of six QTL associated with shoot fresh weight were detected on chromosomes 1, 3, 5, 6, 7, and 12. Chromosomes 7D has QTL for total chlorophyll and chlorophyll a.

We could conclude that in this population, seven markers were mapped on the A genome, three markers in the B genome, and 24 markers in the D genome of the wheat. Although there was significant variation in the population for most of morpho-physiological traits studied, QTL individually explained up to 50% of the variation present in this population for most of the traits. Further improvement by the addition of more markers is needed in this genetic map to have a more extensive coverage of the genome, which could be helpful in finding QTL with major effects for salt tolerance in wheat.

Evaluation of high temperature tolerance of bread wheat germ plasm.

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To achieve self-sufficiency and sustainable productivity in wheat is of prime importance in the context of food security. The major limiting factors in wheat production are associated with several abiotic and biotic stresses. This problem could be resolved by the development of heat-tolerant wheat genotypes through conventional breeding by incorporating physiological characteristics such as membrane thermostability, and a higher proline accumulating ability. To assure success in this strategy, the coördinated efforts of a plant physiologist, molecular biologist, and crop breeder is imperative. Because high temperature stress is the second most limiting factor hampering wheat productivity in Pakistan, an in vitro and in vivo study was undertaken to screen wheat genotypes for heat tolerance based on physiological parameters at the seedling and pre-anthesis growth stages, evaluate wheat genotypes for phenological characteristics under control and heat stress, and identify heat-shock proteins expressed during heat stress. The effects of heat stress on growth, physiology, and yield were assayed in 24 wheat genotypes differing in heat tolerance. The heat stress was imposed at the seedling (15 days after sowing) and pre-anthesis (80 datys after sowing) stages. Heat stress during seedling stage was imposed by placing the seedlings in an incubator for 6 h at 25–30°C for ten days and at pre-anthesis by placing pots in a glasshouse for 3 h at 45°C for ten days.

Effect of heat stress on proline content (μg/g fresh weight) of leaves in different wheat genotypes. ***Seedling stage.*** The analysis of variance revealed a significant difference among the different wheat genotypes for proline content at $P < 0.05$. In the control treatment, high proline content was observed in genotype 5 followed by 3 and 19; the minimum proline content was observed in genotype 7 (Table 70). Due to heat stress treatment at the seedling stage, a high proline content was observed in genotype 7 followed by 10 and 11 and the minimum in 24 (Table 70). Compared to the control, the highest proline content was observed in genotype 7 (84%) followed by 14 (81%), 15 (75%), and 13 (74%), and lowest was observed in genotype 3. The proline content in genotype 14 was similar to that for genotype 7. However, the magnitude of increase in proline content was relatively higher in genotype 7 than in genotype 14 compared to the control.

Table 70. Interaction between genotypes and treatment for proline content (μg/g fresh weight) at the seedling stage. A heat stress at 25–30ºC was imposed 15 days after sowing for 10 days (LSD (0.05) = 440.6).

Line	Control		Heat stress	
1	1,058.77	BCDEFGHIJ	1,288.93	ABCDEFG
2	524.78	KLMN	1,077.18	BCDEFGHIJ
3	1,224.49	ABCDEFGH	1,224.49	ABCDEFGH
4	874.63	EFGHIJKLM	1,160.04	BCDEFGHI
5	1,307.35	ABCDEF	1,537.51	ABC
6	736.53	HIJKLMN	1,528.31	ABC
7	290.7	MN	1,904.94	AB
8	377.47	MN	1,362.59	ABCDE
9	644.47	IJKLMN	1,509.89	ABC
10	626.05	JKLMN	1,555.93	AB
11	681.29	IJKLMN	1,694.03	A
12	405.09	MN	1,546.72	AB
13	359.06	MN	1,408.62	ABCD
14	294.61	N	1,537.51	ABC
15	377.47	MN	1,537.51	ABC
16	1,051.37	BCDEFGHIJ	1,236.40	ABCDEFGH
17	1,100.18	BCDEFGHIJ	1,369.00	ABCDE
18	1,021.00	CDEFGHIJK	1,424.23	ABCD
19	1,155.40	BCDEFGHI	1,496.03	ABC
20	970.33	DEFGHIJKL	1,543.00	ABC
21	835.90	FGHIJKLM	1,310.03	ABCDEF
22	1,107.50	BCDEFGHIJ	1,441.70	ABCD
23	773.33	GHIJKLMN	1,489.57	ABCD
24	595.60	JKLMN	777.00	GHIJKLMN

Pre-anthesis stage. At pre-anthesis, statistically significant differences were observed among the wheat genotypes. The analysis of variance revealed significant differences among different wheat genotypes for proline content at $P < 0.05$. Under control conditions, high proline content was observed in genotype 8 followed by 10 and 11, and the least content was in 24 (Table 71, p. 215). Heat stress imposed at pre-anthesis stage increased proline content in wheat genotypes. A high proline content under heat stress was found in genotype 9, followed by 4 and 8, whereas genotype 16 showed the least proline content at pre-anthesis stage (Table 71, p. 215). Compared to the control, the highest proline content was observed in genotype 7 (90%) and genotype 16 the least. The percentage increase in proline content was greater at the preanthesis growth stage than that at the seedling stage.

Effect of heat stress on total soluble protein content (μg/g) of leaves in different wheat genotypes. ***Seedling stage.*** The results showed that the interaction between treatments and genotypes was significant for protein content at $P < 0.05$.

Heat stress at the seedling stage significantly increased protein content in different wheat genotypes. The highest protein content under normal condition was observed in genotype 1 followed by 4 and 5, and the lowest protein content was observed in genotype 21 (Table 72). Nevertheless, high protein content under heat stress treatment at seedling stage was observed in genotype 7 followed by 8 and 15 and the minimum was observed in genotype 1 (Table 72). Compared to the control, the highest protein content was recorded in genotype 7 (24%) followed by 19, 15, 21, and 8. The ;owest protein content was found in genotype 1. The protein content in genotype 19 was near that of 7, conversely, the magnitude of increase in protein content was relatively higher in genotype 7 than 19 compared to the control.

Pre-anthesis stage.

At pre-anthesis, a statistically significant difference was observed among wheat genotypes. The analysis of variance revealed significant difference among different wheat genotypes for protein content at $P < 0.05$. Genotype 13 showed high protein content under control conditions followed by 15 and 16; the minimum under control conditions was in genotype 18 (Table 73, p. 216). Under heat stress treatment, high protein accumulation was observed in genotype 7 followed by 15 and 8 and a minimum in genoytpe 10. Compared to the control, the highest protein content was observed in genotype 7 (73%) and the least observed in genotype 10. The protein content in genotype 14 was similar to that of genotype 7. However, the extent of the increase in protein content was relatively higher in 7 than in 14 compared to the control. The results indicated that the percent increase in soluble protein content of leaves was greater at pre-anthesis stage in wheat genotypes than that at the seedling stage.

Table 72. Interaction between genotypes and treatment for protein contents (μg /g) at the seedling stage. A heat stress at 25–30ºC was imposed at 15 days after sowing for 10 days. LSD (0.05) = 117.7.

Line	Control		Heat stress	
1	1,367.83	A	351.10	AB
2	1,169.93	FGHIJKLMN	1,253.60	ABCDEFGHIJ
3	1,320.27	ABCDE	1,301.77	ABCDEF
4.	1,356.67	A	1,278.57	ABCDEFGHJI
5	1,342.93	ABC	1,290.00	ABCDEFGH
6	1,250.93	ABCDEFGHIJK	1,288.57	ABCDEFGH
7	1,059.87	MNO	1,403.07	IJKL
8	1,167.57	FGHIJKLMN	1,369.00	A
9	1,191.67	DEFGHIJKLM	1,296.77	ABCDEF
10	1,149.97	HIJKLMNO	1,290.63	ABCDEFG
11	1,203.70	CDEFGHIJKL	1,327.30	ABCD
12	1,197.23	DEFGHIJKL	1,311.17	ABCDE
13	1,203.40	CDEFGHIJKL	1,305.00	ABCDEF
14	1,151.13	GHIJKLMNO	1,338.47	ABC
15	1,140.00	IJKLMNNO	1,355.23	A
16	1,079.73	LMNO	1,180.80	EFGHIJKLM
17	1,053.06	MNO	1,168.74	FGHIJKLMN
18	1,111.80	KLMNO	1,192.27	DEFGHIJKLM
19	1,033.06	NO	1,349.37	AB
20	1,129.67	JKLMNO	1,188.16	DEFGHIJKLM
21	1,015.46	O	1,194.89	DEFGHIJKL
22	1,183.18	EFGHIJKLM	1,195.17	DEFGHIJKL
23	1,077.12	LMNO	1,209.57	CDEFGHIJKL
24	1,133.23	JKLMNO	1,212.20	BCDEFGHIJKL

Table 71. Interaction between genotypes and treatment for proline content (μg/g fresh weight) at pre-anthesis. A heat stress at 40–45ºC was imposed 80 days after sowing for 10 days (LSD (0.05) = 1,291).

Line	Control		Heat stress	
1	785.73	HIJKL	2,114.73	EFGHI
2	767.80	HIJKL	1,793.40	EFGHIJ
3	416.10	JKL	255.90	KL
4	677.07	HIJKL	5,164.90	C
5	1,171.50	FGHIJKL	2,132.20	EFGHI
6	1,332.60	FGHIJKL	1,565.07	EFGHIJKL
7	444.28	JKL	4,290.37	C
8	2554.33	EF	4,595.90	C
9	888.87	GHIJKL	8,877.00	A
10	2,345.80	EFG	1,740.00	EFGHIJK
11	1,994.10	EFGHI	4,072.03	CD
12	1,637.37	EFGHIJKL	6,921.50	B
13	1,708.73	EFGHIJKL	2,139.60	EFGH
14	1,761.63	EFGHIJK	1,329.40	FGHIJKL
15	1,752.90	EFGHIJK	1,040.27	FGHIJKL
16	1,604.23	EFGHIJKL	189.60	L
17	492.37	FGHIJKL	350.73	JKL
18	31.97	HIJKL	1658.07	EFGHIJKL
19	349.37	JKL	1,065.17	FGHIJKL
20	374.20	JKL	194.23	L
21	933.97	GHIJKL	1201.40	FGHIJKL
22	453.37	JKL	1,876.30	EFGHIJ
23	1,136.03	FGHIJKL	847.90	GHIJKL
24	376.97	JKL	596.53	IJKL

Effect of heat stress on superoxide dismutase activity (units/g fresh weight) in different wheat genotypes. ***Seedling stage.*** Superoxide dismutase activity (SOD) was measured in wheat genotypes at the seedling stage (Table 74, p. 216). The analysis of variance revealed significant differences among the different wheat genotypes for SOD activity at the seedling stage at a $P < 0.05$. The highest SOD activity under control conditions was observed in genotype 24 followed by 18 and 20; the minimum in genotype 12. Under heat stress, the maximum SOD activity was observed in genotype 24 and the minimum in geno-

Table 73. Interaction between genotypes and treatment for protein contents (μg/g) at pre-anthesis. A heat stress at 40-45ºC was imposed at 80 days after sowing for 10 days. LSD (0.05) = 99.95.

Line	Control		Heat stress	
1	165.40	CDEFG	203.73	BCDEF
2	137.53	EFG	212.00	BCDEF
3	189.23	BCDEF	139.46	DEFG
4	190.97	BCDEF	234.60	BCDEF
5	164.10	CDEFG	209.07	BCDEF
6	139.73	DEFG	258.10	BCD
7	130.30	FG	476.55	A
8	168.97	CDEFG	304.17	B
9	158.40	CDEFG	214.63	BCDEF
10	163.83	CDEFG	21.70	H
11	147.93	DEFG	52.80	GH
12	153.97	DEFG	431.03	A
13	212.87	BCDEF	244.60	BCDE
14	129.43	EFGH	439.27	A
15	201.37	BCDEF	412.27	A
16	202.27	BCDEF	143.00	DEFG
17	173.53	CDEF	241.33	BCDE
18	118.30	FGH	185.87	BCDEF
19	194.50	BCDEF	200.23	BCDEF
20	173.97	CDEF	153.57	DEFG
21	164.70	CDEFG	177.63	CDEF
22	154.57	DEFG	165.00	CDEFG
23	170.57	CDEFG	182.30	CDEF
24	142.40	DEFG	203.73	BCDEF

Table 74. Interaction between genotypes and treatment for superoxide dismutase activity (units/g fresh weight) at the seedling stage. A heat stress of 25–30ºC was imposed 15 days after sowing for 10 days (LSD (0.05) =1.942).

Line	Control		Heat stress	
1	1.30	JKL	3.80	CDEFGHI
2	2.90	CDEFGHI	3.76	CDEFGHI
3	2.28	CDEFGHIJK	4.06	CDEFG
4	1.63	IJKL	4.34	CDE
5	2.46	EFGHIJKL	3.75	CDEFGHI
6	1.85	GHIJKL	4.11	CDEFG
7	2.55	DEFGHIJKL	4.91	C
8	1.97	FGHIJKL	3.86	CDEFGHI
9	0.81	L	3.39	CDEFGHIJ
10	1.25	JKL	4.47	CDE
11	0.83	L	3.49	CDEFGHI
12	0.99	KL	3.49	CDEFGHIJ
13	2.73	CDEFGHIJKL	4.15	CDEFG
14	1.41	JKL	4.82	CD
15	2.46	EFGHIJKL	4.50	CDE
16	3.86	CDEFGHI	7.91	B
17	1.00	KL	4.68	CDE
18	4.84	CD	8.15	B
19	1.72	HIJKL	2.72	CDEFGHIJKL
20	4.20	CDEF	2.74	CDEFGHIJKL
21	3.85	CDEFGHI	4.44	CDE
22	3.98	CDEFGH	5.00	C
23	3.53	CDEFGHIJ	7.08	B
24	9.00	B	14.08	A

type 19. The highest SOD activity was observed in genotype 17 (79%) followed by 11, 9, and 10, whereas the least SOD activity was observed in genotype 20, compared to the control. The SOD activity in genotype 11 was close to that of 17, however, the magnitude of increase was relatively higher in genotype 17 than in 11.

Pre-anthesis stage. Nonsignificant differences were present among different wheat genotypes for SOD activity at $P > 0.05$. Under control conditions, the maximum SOD activity was shown by genotype 9 followed by 5 and 21 and the minimum by genotype 17 (Table 75, p. 217). Under heat stress treatment, high SOD activity was observed in genotype 7 followed by 8 and 21 (Table 75, p. 217). Compared to the control, the highest SOD activity was observed in genotype 17 followed by 11, 16, and 14 with the least SOD activity observed in genotype 5. Genotype 17 showed a 79% increase in SOD activity under heat stress imposed at pre-anthesis compared to the control.

Effect of heat stress on peroxidase (POD) activity (μmol/g fresh weight) in different wheat genotypes. ***Seedling stage.*** The analysis of variance revealed significant difference among different wheat genotypes for POD activity at $P < 0.05$. The highest POD activity under control condition was observed in genotype 1 followed by 17 and 23. The least POD activity under control condition was observed in genotype 9. Under heat stress, the maximum POD activity was recorded in genotype 21 followed by 11 and 22 and minimum in genotype 9 (Table 76, p. 217). The highest POD activity was observed in genotype 11 (36%) and the least activity in genotype 1 compared to the control. The POD activity in genotype 15 was similar to that of genotype 11, however, the degree of increase was relatively higher in 11 than in 15 compared to the control.

Pre-anthesis stage. At pre-anthesis, statistically significant differences were recorded among wheat genotypes. The analysis of variance revealed significant difference among different wheat genotypes for POD activity at $P < 0.05$. The maximum POD activity under control conditions was recorded in genotype 17 followed by 16 and 19 and the minimum

Table 75. Interaction between genotypes and treatment for superoxide dismutase activity (units/g fresh weight) at pre-anthesis stage. A heat stress of 40–45ºC was imposed at 80 days after sowing for 10 days (LSD (0.05) = 18.66).

Line	Control		Heat stress	
1	12.33	CDEFGH	25.16	ABCDEFGH
2	10.80	CDEFGH	26.28	ABCDEFGH
3	10.67	CDEFGH	24.44	ABCDEFGH
4	11.27	CDEFGH	23.36	ABCDEFGH
5	16.60	ABCDEFGH	21.07	ABCDEFGH
6	14.87	ABCDEFGH	26.74	ABCDEFGH
7	12.63	BCDEFGH	35.33	A
8	13.93	ABCDEFGH	34.79	AB
9	20.33	ABCDEFGH	23.93	ABCDEFGH
10	7.77	FGH	23.54	FGH
11	6.40	GH	26.67	ABCDEFGH
12	8.20	EFGH	25.07	ABCDEFGH
13	11.17	CDEFGH	27.36	ABCDEFGH
14	8.50	DEFGH	30.03	ABCDE
15	9.73	CDEFGH	29.45	ABCDEF
16	6.03	H	21.93	ABCDEFGH
17	5.23	H	25.08	ABCDEFGH
18	12.23	CDEFGH	23.73	ABCDEFGH
19	10.67	CDEFGH	24.13	ABCDEFGH
20	8.37	DEFGH	20.47	ABCDEFGH
21	16.20	ABCDEFGH	30.97	ABC
22	14.97	ABCDEFGH	28.37	ABCDEFG
23	11.63	CDEFGH	28.80	ABCDEF
24	15.40	ABCDEFGH	30.53	ABCD

Table 76. Interaction between genotypes and treatment for peroxidase activity (μmol/g fresh weight) at the seedling stage. A heat stress of 25–30ºC was imposed at 15 days after sowing for 10 days (LSD (0.05) = 0.2838).

Line	Control		Heat stress	
1	1.21	A	0.57	DEF
2	0.76	BCDEF	0.86	BCDE
3	0.77	BCDEF	0.84	BCDEF
4	0.84	BCDEF	0.76	BCDEF
5	0.71	BCDEF	0.70	BCDEF
6	0.66	BCDEF	0.79	BCDEF
7	0.72	BCDEF	0.85	BCDEF
8	0.69	BCDEF	0.55	EF
9	0.52	F	0.64	BCDEF
10	0.55	EF	0.80	BCDEF
11	0.58	CDEF	0.91	BC
12	0.70	BCDEF	0.60	CDEF
13	0.57	DEF	0.72	BCDEF
14	0.68	BCDEF	0.83	BCDEF
15	0.55	EF	0.84	BCDEF
16	0.87	BCDE	0.61	BCDEF
17	0.89	BCD	0.83	BCDEF
18	0.76	BCDEF	0.85	BCDEF
19	0.84	BCDEF	0.74	BCDEF
20	0.82	BCDEF	0.65	BCDEF
21	0.67	BCDEF	0.94	AB
22	0.81	BCDEF	0.89	BCD
23	0.88	BCDE	0.82	BCDEF
24	0.71	BCDEF	0.74	BCDEF

in genotype 22. Under heat stress, the highest activity was observed in genotype 23 followed by 18 and 11 and the minimum in genotype 2 (Table 77, p. 218). The highest POD activity was in genotype 23 (65%) followed by 1, 22, and 8; the least activity was in genotype 17 compared to the control, indicating that the percentage increase in POD activity was greater at pre-anthesis than at the seedling stage.

Effect of heat stress on chlorophyll a content (mg/g) in different wheat genotypes. ***Seedling stage***.
Heat stress has remarkable effect on chlorophyll *a* content. The analysis of variance revealed significant differences among the different wheat genotypes for chlorophyll a content at $P < 0.05$. The maximum chlorophyll a degradation under control conditions was observed in genotype 9 followed by 2 and 11. Genotype 19 showed minimum degradation under control conditions. However, under heat stress, the maximum degradation was recorded in genotype 17 followed by 24 and the minimum degradation was observed in genotypes 20 and 21 (Table 78, p. 218). Chlorophyll a content decreased at the seedling stage in all genotypes. Genotype 18 showed an 86% decrease in chlorophyll a content followed by 8, 13, and 21, compared to the control, and the minimum decrease was in genotypes 17 and 7, compared to the control.

Pre-anthesis stage. At pre-anthesis, statistically significant differences were found among wheat genotypes. The greatest decrease in chlorophyll a content under control conditions was observed in genotype 1 and the minimum in genotype 8, whereas under heat stress, maximum degradation was in genotype 7 followed by 6 and 5, and the minimum in genotypes 9 and 23 (Table 79, p. 218). When the heat-stress treatment was compared with the control, the largest decrease in chlorophyll a content at pre-anthesis was in genotype 22 (67%) and the minimum was observed in genotypes 2, 3, 7, 8, 13, 14, 15, 17, 18, 19, 20, 21, 23, 24, which all are statistically similar. Our data show that the percentage decrease in chlorophyll a content was greater at seedling stage than at pre-anthesis.

Table 77. Interaction between genotypes and treatment for peroxidase activity (μ mol/g fresh weight) at pre-anthesis. A heat stress of 40–45ºC was imposed 80 days after sowing for 10 days (LSD (0.05) = 0.6349).

Line	Control		Heat stress	
1	0.52	HI	1.23	DEFGHI
2	0.52	HI	0.73	EFGHI
3	0.82	DEFGHI	0.72	EFGHI
4	0.59	EFGHI	1.14	DEFGHI
5	0.90	DEFGHI	1.26	DEFGH
6	0.84	DEFGHI	1.12	DEFGHI
7	0.80	DEFGHI	0.97	DEFGHI
8	0.58	FGHI	1.31	DEFG
9	0.87	DEFGHI	0.84	DEFGHI
10	1.03	DEFGHI	1.20	DEFGHI
11	0.84	DEFGHI	1.34	DE
12	0.58	FGHI	1.21	DEFGHI
13	0.94	DEFGHI	1.26	DEFGH
14	0.68	EFGHI	1.27	DEFGH
15	0.56	GHI	1.26	DEFGH
16	2.92	AB	1.11	DEFGHI
17	3.38	A	1.19	DEFGHI
18	1.20	DEFGHI	1.50	D
19	2.29	BC	1.18	DEFGHI
20	0.52	HI	1.11	DEFGHI
21	0.87	DEFGHI	1.32	DEF
22	0.48	I	1.12	DEFGHI
23	0.77	DEFGHI	2.21	C
24	0.61	EFGHI	0.95	DEFGHI

Table 78. Interaction between genotypes and treatment for chlorophyll a content (mg/g) at the seedling stage. A heat stress of 25–30ºC was imposed 15 days after sowing for 10 days (LSD (0.05) = 0.2051).

Line	Control		Heat stress	
1	0.66	AB	0.22	FGHIJ
2	0.76	A	0.22	FGHIJ
3	0.63	ABC	0.18	GHIJ
4	0.72	A	0.18	GHIJ
5	0.60	ABCD	0.19	GHIJ
6	0.63	ABC	0.19	GHIJ
7	0.42	CDEFG	0.27	FGHIJ
8	0.44	BCDEF	0.10	IJ
9	0.77	A	0.23	FGHIJ
10	0.67	AB	0.21	FGHIJ
11	0.73	A	0.24	FGHIJ
12	0.65	ABC	0.25	FGHIJ
13	0.69	A	0.17	HIJ
14	0.37	DEFGH	0.20	FGHIJ
15	0.56	ABCDE	0.20	FGHIJ
16	0.64	ABC	0.25	FGHIJ
17	0.38	DEFGH	0.31	FGHI
18	0.42	CDEFG	0.06	J
19	0.24	FGHIJ	0.10	IJ
20	0.31	FGHI	0.09	IJ
21	0.35	EFGH	0.09	IJ
22	0.37	DEFGH	0.20	FGHIJ
23	0.62	ABC	0.16	HIJ
24	0.59	ABCD	0.30	FGHIJ

Effect of heat stress on chlorophyll b content (mg/g) in different wheat genotypes. ***Seedling stage.*** The analysis of variance revealed nonsignificant differences among different wheat genotypes for chlorophyll b content at $P > 0.05$. The greatest decrease in chlorophyll b content under control conditions was in genotype 17 and the minimum in genotype 2. Under heat stress at the seedling stage, the maximum effect was observed in genotype 21 and the lowest in genotype 2 (Table 80, p. 219). All genotypes showed a decrease in chlorophyll b content. The greatest decrease was in genotype 1 (65%) and the least in genotype 7, compared to the control.

Pre-anthesis stage. The chlorophyll b content at pre-anthesis analysis of variance revealed nonsignificant differences among different wheat genotypes at $P > 0.05$. Under controled conditions, the maximum decrease in chlorophyll b was observed in genotypes 7, 12, 15, and 17, and under heat stress the maximum decrease was in genotype 7 and the minimum (Table 81, p. 220). Compared to the control, the maximum decrease in chlorophyll b content was in genotype 12 (50%) followed by 17 and 24; all the remaining genotypes showed a minimum decrease at pre-anthesis, indicating that the percent decrease was greater at the seedling stage.

The effect of heat stress on fresh shoot weight (g). Heat stress imposed a decrease fresh shoot weight at the seedling stage. Under controled conditions, the maximum fresh shoot weight was in genotype 16 and the minimum in genotype 18. Under heat-stress conditions, the maximum shoot weight was observed in genotype 4 and the minimum in genotype 18 (Table 82, p. 220). The wheat genotypes did not significantly differ with respect to fresh root weight. When heat stress was compared with the control treatment, a maximum (75%) reduction was observed in genotype 20 and a minimum reduction was observed in genotype 7 followed by 17 and 24.

Effect of heat stress on fresh shoot length (cm). An analysis of variance revealed that the interaction between genotypes and treatment was significant for fresh shoot length. Under control conditions, the maximum fresh shoot length was in genotype 5 followed by 15 and 23. Under heat stress, the maximum length was observed in genotype 7 followed

Table 79. Interaction between genotypes and treatment for chlorophyll a content (mg/g) at pre-anthesis. A heat stress of 40–45ºC was imposed 80 days after sowing for 10 days (LSD (0.05) = 0.01034).

Line	Control		Heat stress	
1	0.04	A	0.03	AB
2	0.03	AB	0.03	AB
3	0.03	AB	0.03	AB
4	0.04	A	0.03	AB
5	0.04	A	0.02	BC
6	0.04	A	0.03	AB
7	0.04	A	0.04	A
8	0.02	BC	0.02	BC
9	0.02	BC	0.01	C
10	0.04	A	0.02	BC
11	0.04	A	0.02	BC
12	0.03	AB	0.02	BC
13	0.03	AB	0.03	AB
14	0.04	AB	0.04	A
15	0.04	AB	0.04	A
16	0.03	AB	0.02	BC
17	0.04	A	0.04	A
18	0.04	AB	0.04	A
19	0.04	AB	0.04	A
20	0.04	AB	0.04	A
21	0.03	AB	0.03	AB
22	0.03	AB	0.03	AB
23	0.03	AB	0.01	C
24	0.03	AB	0.03	AB

Table 80. Interaction between genotypes and treatment for chlorophyll b content (mg/g) at the seedling stage. A heat stress of 25–30ºC was imposed 15 das after sowing for 10 days (LSD (0.05) = 0.09406).

Line	Control		Heat stress	
1	0.26	ABCD	0.09	IJKL
2	0.24	ABCDEF	0.10	HIJKL
3	0.77	ABC	0.84	JKL
4	0.84	ABC	0.76	IJKL
5	0.71	ABCD	0.70	JKL
6	0.66	ABCD	0.79	IJKL
7	0.58	ABCDE	0.91	GHIJKL
8	0.69	ABCDEFGHI	0.55	L
9	0.52	A	0.64	HIJKL
10	0.55	ABCDEF	0.80	IJKL
11	0.72	ABCDE	0.85	JKL
12	0.70	ABCDE	0.60	HIJKL
13	0.57	ABC	0.72	IJKL
14	0.68	ABCD	0.83	HIJKL
15	0.55	ABC	0.84	HIJKL
16	0.87	ABCDEF	0.61	FGHIJKL
17	0.89	CDEFGHIJK	0.83	FGHIJKL
18	0.76	ABCDEF	0.85	L
19	0.84	DEFGHIJKL	0.74	KL
20	0.82	BCDEFGHIJ	0.65	KL
21	0.67	BCDEFGHIJ	0.94	JKL
22	0.81	ABCDEFGH	0.89	GHIJKL
23	0.88	ABCDEFG	0.82	DEFGHIJKL
24	0.71	AB	0.74	EFGHIJKL

by 4 and 10 and the minimum in genotype 18 (Table 83, p. 221). When heat stress was compared with the control, the maximum (41%) reduction for fresh shoot length was shown by genotypes 20 and the minimum in genotype 21 followed by 7 and 17. These genotypes show tolerance to heat stress, which will be confirmed by comparing the yield of genotypes.

Effect of heat stress on fresh root weight (g). Statistical analysis of the data showed that at the seedling stage, interaction among the various wheat genotypes and treatments was significant for fresh root weight. The maximum root weight under control conditions was recorded in genotype 19 followed by 20 and 4 and the minimum was in genotype 2. Under heat stress, the maximum fresh root weight was observed in genotype 22, followed by 23 and 7 and the minimum in genotype 2 (Table 84, p. 221). Heat stress imposed at seedling stage caused a reduction in root weight. Compared to the control, the maximum (82%) reduction was in genotype 19 and the minimum in genotypes 7 and 17.

Effect of heat stress on fresh root length (cm). The analysis of variance shows that interaction among genotypes and treatment at seedling stage for root length was not significant. The maximum root length in control conditions was observed in genotype 4 followed by 21 and 11 and the minimum in genotype 18. Under heat stress, genotype 11 had the maximum root length followed by genotypes 8 and 4 (Table 85, p. 222). Similar to fresh root weight, root length decreased due to heat stress imposed at the seedling stage in the different wheat genotypes. However, when heat-stress genotypes are compared with the control, the maximum reduction of root length observed was in genotype 14 and the minimum effect of heat stress on root length was observed in genotypes 13, 18, and 1.

Effect of heat stress on plant height (cm). The interaction between treatment and genotypes for plant height was highly significant at $P < 0.05$. Maximum plant height under controled conditions was observed in genotype 8, followed by 23 and 5 and the minimum was recorded in genotype 17. With a heat-stress treatment, the maximum plant height

Table 81. Interaction between genotypes and treatment for chlorophyll b content at pre-anthesis. A heat stress of 40–45ºC was imposed 80 days after sowing for 10 days (LSD (0.05) = 0.006607).

Line	Control		Heat stress	
1	0.01	B	0.01	B
2	0.01	B	0.01	B
3	0.01	B	0.01	B
4	0.01	B	0.01	B
5	0.01	B	0.01	B
6	0.01	B	0.01	B
7	0.02	A	0.02	A
8	0.01	B	0.01	B
9	0.01	B	0.01	B
10	0.01	B	0.01	B
11	0.01	B	0.01	B
12	0.02	A	0.01	B
13	0.01	B	0.01	B
14	0.01	B	0.01	B
15	0.02	A	0.01	B
16	0.01	B	0.01	B
17	0.02	A	0.01	B
18	0.01	B	0.01	B
19	0.01	B	0.01	B
20	0.01	B	0.01	B
21	0.01	B	0.01	B
22	0.01	B	0.01	B
23	0.01	B	0.01	B
24	0.01	B	0.01	B

Table 82. Interaction between genotypes and treatments for fresh shoot weight (g). A heat stress of 25–30ºC was imposed 15 days after sowing for 10 days (LSD (0.05) =20.53).

Line	Control		Heat stress	
1	0.3480	B	0.3087	B
2	0.2907	B	0.1880	B
3	0.3043	B	0.2070	B
4	0.5570	B	0.4583	B
5	0.3317	B	0.2143	B
6	0.4377	B	0.2433	B
7	0.3437	B	0.3253	B
8	0.2970	B	0.2247	B
9	0.3127	B	0.2817	B
10	0.3400	B	0.2897	B
11	0.5083	B	0.4067	B
12	0.4073	B	0.3080	B
13	0.4290	B	0.2597	B
14	0.2060	B	0.1480	B
15	0.4220	B	0.2553	B
16	0.7447	B	0.2140	B
17	0.3293	B	0.3057	B
18	0.1753	B	0.0506	B
19	0.2431	B	0.0867	B
20	0.2459	B	0.0603	B
21	0.2700	B	0.1940	B
22	0.3806	B	0.1700	B
23	0.3316	B	0.2567	B
24	0.3212	B	0.2970	B

was observed in genotype 8 followed by 12 and 19 and the minimum was in genotype 3 (Table 86, p. 222). Heat stress imposed at pre-anthesis caused a significant decrease in plant height. The maximum effect of heat stress on plant height was observed in genotypes 21, 20, and 11 compared to the control. In genotype 21, heat stress imposition caused a reduction of 24% in plant height, followed by genotypes 20 and 10, and the minimum effect was observed in genotypes 17, 7, 12, 19, and 24 compared to the control. In genotype 17, no effect of heat stress on plant height compared to the control was observed, and genotypes 7 (1%) and 12 (2%) had a slight decrease compared to control. We concluded that heat stress imposed at pre-anthesis has minimum effect on plant height.

Effect of heat stress on spike length (cm). An analysis of variance revealed that the wheat genotypes significantly differed with respect to spike length at $P < 0.05$. Under normal conditions, the maximum spike length was observed in genotype 10 and the minimum in genotype 16. With a heat treatment, the maximum length observed was in genotype 7 followed by 17 and the minimum in genotype 23 (Table 87, p. 223). Heat stress significantly decreases the spike length in the different wheat genotypes. Maximum reduction in spike length was shown in genotype 21 (49%) compared to the control, and the minimum effect of heat stress was observed in genotypes 2, 4, 12, 19, 17, and 7 compared to the control. In these genotypes, heat stress effects ranged from 0 to 3%, indicating that heat stress imposed at pre-anthesis influences spike length.

Effect of heat stress on the number of spikelets/spike. Wheat genotypes were significantly different for number of spikelets/spike at $P < 0.05$. The maximum number of spikelets/spike under control conditions was in genotypes 1 and 3 followed by 2 and 4, the minimum number was in genotype 16. Genotype 1 produced the maximum number of spikelets/spike under heat stress, followed by genotypes 3 and 5, and the minimum number observed in genotype 21 (Table 88, p. 223). When compared with the control, the maximum reduction in the number of spikelets/spike was in genotype 22 (23%) and the minimum by genotype 7 followed by genotypes 17, 19, and 12, indicating that heat stress imposed at pre-anthesis causes a reduction in the number of spikelets/spike.

Table 83. Interaction between genotypes and treatment for fresh shoot length (cm) at the seedling stage. A heat stress of 25–30ºC was imposed 15 days after sowing for 10 days (LSD (0.05) = 6.746).

Line	Control		Heat stress	
1	28.6	ABCD	25.9	ABCDEF
2	28.8	ABCD	22.3	CDEFGHI
3	29.8	ABC	26.2	ABCDEF
4	29.5	ABC	28.4	ABCD
5	31.8	A	24.6	ABCDEFGH
6	27.9	ABCD	25.9	ABCDEF
7	30.0	ABC	28.8	ABCD
8	30.4	AB	23.7	BCDEFGHI
9	29.6	ABC	26.4	ABCDEF
10	28.5	ABCD	27.5	ABCD
11	21.3	DEFGHI	13.9	JK
12	27.6	ABCD	23.9	ABCDEFGH
13	30.7	AB	26.2	ABCDEF
14	24.6	ABCDEFGH	22.2	BCDEFGHI
15	30.5	AB	19.3	EFGHIJK
16	24.3	ABCDEFGH	16.7	HIJK
17	25.5	ABCDEF	25.2	ABCDEFG
18	18.6	FGHIJK	12.0	K
19	22.8	BCDEFGHI	17.3	GIJK
20	26.9	ABCDE	15.9	IJK
21	21.0	DEFGHIJ	21.0	DEFGHIJ
22	28.4	ABCD	17.1	HJK
23	30.4	AB	23.8	BCDEFGHI
24	28.1	ABCD	22.9	BCDEFGHI

Table 84. Interaction between genotypes and treatment for fresh root weight (g) at the seedling stage. A heat stress of 25–30ºC was imposed 15 days after sowing for 10 days (LSD (0.05) = 0.1722).

Line	Control		Heat stress	
1	0.1503	ABCDE	0.0663	DE
2	0.0510	DE	0.0443	E
3	0.0580	DE	0.0473	E
4	0.3107	AB	0.0750	DE
5	0.1777	ABCDE	0.0487	DE
6	0.0843	DE	0.0607	DE
7	0.1460	ABCDE	0.1402	ABCDE
8	0.1370	ABCDE	0.1030	CDE
9	0.1677	ABCDE	0.0953	CDE
10	0.1790	ABCDE	0.0810	DE
11	0.2533	ABCD	0.1250	BCDE
12	0.2103	ABCDE	0.1040	CDE
13	0.2523	ABCD	0.0903	CDE
14	0.2917	ABC	0.1257	BCDE
15	0.2003	ABCDE	0.1293	BCDE
16	0.0853	DE	0.0623	DE
17	0.0964	CDE	0.0897	CDE
18	0.0967	CDE	0.0431	E
19	0.3402	A	0.0620	DE
20	0.2920	ABC	0.0577	DE
21	0.1373	ABCDE	0.1007	CDE
22	0.0913	CDE	0.1428	ABCDE
23	0.1735	ABCDE	0.1420	ABCDE
24	0.1540	ABCDE	0.1213	BCDE

Effect of heat stress on the number of grains/spike. Heat significantly decreases the number of grains/spike. The analysis of variance revealed that wheat genotypes were not significantly different with respect to the number of seeds/spike at $P > 0.05$. Under controled conditions, the maximum number of grains/spike was in genotype 2 and the minimum in genotype 14. Under heat stress, the maximum number of grains/spike was observed in genotype 3 and minimum in genotype 14 (Table 89, p. 224). A minimum effect of heat stress on number of grains/spike was observed in genotypes 7, 12, 17, and 24, compared to the control, indicating that heat stress imposed at pre-anthesis reduces the number of grains/spike.

We concluded that heat stress imposed at pre-anthesis stage causes a decrease in yield by reducing the number of grains/spike.

Effect of heat stress on the number of florets/spike. The analysis of variance showed a significant interaction for the number of florets/spike between genotypes and treatments. The maximum number of florets/spike under control conditions was observed in genotype 1, followed by genotypes 2 and 4, and the minimum in genotype 19. The maximum number of number of florets/spike under hgeat stress was recorded in genotype 1, followed by genotypes 3 and 4, and the minimum number in genotype 11 (Table 90. p. 224). Heat stress at pre anthesis significantly decreases the number of florets/spike compared to the control treatment. The maximum reduction for number of florets/spike was in genotype 21 (34%) and the minimum in genotype 1, followed by 7, 17, and 19. These results indicate that a heat stress imposed at pre-anthesis reduces the number of florets/spike.

Effect of heat stress on biomass/plant. Heat stress imposed at pre-anthesis decreases plant biomass, and the interaction among the wheat genotypes and treatments for biomass/plant was highly significant. We observed that heat stress imposed at pre-anthesis growth stage significantly decreases biomass per plant at $P < 0.05$. Under the control conditions, the maximum biomass/plant was recorded in genotype 6 and minimum in genotype 18, under heat stress, the maximum

Table 85. Interaction between genotypes and treatment for fresh root length (cm) at the seedling stage. A heat stress of 25–30ºC was imposed 15 days after sowing for 10 days (LSD (0.05) = 3.891).

Line	Control		Heat stress	
1	5.1	BCD	5.1	BCD
2	6.8	ABCDE	4.6	BCD
3	7.4	ABCDE	4.5	BCDE
4	10.7	A	6.5	ABCDE
5	6.3	ABCDE	4.6	BCDE
6	6.1	ABCDE	4.7	BCDE
7	6.0	BCDE	3.8	DE
8	7.7	ABCDE	6.6	ABCDE
9	7.0	ABCDE	5.5	BCDE
10	6.2	ABCDE	5.3	BCDE
11	8.6	ABC	8.1	ABCD
12	7.2	ABCDE	5.9	BCDE
13	6.0	BCDE	6.0	BCDE
14	7.9	ABCDE	4.8	BCDE
15	4.2	CDE	3.3	E
16	4.8	BCDE	3.7	DE
17	6.3	ABCDE	4.2	CDE
18	3.6	DE	3.6	DE
19	5.0	BCDE	4.7	BCDE
20	5.5	BCDE	5.0	BCDE
21	9.0	AB	5.8	BCDE
22	6.5	ABCDE	4.4	BCDE
23	6.9	ABCDE	5.7	BCDE
24	7.3	ABCDE	5.2	BCDE

Table 86. Interaction between genotypes and treatment for plant height (cm) at pre-anthesis. A heat stress of 40–45ºC was imposed 80 days after sowing for 10 days (LSD (0.05) = 10.93).

Line	Control		Heat stress	
1	60.3	BCDEFGHIJK	54.0	EFGHIJKL
2	55.3	DEFGHIJKL	51.0	IJKL
3	59.0	CEFGHIJKL	48.0	KL
4	63.0	BCDEFGHI	60.3	BCDEFGHIJK
5	68.6	ABC	58.3	BCDEFGHIJKL
6	66.6	ABCDE	59.3	BCDEFGHIJKL
7	66.0	ABCDEFG	65.3	ABCDEFG
8	76.3	A	68.0	ABCD
9	53.0	GHIJKL	48.3	KL
10	66.0	ABCDEFG	52.0	HIJKL
11	66.3	ABCDEF	51.6	HIJKL
12	64.6	ABCDEFGH	63.6	ABCDEFGHI
13	59.3	CDEFGHIJKL	53.3	FGHIJKL
14	51.0	IJKL	49.6	JKL
15	58.0	DEFGHIJKL	49.3	JKL
16	63.3	BCDEFGHI	55.0	DEFGHIJKL
17	46.6	L	46.6	L
18	60.0	BCDEFGHIJK	52.0	HIJKL
19	65.8	ABCDEFG	62.3	BCDEFGHIJ
20	62.0	BCDEFGHIJ	48.0	KL
21	68.0	ABCD	52.0	HIJKL
22	63.3	BCDEFGHI	54.6	EFGHIJKL
23	71.0	AB	60.5	BCDEFGHIJK
24	60.6	BCDEFGHIJK	57.0	CDEFGHIJKL

was in genotype 8 and the minimum in genotype 18 (Table 91, p. 224). The maximum decrease in biomass/plant was found in genotype 18 (48%) and the minimum was observed in genotypes 7, 17, 12, and 24, compared to the control.

Effect of heat stress on 100-kernel weight. Heat stress imposed at pre-anthesis stage significantly decreases 100-kernel weight at $P < 0.05$. Under controled conditions, the maximum 100-kernel weight was observed in genotype 2 and the minimum in genotype 22. Under heat stress, the maximum 100-kernel weight was recorded in genotype 17 and the minimum in genotype 18 (Table 92, p. 225). Heat stress significantly decreased 100-kernel weight in the different wheat genotypes. Genotype 18 showed the maximum reduction (36%) due to high temperature imposed at pre-anthesis and genotypes 7, 12, 17, and 19 the minimum compared to the control. These results indicate that a heat stress imposed at pre-anthesis causes reduction in crop yield.

Identification of heat-shock proteins. ***Effect of heat-stress treatment on the change in the protein banding patterns of various wheat genotypes.*** Changes in the protein patterns were observed in wheat grown under control and heat-stressed conditions at the seedling stage of the wheat genotypes (Table 93, p. 225). Proteins extracted from the leaves were separated by SDS–PAGE. The appearance or disappearance of proteins was identified visually in control and heat stressed plants. The position of the proteins bands against those of known molecular weight markers were carefully measured with a ruler. The soluble protein from the leaves revealed the presence of 55-kDa polypeptides in both control and heat-stress treatments of all the ten genotypes except the genotype 8 control, and a 43-kDa band appeared in ten genotypes in both control and heat-stressed plants. A 72-kDa band appears under heat stress in nine genotypes, but the same polypeptide band was present in genotype 10 in both control and heat-stressed plants (Table 93, p. 225). However, a new protein of 35 kDa appeared in genotype 7 only.

Table 87. Interaction between genotypes and treatment for spike length (cm) at pre-anthesis. A heat stress of 40–45ºC was imposed 80 days after sowing for 10 days (LSD (0.05) = 2.083).

Line	Control		Heat stress	
1	10.6	ABCDE	9.3	BCDEFGH
2	9.6	ABCDEFG	9.6	ABCDEFG
3	11.0	ABCD	8.3	EFGH
4	10.0	ABCDEF	10.0	ABCDEF
5	10.0	ABCDEF	9.3	BCDEFGH
6	10.6	ABCDE	9.3	BCDEFGH
7	11.6	ABC	11.3	ABCDE
8	10.6	ABCDE	10.3	ABCDE
9	9.0	CDEFGH	8.6	DEFGH
10	12.0	A	9.3	BCDEFGH
11	10.6	ABCDE	9.0	CDEFGH
12	9.6	ABCDEFG	9.6	ABCDEFG
13	11.0	ABCD	8.6	DEFGH
14	9.6	ABCDEFG	8.6	DEFGH
15	9.3	BCDEFGH	9.0	CDEFGH
16	8.3	EFGH	7.6	FGH
17	11.0	AB	10.8	ABCDEF
18	9.6	ABCDEFG	9.3	BCDEFGH
19	9.3	BCDEFGH	9.3	BCDEFGH
20	9.6	ABCDEFG	8.3	EFGH
21	10.3	ABCDE	5.3	I
22	9.3	BCDEFGH	7.3	GHI
23	9.3	BCDEFGH	7.0	HI
24	10.6	ABCDE	9.3	BCDEFGH

Table 88. Interaction between genotypes and treatment for number of spikelets/spike at pre-anthesis. A heat stress of 40–45ºC was imposed 80 days after sowing for 10 days (LSD (0.05) = 3.439).

Line	Control		Heat stress	
1	22.3	A	21.6	AB
2	21.6	AB	20.3	AB
3	22.3	A	21.0	AB
4	21.3	AB	20.3	AB
5	21.0	AB	20.3	AB
6	21.6	AB	21.0	AB
7	20.3	AB	20.3	AB
8	19.6	ABC	17.6	BCDE
9	19.6	ABC	17.6	BCDE
10	21.0	AB	19.0	ABCD
11	20.3	AB	14.3	EF
12	19.6	ABC	19.0	ABCD
13	19.6	ABC	18.3	ABCDE
14	19.6	ABC	17.6	BCDE
15	20.3	AB	19.6	ABC
16	17.6	BCDE	15.6	CDEF
17	18.3	ABCDE	18.3	ABCDE
18	18.3	ABCDE	17.6	BCDE
19	17.6	BCDE	17.6	BCDE
20	21.0	AB	19.0	ABCD
21	19.6	ABC	13.0	F
22	19.6	ABC	15.0	DEF
23	18.3	ABCDE	14.3	EF
24	19.0	ABCD	15.6	CDEF

In all ten genotypes 11–20, SDS-PAGE analysis of the soluble protein from leaves revealed that polypeptides of 26 kDa, 43 kDa, and 55 kDa appeared during both control and heat-stress conditions. Similarly, a protein band of 55 kDa also appeared during both control and heat stress in the four wheat genotypes 21–24, whereas band of 72 kDa appeared in genotypes 21 and 22 under both control and heat stress plants. A new 20-kDa protein appeared in genotype 17 and a new 25-kDa protein band appeared in genotype 24 under heat stress treatment at the seedling stage.

Genetic diversity in wheat genotpes using SSR markers. A set of 50 simple sequence repeat (SSR) primers was used to detect genetic diversity at DNA level in the 24 wheat genotypes. Using the discrimination among genotypes based on these SSR markers, a dendrogram was prepared using Nei and Lei co-efficient. In order to elucidate genetic diversity at each locus, the polymorphic information content (PIC) value was calculated (Table 94, p. 226). The highest PIC value was 0.87 exhibited by D genome, whereas the lowest PIC value of 0 was shown by the A and D genomes.

A total of 179 alleles were detected in the 24 wheat genotypes using 50 pairs of primers that produced clearly polymorphic fragments. The maximum number of alleles (10) were amplified by primer WMC-42 and the minimum (1) by WMC-15, WMC-17, WMC-22, WMC-23, and WMC-26. Most of the SSR loci in the wheat genotypes contained dinucleotide repeats, with a much smaller fraction containing trinucleotide repeats. The CA repeat was the most common type.

The mean PIC values among the genomes were 0.38 (A), 0.51 (B), and 0.40 (D). The highest PIC values were 0.87 (WMC-42) on chromosome 7D, 0.84 (WMC-31) on 1B, and 0.83 (WMC-45) on 3A. In general, the variance of the PIC mean values was similar among the A, B, and D genomes with B > D > A.

Table 89. Interaction between genotypes and treatments for number of grains/spike at pre-anthesis. A heat stress of 40–45ºC was imposed 80 days after sowing for 10 days (LSD (0.05) = 19.59).

Line	Control		Heat stress	
1	44.0	ABCD	18.0	GHIJKL
2	53.6	A	40.6	ABCDEFGH
3	49.6	AB	45.6	ABC
4	51.6	A	39.6	ABCDEFGH
5	34.0	ABCDEFGHI	32.0	ABCDEFGHIJ
6	49.3	AB	45.3	ABC
7	48.2	ABCDEFG	47.2	ABCDE
8	38.0	ABCDEFGHI	21.3	DEFGHIJKL
9	36.0	ABCDEFGHI	32.0	ABCDEFGHIJ
10	41.6	ABCDEF	25.6	CDEFGHIJK
11	20.3	EFGHIJKL	20.3	EFGHIJKL
12	41.3	ABCDEFG	40.3	ABCDEF
13	34.0	ABCDEFGHI	17.6	HIJKL
14	2.0	L	1.6	L
15	44.0	ABCD	33.0	ABCDEFGHI
16	9.0	JKL	5.0	KL
17	38.0	ABCDEFGHI	37.0	ABCDEFGH
18	42.0	ABCDE	23.6	CDEFGHIJKL
19	32.6	ABCDEFGHI	26.6	BCDEFGHIJK
20	39.0	ABCDEFGH	18.3	FGHIJKL
21	25.3	CDEFGHIJK	18.6	FGHIJKL
22	23.3	CDEFGHIJKL	15.3	IJKL
23	23.0	CDEFGHIJKL	17.6	HIJKL
24	34.3	ABCDEFGHI	33.0	ABCDEFGHI

Table 90. Interaction between genotypes and treatments for the number of florets/spike at pre-anthesis. A heat stress of 40–45ºC was imposed 80 days after sowing for 10 days (LSD (0.05) = 10.30).

Line	Control		Heat stress	
1	67.0	A	65.0	AB
2	65.0	AB	61.0	AB
3	67.0	A	63.0	AB
4	63.0	AB	61.0	AB
5	63.0	AB	61.0	AB
6	65.0	AB	63.0	AB
7	61.0	AB	61.0	AB
8.	59.0	ABC	53.0	BCDE
9	59.0	ABC	53.0	BCDE
10	63.0	AB	57.0	ABCD
11	61.0	AB	43.0	EF
12	59.0	ABC	57.0	ABCD
13	59.0	ABC	55.0	ABCDE
14	59.0	ABC	53.0	BCDE
15	61.0	AB	59.0	ABC
16	53.0	BCDE	47.0	CDEF
17	55.0	ABCDE	55.0	ABCDE
18	55.0	ABCDE	53.0	BCDE
19	53.0	BCDE	53.0	BCDE
20	63.0	AB	57.0	ABCD
21	59.0	ABC	39.0	F
22	59.0	ABC	45.0	DEF
23	55.0	ABCDE	46.3	DEF
24	57.0	ABCD	47.0	CDEF

Table 91. Interaction between genotypes and treatments for biomass/plant at pre-anthesis. A heat stress at 40–45ºC was imposed 80 days after sowing for 10 days (LSD (0.05) = 0.7514).

Line	Control		Heat stress		Line	Control		Heat stress	
1	4.4	BCDEFG	4.0	DEFGHI	13	4.9	ABC	3.5	HIJKL
2	4.3	CDEFGH	3.2	IJKLM	14	4.8	ABCD	3.6	GHIJKL
3	3.2	IJKLM	2.5	MN	15	4.3	CDEFGH	3.6	GHIJKL
4	3.9	EFGHIJ	3.5	HIJKL	16	3.9	EFGHIJ	3.0	KLM
5	3.8	EFGHIJK	3.6	HIJKL	17	4.7	ABCDE	4.5	BCDEF
6	5.2	AB	4.4	BCDEFG	18	4.0	DEFGHI	2.1	N
7	3.8	EFGHI	3.6	HIJKL	19	3.1	JKLM	2.9	LM
8	5.4	A	4.6	ABCDEF	20	4.3	CDEFGH	3.6	GHIJKL
9	4.6	ABCDEF	4.3	CDEFGH	21	5.0	ABC	4.3	CDEFGH
10	3.6	GHIJKL	3.2	IJKLM	22	3.9	EFGHIJ	3.4	IJKL
11	4.7	ABCDE	3.4	IJKL	23	3.8	FGHIJK	3.4	IJKL
12	4.6	ABCDEF	4.4	BCDEFG	24	3.8	FGHIJK	3.6	GHIJKL

Conclusion. On the basis of yield, physiological, and molecular attributes, wheat genotypes 7 and 17 were tolerant to high temperature. Genotypes 7 and 17 exhibited better osmoregulation by accumulation of compatible solutes, such as prolines and induced heat-shock proteins. High-temperature stress triggered an antioxidant defense mechanism in these two cultivars that helped them survive under heat stress. An urgent need is to fully explore the genetic diversity among the present wheat germ plasm in field under heat stress. The information generated in this investigation will be helpful for the plant breeders when including these traits in a breeding program for

Table 92. Interaction between genotypes and treatments for 100-kernel weight at pre-anthesis. A heat stress of 40–45ºC was imposed 80 days after sowing for 10 days (LSD (0.05) = 0.4384).

Line	Control		Heat stress		Line	Control		Heat stress	
1	3.693	ABCD	2.432	JKL	13	3.307	BCDEF	3.140	DEFGH
2	3.913	A	2.643	HIJKL	14	3.680	ABCD	2.757	GHIJKL
3	3.640	ABCD	2.467	JKL	15	3.617	ABCD	2.947	EFGHIJ
4	3.850	AB	2.650	HIJKL	16	3.127	DEFGH	2.870	FGHIJK
5	3.140	DEFGH	2.667	HIJKL	17	3.600	ABCDE	3.500	ABCDE
6	3.293	CDEFG	2.183	L	18	3.427	ABCDE	2.121	L
7	3.497	ABCDE	3.383	ABCDE	19	3.440	ABCDE	3.402	ABCDE
8	3.413	ABCDE	3.070	EFGH	20	3.407	ABCDE	2.780	GHIJK
9	3.150	DEFGH	2.463	JKL	21	3.150	DEFGH	2.397	KL
10	3.317	BCDEF	2.413	JKL	22	3.090	DEFGH	2.377	KL
11	3.777	ABC	2.543	IJKL	23	3.640	ABCD	2.790	GHIJK
12	3.227	DEFG	3.100	DEFGH	24	3.163	DEFG	3.005	EFGHI

Table 93. Distribution pattern of protein bands in wheat genotypes under control and heat stress conditions at the seedling stage (+ = band present, – = band absent).

	Marker (kDa)	Genotype 1	2	3	4	5	6	7	8	9	10
Control	130	–	–	–	–	–	–	–	–	–	–
	95	–	–	–	–	–	–	–	–	–	–
	72	–	–	–	–	–	–	–	–	–	+
	55	+	+	+	+	+	+	+	–	+	+
	43	+	+	+	+	+	+	+	+	+	+
	34	–	–	–	–	–	–	–	–	–	–
Heat stress	130	–	–	–	–	–	–	–	–	–	–
	95	–	–	–	–	–	–	–	–	–	–
	72	+	+	+	+	+	+	+	+	+	+
	55	+	+	+	+	+	+	+	+	+	+
	43	+	+	+	+	+	+	+	+	+	+
	34	–	–	–	–	–	–	–	–	–	–

	Marker (kDa)	Genotype 11	12	13	14	15	16	17	18	19	20
Control	72	–	–	–	–	–	–	–	–	–	–
	55	+	+	+	+	+	+	+	+	+	+
	43	+	+	+	+	+	+	+	+	+	+
	34	–	–	–	–	–	–	–	–	–	–
	26	+	+	+	+	+	+	+	+	+	+
	17	–	–	–	–	–	–	–	–	–	–
Heat stress	72	–	–	–	–	–	–	–	–	–	–
	55	+	+	+	+	+	+	+	+	+	+
	43	+	+	+	+	+	+	+	+	+	+
	34	–	–	–	–	–	–	–	–	–	–
	26	+	+	+	+	+	+	+	+	+	+
	17	–	–	–	–	–	–	–	–	–	–

	Marker (kDa)	Genotype 21	22	23	24
Control	95	–	–	–	–
	72	+	+	–	–
	55	+	+	+	+
	43	–	–	–	–
	34	–	–	–	–
	26	–	–	–	–
	17	–	–	–	–
Heat stress	95	–	–	–	–
	72	+	+	–	–
	55	+	+	+	+
	43	–	–	–	–
	34	–	–	–	–
	26	–	–	–	–
	17	–	–	–	–

the development of heat-tolerant wheat cultivars. Genotypes 7 and 17 should be grown in the warmer areas to obtain economic yield.

Table 94. Genetic diversity among the A, B, and D genomes. The number of alleles and polymorphic information content (PIC) detected in 24 wheat genotypes are presented.

Genome	Line	Primer	Locus designation	Alleles amplified	PIC value
A	1	WMC-9	*Xwmc*9-1A	5	0.68
	2	WMC-11	*Xwmc* 11-3A	4	0.62
	3	WMC-13	*Xwmc* 13-7A	2	0.19
	4	WMC-15	*Xwmc* 15-4A	1	0.00
	5	WMC-17	*Xwmc* 17-7A	1	0.00
	6	WMC-21	*Xwmc* 21-6A	3	0.40
	7	WMC-24	*Xwmc* 24-1A	6	0.72
	8	WMC-29	*Xwmc* 29-7A	3	0.26
	9	WMC-30	*Xwmc* 30-5A	2	0.04
	10	WMC-33	*Xwmc* 33-6A	2	0.08
	11	WMC-39	*Xwmc* 39-1A	4	0.62
	12	WMC-40	*Xwmc* 40-4A	3	0.62
	13	WMC-45	*Xwmc* 45-3A	7	0.83
B	1	WMC-1	*Xwmc* 1-3B	3	0.32
	2	WMC-3	*Xwmc*3-4B	5	0.37
	3	WMC-5	*Xwmc* 5-5B	3	0.16
	4	WMC-7	*Xwmc* 7-3B	3	0.43
	5	WMC-10	*Xwmc* 10-7B	6	0.73
	6	WMC-16	*Xwmc* 16-4B	2	0.50
	7	WMC-19	*Xwmc* 19-6B	3	0.40
	8	WMC-20	*Xwmc* 20-7B	4	0.53
	9	WMC-27	*Xwmc* 27-2B	5	0.73
	10	WMC-28	*Xwmc* 28-5B	4	0.61
	11	WMC-31	*Xwmc* 31-1B	8	0.84
	12	WMC-35	*Xwmc* 35-2B	5	0.74
	13	WMC-37	*Xwmc* 37-7B	3	0.65
	14	WMC-44	*Xwm44*-1B	4	0.73
	15	WMC-46	*Xwmc* 46-2B	3	0.59
	16	WMC-53	*Xwmc* 53-4B	3	0.16
	17	WMC-54	*Xwmc* 54-3B	3	0.58
	18	WMC-55	*Xwmc55* -1B	2	0.5
	19	WMC56	*Xwmc56*-1B	2	0.16
	20	WMC-57	*Xwmc* 57-1B	2	0.48
D	1	WMC-4	*Xwmc* 4-3D	2	0.09
	2	WMC-6	*Xwmc* 6-5D	3	0.16
	3	WMC-8	*Xwmc* 8-4D	6	0.76
	4	WMC-14	*Xwmc* 14-7D	5	0.68
	5	WMC-18	*Xwmc* 18-2D	3	0.45
	6	WMC-22	*Xwmc* 22-5D	1	0.00
	7	WMC-23	*Xwmc* 23-6D	1	0.00
	8	WMC-25	*Xwmc*25- 2D	5	0.75
	9	WMC-26	*Xwmc* 26-1D	5	0.00
	10	WMC-32	*Xwmc* 32-5D	4	0.60
	11	WMC-34	*Xwmc*34- 6D	2	0.05
	12	WMC-38	*Xwmc* 38-7D	6	0.74
	13	WMC-41	*Xwmc* 41-2D	5	0.56
	14	WMC-42	*Xwmc* 42-7D	10	0.87
	15	WMC-43	*Xwmc* 43-3D	3	0.63
	16	WMC-58	*Xwmc* 58-7D	2	0.18

Salinity tolerance potential of some durum cultivars and their derived D-genome synthetic hexaploid wheats.

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Among the agricultural crops, wheat is an extremely important source of food for human beings. Wheat originated about 10,000 years ago in the Fertile Crescent, one of the most diversified region in the world, comprising a wide array of habitats. Bread wheat originated about 8,000 years ago by hybridization of a tetraploid *T. turgidum* species with diploid donor of D genome, *Ae. tauschii*. The A and B genomes were most likely provided by *T. tugidum* itself, presumably formed from the wild diploid *T. urartu* (A genome) and the donor of B genome *Ae. speltoides*.

Bread wheat is moderately tolerant to the stress and durum wheat is more susceptible. One reason for this is absence of trait for enhanced K^+:Na^+ discrimination in durum wheat, which is carried on long arm of chromosome 4D in bread wheat and is also present in D-genome ancestors of wheat. The D genome of *Ae. tauschii* is homologous to the D genome of bread wheat. For wheat improvement, one route is therefore via bridge crosses that utilize synthetic hexaploids (SH), which are produced by crossing *T. turgidum* (2n=4x=28, AABB) with *Ae. tauschii* (2n=2x=14, DD). *T. turgidum* is generally used as a female parent. The result of the cross is an F_1 hybrid with 21 chromosomes (ABD), which are doubled with a colchicine treatment to produce 42 chromosomes SH (AABBDD) wheats. The hexaploid formation also could be spontaneous.

Over 1,000 new SH wheats have been produced from more than 600 *Ae. tauschii* accessions at CIMMYT, Mexico. The SHs are agronomically poor, difficult to thresh, generally tall, low yielding, and frequently have poor quality. However, they do carry useful and new variation for a range of economically important characters. Potentially, new genetic variation among primary synthetics also have been found for tolerance to drought and salinity. These primary synthetics have been crossed to adapted wheat and agronomically improved materials have been developed with superior yield performance compared to check cultivars under stress.

Salinity is a world-wide problem for many crops, including wheat, and it reduces yield. The yield reduction is due to disturbed metabolic processes. Despite reasonable work done in this regard, the mechanism of salinity tolerance is still not yet fully explored and abundance of genetic diversity remains elusive.

Table 95. Pedigrees of wheat synthetic hexaploid (SH) lines.

SH #	Pedigree
4	CETA/*Ae. tauschii* (540)
5	D67.2/P66.270//*Ae. tauschii* (213)
6	GARZA/BOY//*Ae. tauschii* (268)
10	D67.2/P66.270//*Ae. tauschii* (308)
11	CETA /*Ae. tauschii* (1016)
12	D67.2/P66.270//*Ae. tauschii* (221)
13	DVERD_2/*Ae. tauschii* (1027)
14	68.111/RGB-U//WARD/3/*Ae. tauschii* (329)
15	GARZA/BOY//*Ae. tauschii* (467)
16	DVERD_2/*Ae. tauschii* (221)
17	DVERD_2/*Ae. tauschii* (214)
20	CETA/*Ae. tauschii* (327)
21	D67.2/P66.270//*Ae. tauschii*
28	CPI/GEDIZ/3/GOO//JO6/CRA/4/*Ae. tauschii* (215)
30	ALTAR 84/*Ae. tauschii* (333)
32	GAN/*Ae. tauschii* (182)
35	CETA /*Ae. tauschii* (661)
36	DVERD_2/*Ae. tauschii* (402)
37	CETA /*Ae. tauschii* (174)
38	CETA /*Ae. tauschii* (1024)
39	CROC_1/*Ae. tauschii* (886)
40	CROC_1/*Ae. tauschii* (444)
41	CROC_1/*Ae. tauschii* (518)
42	CETA /*Ae. tauschii* (256)
43	6.111/RGB-U//WARD/3/*Ae. tauschii* (325)
44	DOY 1/*Ae. tauschii* (188)
46	DVERD_2/*Ae. tauschii* (1022)
48	GAN/*Ae. tauschii* (236)
52	ALTAR 84/*Ae. tauschii* (332)
53	GAN/*Ae. tauschii* (180)
54	DOY 1/*Ae. tauschii* (255)
58	D67.2/P66.270//*Ae. tauschii* (217)
60	CROC_1/*Ae. tauschii* (170)
61	DVERD_2/*Ae. tauschii* (1031)
62	CROC_1/*Ae. tauschii* (213)
63	ALTAR 84/*Ae. tauschii* (304)
66	ALTAR 84/*Ae. tauschii* (507)
68	GAN/*Ae. tauschii* (163)
72	GAN/*Ae. tauschii* (201)
76	GAN/*Ae. tauschii* (285)
77	DOY 1/*Ae. tauschii* (333)
78	ALTAR 84/*Ae. tauschii* (219)
79	CPI/GEDIZ/3/GOO//JO6/CRA/4/*Ae. tauschii* (208)
80	DOY 1/*Ae. tauschii* (1030)
81	DOY 1/*Ae. tauschii* (515)
82	CPI/GEDIZ/3/GOO//JO6/CRA/4/*Ae. tauschii* (637)
83	ALTAR 84/*Ae. tauschii* (502)
84	DOY 1/*Ae. tauschii* (517)
85	CROC_1/*Ae. tauschii* (224)
86	GAN/*Ae. tauschii* (890)
87	DOY 1/*Ae. tauschii* (458)
88	DVERD_2/*Ae. tauschii* (1029)
89	ALTAR 84/*Ae. tauschii* (211)
90	CROC_1/*Ae. tauschii* (879)

Screening for salt stress has been done at germination and at the adult-plant stage, because tolerance varies at both stages. Keeping in view the economic characteristics of wheat, we plan the following research work to screen for salinity tolerant lines: explore the salinity tolerance potential of some durum cultivars and their derived D-genome SHs and evaluate the performance of different *Ae. tauschii* accessions in similar durum backgrounds for which ten groups are to be studied.

Germ plasm. From the set of SH lines, germ plasm was selected for estimating the salt-tolerance potential where the study structure was classed in to same durum with diverse *Ae. tauschii* accessions. Ten such groups were utilized with 54 entries (Table 95, p. 227). Seed of the 54 lines was provided by Laboratory of Wheat Wide Crosses and Cytogenetics, NARC, Islamabad. Two salt-tolerant, bread wheat checks, Shorawaki and Kharchia 65, and one salt-susceptible durum cultivar, PDW 34, also were evaluated for their morphological and physiological attributes.

A hydroponic experiment was conducted at control (0 mM NaCl) and stress (75 mM NaCl) conditions to evaluate the performance of the SHs. All 54 lines were subjected to chlorophyll analysis, sugar analysis, shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, and root dry weight at control and stress levels. The K^+:Na^+ discrimination was observed at 75 mM NaCl. The ten best lines were selected at 75 mM on the basis of their K^+:Na^+ discrimination, shoot dry weight, chlorophyll, sugar content, and fresh and dry weight. These ten lines were then further analysed for SOD, protein, proline, shoot dry weight, and K^+:Na^+ at 100 $molm^{-3.}$

An analysis of variance was made of total chlorophyll content, sugar content, and K^+:Na^+ ratio of the 54 wheat synthetic lines along with the two check cultivars, Shorawaki and PDW34, grown under control and stress conditions (Table 96). The stress treatment had significant ($P \leq 0.05$) adverse effects on total chlorophyll content and sugar content increases significantly. The genotypes also differed significantly from each other for both parameters, but the 'treatment × genotype' interactions were not significant for these parameters. There was a significant difference in the genotypes for K^+:Na^+ ratio.

Table 96. Analysis of variance summaries (mean squares) for total chlorophyll content (mg\g), sugar content (mg\g), and K^+:Na^+ ratio of 54 synthetic wheat lines grown at two salinity levels, 0 mM and 75 mM NaCl (* = significant at the 0.05 level; NS = not significant).

Source of variation	df	Total chlorophyll (mg/g)	df	Sugar content (mg/g)	df	K^+:Na^+ discrimination
Treatment	1	7.264*	1	4.82*		
Genotype	55	1.047*	55	0.64*	55	4.75*
Treatment × Genotype	55	0.066 NS	55	0.12 NS		
Error	449	0.082	222	0.14	220	0.27

Results of the basic, descriptive statistics applied on the 54 synthetic wheat lines at both control (0 mM) and stress levels (75 mM NaCl) indicate that there is a significant difference in morphological, physiological, and biochemical attributes of genotypes at both conditions. The mean value of total chlorophyll content of the 54 SH lines under control conditions was 1.111 mg/g (0.208–2.698 mg/g), whereas the mean value the stress level was 0.884 mg/g. The total chlorophyll decrease in saline conditions ranging from 0.111 to 1.889 mg/g. The skewness value was 0.486 under control (0 mM) and 0.292 under stress (75 mM NaCl) conditions. Heritability for total chlorophyll was 0.72 under control and 0.81 under stress conditions (Table 97).

Table 97. Evaluation of 54 synthetic wheat lines for physiological traits under saline and nonsaline conditions.

Parameter	Salt concentration	Mean	S.D	Minimum	Maximum	Skewness	h^2
Total chlorophyll (mg/g)	0 mM	1.111	0.338	0.208	2.698	0.486	0.72
	75 mM	0.884	0.326	0.111	1.889	0.292	0.81
Sugar (mg/g)	0 mM	0.776	0.373	0.137	2.207	1.078	0.64
	75 mM	0.996	0.478	0.069	3.203	0.986	0.57
K^+:Na^+	75 mM	1.338	0.962	0.234	5.652	1.736	0.93

A significant decrease in chlorophyll content in saline conditions is observed, decreasing from 2.698 mg/g to 1.889 mg/g. Genotypes showed a higher skewness value for total chlorophyll at the control conditions (0.486) than at saline conditions (0.292). A positive skewness value showed that the performance of most of the genotypes for this trait was equal to or greater than the mean value. Heritiability for total chlorophyll was greater in stress condition (0.81) than in control condition (0.72) (Table 97, p. 228).

The mean performance of the 54 SH lines for sugar content at the control conditions was 0.776 mg/g, and the range was 0.137 mg/g to 2.207 mg/g. The skewness value in nonsaline conditions was 1.078. Heritability for sugar content at control conditions was 0.641. Sugar content increased under stressed conditions. The mean value of sugar content was 0.996 mg/g (0.069–3.203 mg/g) under saline conditions. Skewness in the genotypes was 0.986 and heritability was 0.577 in saline conditions (Table 97, p. 228).

Sugar content increases significantly in saline conditions; from 0.137 mg/g under control and 3.203 mg/g under saline conditions. A positive skewness value is indicative of the fact that it is equal to or greater than the mean value. The skewness is less in saline conditions than in the control.

The mean value of K^+:Na^+ ratio at stressed condition was 1.33. The K^+:Na^+ ratio ranged from 0.23 to 5.65 in genotypes under stress conditions. The skewness of the K^+:Na^+ ratio was 1.73 and kurtosis was 3.44. The positive value of skewness indicated that most of the lines had a value equal to or greater than the average. The heritability under salt stress conditions was 0.93, which indicates that it can be used as a criteria for selection for salinity tolerance (Table 97, p. 228).

The mean data for total chlorophyll content, sugar content, and K^+:Na^+ discrimination of the 54 wheat synthetic lines and the salt-tolerant and salt-susceptible checks gives a clear picture that salt stress caused a significant reduction in total chlorophyll content in the SHs (Table 98). The highest total chlorophyll content was found in SH-16 (1.42 mg/g), followed by SH-20 (1.28 mg/g). Shorawaki had a total chlorophyll content of 2.75 mg/g. The lowest value for total chlorophyll content was in SH-39 (0.44 mg/g) and SH-41 (0.50 mg/g); all other synthetic lines had intermediate performance.

Sugar content increased under salt stress (Table 98). The maximum sugar

Table 98. Means of total chlorophyll content, sugar content, and K^+:Na^+ ratio of 54 synthetic wheat lines grown at two salinity levels, 0 mM NaCl and 75 mM NaCl.

	Chlorophyll (mg/g)		Sugar (mg/g)		K^+:Na^+
Genotype	0 mM	75 mM	0 mM	75 mM	75 mM
SH-4	1.11±0.13	1.08±0.03	1.14±0.36	1.27±0.23	1.00±0.16
SH-5	0.88±0.15	0.65±0.12	0.70±0.12	0.73±0.03	0.64±0.04
SH-6	0.77±0.20	0.61±0.06	1.20±0.33	1.28±0.41	0.59±0.06
SH-10	1.42±0.26	0.86±0.08	0.97±0.21	1.12±0.15	0.59±0.06
SH-11	0.96±0.21	0.63±0.04	1.21±0.50	1.27±0.07	0.95±0.07
SH-12	1.09±0.15	0.93±0.04	0.78±0.06	0.90±0.04	0.78±0.25
SH-13	1.32±0.07	0.74±0.15	0.33±0.03	1.67±0.39	0.37±0.07
SH-14	0.96±0.11	0.81±0.21	0.65±0.07	0.81±0.09	0.51±0.09
SH-15	1.15±0.21	1.02±0.12	0.84±0.23	0.94±0.13	0.49±0.04
SH-16	1.55±0.18	1.42±0.09	1.17±0.27	1.34±0.19	3.83±0.37
SH-17	1.04±0.03	0.96±0.17	0.68±0.12	1.41±0.40	0.94±0.12
SH-20	1.35±0.08	1.28±0.22	1.15±0.31	1.67±0.78	0.59±0.07
SH-21	1.26±0.08	1.11±0.17	0.87±0.10	0.91±0.05	0.61±0.04
SH-28	0.94±0.14	0.83±0.16	0.97±0.20	1.06±0.34	0.68±0.07
SH-30	1.37±0.10	0.80±0.12	1.04±0.14	1.12±0.13	0.91±0.07
SH-32	1.15±0.04	0.66±0.04	1.09±0.12	1.12±0.09	0.64±0.06
SH-35	1.46±0.10	1.22±0.16	1.20±0.23	1.30±0.15	0.62±0.07
SH-36	1.40±0.07	1.19±0.24	1.00±0.06	1.10±0.04	0.71±0.11
SH-37	1.51±0.29	1.24±0.10	0.88±0.18	1.14±0.05	0.62±0.07
SH-38	1.40±0.19	0.73±0.32	0.52±0.05	0.80±0.11	0.48±0.04
SH-39	0.63±0.04	0.44±0.04	0.39±0.10	0.41±0.03	2.03±0.22
SH-40	0.63±0.05	0.511±0.04	0.83±0.09	1.04±0.07	2.53±0.18
SH-41	0.78±0.03	0.50±0.18	0.62±0.18	0.85±0.45	2.51±0.28
SH-42	0.85±0.07	0.62±0.02	0.89±0.08	1.07±0.11	3.01±0.15
SH-43	0.85±0.09	0.67±0.05	0.95±0.13	1.22±0.10	3.00±0.43
SH-44	1.10±0.11	0.52±0.02	0.82±0.08	1.20±0.11	1.32±0.16
SH-46	0.86±0.10	0.58±0.03	0.47±0.03	1.51±0.23	2.26±0.24
SH-48	0.87±0.06	0.61±0.07	0.33±0.05	0.64±0.02	1.77±0.20
SH-52	1.03±0.04	0.57±0.07	0.39±0.13	0.50±0.25	1.88±0.46
SH-53	1.17±0.16	1.10±0.20	0.96±0.10	1.10±0.10	2.00±0.14
SH-54	1.16±0.16	1.02±0.06	0.50±0.09	0.69±0.04	1.53±0.10
SH-58	1.20±0.07	0.94±0.11	0.75±0.19	0.83±0.26	1.40±0.09
SH-60	0.96±0.09	0.84±0.07	0.72±0.08	0.88±0.13	1.73±0.17
SH-61	1.15±0.04	0.99±0.08	0.50±0.08	1.06±0.33	1.15±0.21
SH-62	0.94±0.08	0.76±0.07	0.94±0.26	1.07±0.06	1.03±0.08
SH-63	1.21±0.07	0.84±0.19	0.95±0.18	1.01±0.40	1.11±0.10
SH-66	1.15±0.07	0.93±0.07	0.63±0.05	1.55±0.22	1.10±0.12
SH-68	1.40±0.08	0.84±0.11	0.68±0.08	0.71±0.05	0.85±0.09
SH-72	1.06±0.05	0.92±0.09	0.54±0.06	0.56±0.17	0.74±0.08
SH-76	0.93±0.06	0.76±0.03	0.80±0.07	0.98±0.66	1.46±0.66
SH-77	1.07±0.13	0.68±0.07	0.39±0.06	0.59±0.25	0.83±0.16
SH-78	1.23±0.09	1.20±0.08	0.81±0.17	1.20±0.24	3.04±0.33
SH-79	1.22±0.09	1.03±0.10	0.78±0.19	1.14±0.49	1.26±0.19
SH-80	1.29±0.11	1.07±0.10	0.50±0.08	0.54±0.14	1.12±0.14
SH-81	1.29±0.05	1.03±0.08	0.30±0.04	0.48±0.11	1.49±0.20
SH-82	1.24±0.19	1.15±0.15	0.72±0.24	1.09±0.10	2.14±0.26
SH-83	1.20±0.08	0.90±0.05	0.25±0.03	0.42±0.07	0.94±0.07
SH-84	1.09±0.12	0.96±0.07	1.04±0.39	1.22±0.26	1.00±0.15
SH-85	1.34±0.35	1.16±0.10	1.16±0.23	1.24±0.15	3.49±0.29
SH-86	1.15±0.06	0.72±0.18	0.34±0.06	0.38±0.11	1.35±0.08
SH-87	0.89±0.25	0.79±0.12	0.97±0.19	1.39±0.35	0.83±0.12
SH-88	1.09±0.04	1.04±0.14	0.76±0.07	0.86±0.04	0.67±0.07
SH-89	0.98±0.04	0.80±0.08	0.91±0.30	0.91±0.03	0.83±0.18
SH-90	1.04±0.05	0.98±0.11	0.94±0.27	1.53±0.20	1.12±0.20
Shorawaki	0.94±0.24	2.75±0.37	1.47±0.18	1.52±0.10	4.96±0.65
PDW 34	1.10±0.23	0.32±0.06	1.47±0.12	2.93±0.25	0.84±0.17

content was observed in PDW 34 (2.93 mg/g), followed by SH-20 (1.67 mg/g), Shorawaki (1.52 mg\g), and SH-16 (1.34 mg/g). Minimum sugar contents were found in SH-86 (0.38 mg\g), SH-72 (0.56 mg\g), and SH-68 (0.71 mg\g). The rest of the genotypes accumulated sugar between these maximum and minimum values.

The highest K^+:Na^+ ratio was recorded in Shorawaki (4.96), followed by SH-16 (3.83) and SH-85 (3.49), and the lowest was in SH-13 (0.32), SH-38 (0.47), and SH-15 (0.48) (Table 98, p. 229).

The analysis of variance in for shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight of the 54 SH lines and two check genotypes grown under control and stress conditions indicates that the treatment (stress) had a significant ($P \leq 0.05$) adverse effect on all these parameters except for root dry weight, where the treatment was non-significant (Table 99). A significant difference between the genotypes was observed for all these traits, whereas the 'treatment x genotype' interactions were not significant for shoot length, root length, and root fresh weight.

Table 99. Analysis of variance summaries (mean squares) of shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight of 54 synthetic wheat lines grown at two salinity levels, 0 mM NaCl and 75 mM NaCl (* = significant at the 0.05 level; NS = not significant).

Source of variation	df	Shoot length (cm)	Root length (cm)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Treatment	1	614.4*	128.16*	0.469*	0.0373*	0.0021*	2.1804 NS
Genotype	55	87.13*	23.3*	0.059*	0.0112*	0.0021*	3.1837*
Treatment × Genotype	55	15.04 NS	4.31 NS	0.0069*	0.0015 NS	1.5581*	1.626*
Error	449	11.99	4.33	0.0043	0.0011	6.2192	6.1744

A significant difference for all the observed morphological attributes under control and stress conditions was observed for the 54 SHs (Table 100). The mean value for shoot length under the control condition was 28.8 cm (18–42 cm) and under the stress condition was 26.9 cm (17–40 cm). The skewness of shoot length was –0.120 under the control condition, negative from the mean values in the control condition, but was 0.139 at the stress condition. Heritability for shoot length was 0.726 in control and 0.890 under salt stress. Shoot length decreased under the stress condition.

Table 100. Evaluation of 54 synthetic wheat lines for morphological traits at saline (75 mM NaCl) and nonsaline (0 mM NaCl) conditions.

Parameter	Salt concentration	Mean	S.D	Minimum	Maximum	Skewness	h^2
Shoot length (cm)	0 mM	28.8	4.38	18	42	–0.120	0.72
	75 mM	26.9	3.80	17	40	0.139	0.73
Shoot fresh weight (g)	0 mM	0.329	0.10	0.12	0.59	0.095	0.86
	75 mM	0.273	0.08	0.08	0.50	0.164	0.87
Root fresh weight (g)	0 mM	0.085	0.048	0.010	0.298	1.043	0.79
	75 mM	0.071	0.035	0.006	0.179	0.427	0.91
Shoot dry weight (g)	0 mM	0.034	0.010	0.010	0.088	0.925	0.78
	75 mM	0.032	0.009	0.010	0.082	1.092	0.77
Root dry weight (g)	0 mM	0.008	0.003	0.001	0.023	1.188	0.68
	75 mM	0.008	0.002	0.002	0.019	1.113	0.82

The mean value of shoot fresh weight in the nonsaline condition was 0.32g and 0.27g in the saline condition. The range of shoot fresh weight was 0.120–0.596 g under the control condition and 0.083–0.500g at the stress level. The skewness of shoot fresh weight in the genotypes in the control condition was 0.095 and 0.164 at the stress condition. Heritability at controls was 0.86 and 0.87 under saline conditions (Table 100).

Shoot fresh weight was higher in the control condition (0.12–0.596g) and reduced in saline (0.083–0.500 g), indicating that salinity stress reduces shoot fresh weight. Positive skewness values at both levels show that the majority of genotypes performed equal to or greater than the mean value for this parameter (Table 100, p. 230).

The mean value for root fresh weight at the control condition was 0.085 g (0.01–0.29 g) and the skewness value was 1.043. At saline stress levels, the mean value was 0.071 g (0.006–0.179 g) and the skewness was 0.427. Heritability for root fresh weight was 0.79 at control and 0.91 at saline conditions. Salt stress has a negative effect on root fresh weight, decreasing in the stress condition. Skewness was positive from mean value under both control and stress conditions (Table 100, p. 230).

The mean value for shoot dry weight under control condition was 0.034 g and 0.032 g under stress condition. Shoot dry weight ranged from 0.010 g to 0.088 g at the control level and 0.012 g to 0.082 g in genotypes under saline conditions. Skewness in the genotypes was 0.925 and 1.092 in control and stress conditions, respectively. Heritability for shoot dry weight was 0.78 under control and 0.77 under salt stress conditions. Shoot dry weight decreases under stress condition. The positive skewness value at both levels shows that the performance of most of the genotypes for this trait was equal to or greater than the mean value (Table 100, p. 230).

The mean value of root dry weight under control and stress condition was 0.008 (0.001–0.023 g) under the nonsaline condition and 0.002–0.010 g under saline condition. Skewness in the genotypes was 1.18 and 1.11 at 0 mM and 75 mM NaCl, respectively. Heritability for root dry weight was 0.68 under control and 0.82 under stress conditions (Table 100, p. 230).

Mean data for morphological traits fo the 54 SH lines was determined (Table 101, p. 232-233). Shoot length decreased under salt stress. The greatest shoot length was found in Shorawaki (40.2 cm), followed by SH-38 (33.4 cm) and SH-20 (31.6 cm), whereas the lowest was in SH-40 (22.4 cm), SH-78 (23.2 cm), and SH-90 (24.1 cm).

Root length increased under salt stress (Table 101, p. 232). The maximum root length was recorded in SH-6 (9.2 cm), followed by SH-16 (9.1 cm) and SH-15 (8.8 cm) and was lowest in SH-30 (3.0 cm), SH-5 (3.7 cm) and SH-17 (4.0 cm). Shoot fresh weight also decreased under salt stress. The greatest shoot fresh weight was observed in Shorawaki (0.47 g), followed by SH-16 (0.42 g) and SH-12 (0.41 g), and SH-52 (0.13 g) and SH-46 (0.14 g) had the lowest weight. Root fresh weight was the maximum in SH-16 (0.13 g), SH-14 (0.12 g), and SH-15 (0.11 g) and the minimum in SH-41 (0.013 g), SH-53 (0.016 g), and SH-41 (0.018 g). Root fresh weight of Shorawaki was 0.11 g and that of PDW34 was 0.06 g. Shoot dry weight and root dry weight decreased under salt stress. The highest shoot dry weight was in SH-16 (0.060 g), followed by SH-6 (0.042 g) and SH-21 (0.041 g), and the lowest in SH-58 (0.020 g), SH-46 (0.022 g), and SH-88 (0.028 g). The greatest root dry weight was found in Shorawaki (0.012 g) and SH-39 (0.011 g) and the lowest in SH-53 (0.004 g), SH-32 (0.005 g), and SH-21 (0.006 g). The dry weight of the shoots of Shorawaki was 0.011 g and of PDW 34 was 0.025 g and of the roots were 0.011 g (Shorawaki) and 0.006 (PDW 34) respectively. All other synthetic lines were intermediate between the two check lines.

Based on the overall performance of the 54 synthetic wheat lines, SH-16, SH-82, and SH-78 performed better with respect to morphological, physiological, and biochemical attributes and were found to be tolerant at 75 mM NaCl stress. On the other hand, SH-13, SH-10, and SH-77 showed poor performance with respect to biomass production and physiological and biochemical traits and were susceptible at 75mM NaCl stress.

A reduction in chlorophyll content is to be expected under stress. Being membrane bound, chlorophyll content is dependent on membrane stability, which under saline conditions seldom remains intact. Salinity stress is well known to cause significant reduction in leaf chlorophyll concentration. Reduction in chlorophyll content is probably due to the inhibitory effect of the accumulated ions of various salts on the biosynthesis of different chlorophyll fractions. In tolerant wheat lines, there was less reduction in total chlorophyll content compare to the susceptible DH lines. The highest chlorophyll content and them minimum reduction (1.55–1.42 mg/g) under salt stress was found in SH-16.

The accumulation of solutes, especially proline, glycine-betaine, and sugar, is a common observation under stress conditions. During salt stress, an increase in sugar concentration has been reported in many species, possibly due to inhibitory effects of salinity stress on the translocation of assimilates. The increased content of sucrose with salinity (but to a lesser extent in tolerant wheat cultivars) is probably due to starch hydrolysis by enhanced activity of α-amylase under salinity. Sugar may contribute to salt-stress tolerance either by serving as osmotica or as respiratory substrate. High sugar under salt stress prevents plants from oxidative damage and maintains structure of different proteins and

Table 101. Means of shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight of 54 synthetic wheat lines grown at two salinity levels, 0 mM NaCl and 75 mM NaCl.

Genotype	Shoot length (cm)		Root length (cm)		Shoof fresh weight (g)		Root fresh weight (g)	
	0 mM	75 mM	0 mM	75 mM	0 mM	75 mM	0 mM	75 mM
SH-4	29.3±0.56	26±1.45	6±1.04	7.74±0.77	0.49±0.02	0.32±0.05	0.15±0.017	0.13±0.022
SH-5	26.64±0.83	26.08±0.90	3.68±0.61	3.78±0.41	0.38±0.01	0.30±0.02	0.090. ±01	0.07±0.006
SH-6	32±0.74	27±1.05	8±1.36	9±2.27	0.50±0.02	0.32±0.05	0.14±0.01	0.12±0.016
SH-10	24.4±2.17	21±2.18	7±2.80	9±2.80	0.22±0.03	0.17±0.03	0.15±0.03	0.13±0.016
SH-11	30.42±0.88	27±1.60	6±1.93	8.18±1.78	0.42±0.041	0.36±0.05	0.09±0.009	0.08±0.004
SH-12	30.04±0.25	30±0.34	6±0.53	7.82±0.95	0.45±0.02	0.41±0.02	0.10±0.01	0.08±0.004
SH-13	27.5±1.36	26±0.729	5±1.27	9±0.49	0.43±0.02	0.29±0.03	0.12±0.01	0.09±0.01
SH-14	29.44±1.05	28±1.43	6±0.85	7.74±0.74	0.419±0.017	0.35±0.04	0.18±0.02	0.12±0.01
SH-15	32±2.10	27±1.38	6±0.14	8.8±0.79	0.421±0.053	0.31±0.05	0.19±0.03	0.11±0.01
SH-16	28±1.30	27±1.57	7±1.05	9.1±1.28	0.50±0.027	0.42±0.03	0.15±0.013	0.13±0.006
SH-17	33±1.12	31±3.20	5±0.33	4±0.67	0.39±0.04	0.27±0.03	0.06±0.01	0.060.008
SH-20	34±1.64	311±.41	5±0.48	4±0.85	0.35±0.03	0.30±0.02	0.07±0.02	006±0.003
SH-21	32±0.93	31±0.41	5±1.22	5±1.16	0.34±0.006	0.32±0.02	0.08±0.006	0.07±0.01
SH-28	30±0.54	29±0.22	5±0.37	6±0.32	0.25±0.03	0.39±0.02	0.084±0.016	0.07±0.002
SH-30	31±0.27	30±0.51	3±0.20	3±0.17	0.32±0.02	0.26±0.02	0.06±0.001	0.05±0.004
SH-32	29±1.69	29±0.84	3±0.40	7±0.23	0.279±0.04	0.21±0.01	0.06±0.008	0.05±0.003
SH-35	29±1.49	28±0.81	5±0.22	5±0.38	0.30±0.03	0.24±0.02	0.08±0.01	0.06±0.01
SH-36	30±1.75	25±2.0	4±0.0	7±1.05	0.34±0.01	0.16±0.011	0.10±0.002	0.04±0.01
SH-37	31±1.47	30±0.31	4±0.33	6±0.70	0.30±0.04	0.24±0.05	0.05±0.01	0.05±0.01
SH-38	34±2.63	33±1.78	6.9±1.005	8±0.66	0.37±0.05	0.30±0.01	0.06±0.01	0.04±0.01
SH-39	29±1.84	24±0.45	6±0.94	8±0.66	0.19±0.03	0.16±0.01	0.05±0.03	0.03±0.01
SH-40	23±1.29	22±0.70	6±1.58	9±1.11	0.24±0.01	0.22±0.02	0.03±0.01	0.036±0.005
SH-41	31±2.12	24±1.72	5±0.73	9±1.06	0.24±0.04	0.18±0.03	0.02±0.005	0.01±0.002
SH-42	31±1.47	30±0.59	6±0.75	7±1.77	0.23±0.02	0.19±0.01	0.03±0.01	0.02±0.003
SH-43	30±2.62	23±1.48	6±1.05	7±0.88	0.26±0.01	0.23±0.01	0.05±0.002	0.04±0.01
SH-44	27±1.67	26±1.52	6±0.47	8±0.99	0.18±0.005	0.15±0.01	0.05±0.002	0.04±0.01
SH-46	29±2.0	25±0.83	3±0.33	6±0.61	0.18±0.004	0.13±0.01	0.04±0.002	0.02±0.004
SH-48	27±3.12	27±1.90	7±1.86	8±1.82	0.19±0.03	0.17±0.03	0.07±0.005	0.06±0.02
SH-52	28±0.58	28±1.73	6±0.64	6±0.98	0.14±0.01	0.13±0.01	0.02±0.002	0.02±0.004
SH-53	27±1.37	26±0.20	5±0.8	7±0.44	0.20±0.003	0.18±0.03	0.01±0.001	0.01±0.002
SH-54	33±2.80	26±2.38	6±0.61	8±1.17	0.45±0.05	0.28±0.02	0.13±0.02	0.09±0.01
SH-58	27±1.70	23±0.37	4±0.65	6±0.58	0.28±0.03	0.20±0.01	0.11±0.01	0.09±0.01
SH-60	31±1.10	28±1.49	5±0.76	8±0.51	0.35±0.05	0.31±0.03	0.09±0.03	0.09±0.002
SH-61	32±0.83	29±1.74	6±0.72	7±0.92	0.37±0.03	0.33±0.01	0.08±0.01	0.07±0.005
SH-62	29±1.69	25±1.42	6±1.07	7±0.87	0.33±0.03	0.27±0.05	0.10±0.01	0.08±0.01
SH-63	32±2.43	25±1.06	5±0.75	6±0.54	0.41±0.04	0.28±0.01	0.10±0.02	0.09±0.01
SH-66	25±2.23	25±3.55	6±1.62	5±1.12	0.31±0.05	0.24±0.05	0.09±0.01	0.08±0.002
SH-68	28±1.96	28±1.91	5±0.59	6±0.36	0.34±0.01	0.34±0.04	0.12±0.02	0.08±0.02
SH-72	27±1.46	27±0.99	5±0.41	6±0.48	0.30±0.03	0.29±0.01	0.07±0.01	0.06±0.002
SH-76	29±2.63	27±2.99	5±0.82	6±0.42	0.32±0.06	0.23±0.04	0.09±0.005	0.08±0.01
SH-77	28±2.30	25±0.10	4±0.60	7±0.85	0.35±0.07	0.21±0.01	0.10±0.02	0.07±0.004
SH-78	23±2.84	23±1.03	5±1.0	6±0.78	0.30±0.03	0.28±0.01	0.10±0.021	0.06±0.01
SH-79	28±1.12	26±1.21	5±0.32	5±0.82	0.34±0.03	0.25±0.02	0.16±0.02	0.10±0.01
SH-80	25±2.80	24±0.42	5±0.83	5±1.10	0.37±0.024	0.31±0.01	0.13±0.06	0.10±0.02
SH-81	26±1.11	25±1.53	4±0.76	5±0.85	0.34±0.02	0.28±0.03	0.08±0.02	0.07±0.002
SH-82	29±0.73	271.03	5±0.42	5±0.67	0.39±0.002	0.37±0.02	0.095±0.005	0.08±0.01
SH-83	26±1.40	26±1.27	6±1.27	8±1.91	0.41±0.03	0.40±0.02	0.07±0.02	0.09±0.02
SH-84	26±0.49	25±0.58	6±0.41	6±0.86	0.33±0.05	0.30±0.03	0.08±0.01	0.07±0.01
SH-85	26±0.92	25±0.28	4±0.49	4±1.25	0.40±0.02	0.32±0.05	0.08±0.01	0.07±0.003
SH-86	25±0.80	24±0.30	6±0.83	6±0.72	0.34±0.01	0.30±0.02	0.10±0.01	0.09±0.003
SH-87	32.16±2.11	28.2±1.47	5±0.34	7.52±1.26	0.37±0.05	0.29±0.03	0.05±0.004	0.04±0.01
SH-88	30.90±1.95	27.38±3.16	5.36±0.71	3.44±0.45	0.31±0.03	0.26±0.03	0.08±0.01	0.05±0.01
SH-89	31.5±0.85	31±0.42	7±1.25	6.9±0.59	0.36±0.01	0.35±0.01	0.06±0.01	0.13±0.016
SH-90	24±2.78	24±2.74	6±1.17	6±0.99	0.29±0.05	0.28±0.05	0.043±0.012	0.08±0.014
Shorawaki	420.99	400.41	130.50	140.48	0.550.01	0.470.03	0.130.004	0.110.003

Table 101. Means of shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight of 54 synthetic wheat lines grown at two salinity levels, 0 mM NaCl and 75 mM NaCl.

Genotype	Shoot dry weight (g)		Root dry weight (g)	
	0 mM	75 mM	0 mM	75 mM
SH-4	0.042±0.001	0.04±0.005	0.009±0.001	0.008±0.001
SH-5	0.04±0.003	0.036±0.003	0.008±0.001	0.007±0.001
SH-6	0.05±0.003	0.04±0.003	0.01±0.0003	0.008±0.001
SH-10	0.036±0.005	0.028±0.005	0.01±0.002	0.007±0.002
SH-11	0.04±0.004	0.04±0.006	0.01±0.001	0.01±0.001
SH-12	0.04±0.002	0.03±0.004	0.01±0.001	0.01±0.001
SH-13	0.043±0.003	0.029±0.004	0.01±0.001	0.007±0.001
SH-14	0.04±0.002	0.03±0.005	0.01±0.001	0.009±0.001
SH-15	0.04±0.007	0.03±0.004	0.009±0.002	0.007±0.001
SH-16	0.065±0.009	0.060±.007	0.006±0.001	0.008±0.001
SH-17	0.04±0.004	0.03±0.005	0.01±0.001	0.008±0.0011
SH-20	0.04±0.004	0.03±0.004	0.006±0.001	0.005±0.001
SH-21	0.05±0.003	0.03±0.003	0.01±0.001	0.006±0.001
SH-28	0.031±0.003	0.030±0.0002	0.006±0.001	0.005±0.0003
SH-30	0.032±0.001	0.03±0.004	0.01±0.01	0.006±0.0005
SH-32	0.034±0.005	0.028±0.002	0.005±0.001	0.005±0.0002
SH-35	0.032±0.002	0.03±0.004	0.008±0.001	0.007±0.001
SH-36	0.04±0.001	0.02±0.003	0.01±0.001	0.01±0.0003
SH-37	0.036±0.001	0.03±0.004	0.007±0.001	0.007±0.001
SH-38	0.042±0.003	0.04±0.001	0.005±0.001	0.007±0.001
SH-39	0.031±0.002	0.026±0.002	0.01±0.003	0.011±0.001
SH-40	0.042±0.002	0.036±0.004	0.01±0.002	0.01±0.001
SH-41	0.03±0.005	0.025±0.003	0.01±0.001	0.006±0.0
SH-42	0.04±0.003	0.035±0.002	0.01±0.002	0.01±0.001
SH-43	0.030±0.004	0.03±0.001	0.01±0.0004	0.009±0.001
SH-44	0.03±0.002	0.03±0.003	0.01±0.0002	0.009±0.001
SH-46	0.025±0.002	0.022±0.003	0.008±0.001	0.0006±0.0005
SH-48	0.03±0.005	0.029±0.003	0.007±0.002	0.01±0.001
SH-52	0.03±0.003	0.026±0.004	0.008±0.001	0.01±0.001
SH-53	0.031±0.002	0.029±0.005	0.005±0.0004	0.004±0.001
SH-54	0.05±0.004	0.03±0.003	0.01±0.001	0.01±0.001
SH-58	0.024±0.002	0.02±0.001	0.01±0.001	0.01±0.001
SH-60	0.033±0.004	0.032±0.003	0.01±0.002	0.01±0.001
SH-61	0.04±0.003	0.037±0.002	0.01±0.001	0.008±0.001
SH-62	0.033±0.002	0.03±0.005	0.01±0.001	0.009±0.001
SH-63	0.04±0.004	0.03±0.001	0.01±0.002	0.009±0.001
SH-66	0.03±0.005	0.027±0.006	0.01±0.002	0.008±0.001
SH-68	0.034±0.002	0.03±0.003	0.01±0.002	0.009±0.001
SH-72	0.03±0.002	0.03±0.002	0.01±0.001	0.007±0.001
SH-76	0.03±0.007	0.026±0.003	0.01±0.001	0.009±0.002
SH-77	0.034±0.006	0.028±0.002	0.006±0.001	0.006±0.001
SH-78	0.032±0.001	0.03±0.005	0.008±0.001	0.007±0.001
SH-79	0.03±0.003	0.025±0.002	0.01±0.003	0.008±0.001
SH-80	0.031±0.002	0.03±0.001	0.01±0.005	0.01±0.002
SH-81	0.03±0.004	0.03±0.01	0.01±0.001	0.008±0.001
SH-82	0.04±0.002	0.04±0.003	0.009±0.003	0.007±0.001
SH-83	0.03±0.003	0.029±0.0003	0.007±0.001	0.007±0.0003
SH-84	0.03±0.006	0.03±0.001	0.01±0.001	0.008±0.001
SH-85	0.04±0.001	0.04±0.01	0.01±0.002	0.008±0.001
SH-86	0.042±0.001	0.039±0.004	0.01±0.001	0.009±0.0004
SH-87	0.033±0.004	0.03±0.003	0.01±0.001	±0.0080.0003
SH-88	0.03±0.003	0.028±0.003	0.01±0.002	0.008±0.001
SH-89	0.036±0.005	0.03±0.0001	0.009±0.0004	0.009±0.001
SH-90	0.028±0.006	0.030±0.005	0.006±0.0003	0.006±0.0007
Shorawaki	0.16±0.006	0.11±0.005	0.014±0.001	0.011±0.001
PDW 34	0.034±0.002	0.025±0.002	0.02±0.001	0.006±0.001

membranes. Our results show the accumulation of sugar content under salt stress in SH-13 (0.33–1.67mg/g) increased.

K^+:Na^+ discrimination in SHs has been determined by different scientists at different salinity levels. We screened the genotypes at 75 mM NaCl. Line SH-16 was found to have highest K^+:Na^+ ratio, and lowest was in SH-13. Although the salt-tolerant check Shorawaki was higher than SH-16.

The salinity tolerance in wheat is associated with the accumulation of K^+ and exclusion of Na^+ under saline conditions. Sodium competes with K^+ for uptake through common transport system and does this effectively since the Na^+ concentration in saline environment is usually considerably greater than that of K^+. The sensitivity of some crops to salinity is due to the inability to keep Na^+ and Cl^- out of transpiration streams. Plants limiting the uptake of toxic ions or maintaining normal nutrient ion contents could show greater tolerance, which was the case with our study. Uptake mechanisms that discriminate similar ions, such as Na^+ and K^+, could be useful selection criteria for salt tolerance in wheat and breeding for efficient nutrient uptake. All the genotypes showed a decreasing trend in K^+ content due to salinity stress. The decrease in K^+ was due to the presence of excessive Na^+ in the growth medium, because high external Na^+ content is known to have an antagonistic effect on K^+ uptake in plant. Salt tolerance is associated with K^+ content, because of its involvement in osmotic regulation and competition with Na^+. Regulation of K^+ uptake and prevention of Na+ entry and efflux of Na^+ from cell are the strategies commonly used by plants to maintain desirable K^+:Na^+ ratio in the cytosol.

Plants irrigated with saline water show great depression in dry weight. Such a reduction is due to inadequacy of nutrients present in growing media or due to decrease in water entry rate into plants. Because root pressure is reduced under saline conditions causing a decrease in water flow, less water is available for normal growth and development. The decrease in shoot fresh weight may be due to low uptake of water by plants as well as toxicity of Na^+ and Cl^- because of their high concentration in the nutrient solution. A similar trend was noted in the fresh and dry root weights and also in dry shoot weights in the lines under saline conditions by other scientists. We also observed a decrease in

shoot and root fresh and dry weight under salinity stress. Shoot fresh weight was reduced the least in SH-16, SH-78, and SH-82 under salt stress and a smaller decrease in root fresh weight in SH-16 and SH-82 showed their tolerant behavior under salt stress. Shoot fresh weight was reduced significantly in SH-13. Smaller reductions in the shoot and root dry weights of the tolerant genotypes compared to the susceptible genotypes were observed.

Physiological and morphological evaluation of ten selected lines at 75 mM NaCl. The ten best lines (SH-4, SH-16, SH-40, SH-42, SH-43, SH-44, SH-53, SH-78, SH-82, and SH-85) were selected at 75mM on the basis of their K^+:Na^+ discrimination, cholorophyll content, sugar content, and root and shoot fresh and dry weights. For these ten lines, SOD, protein, proline, shoot dry weight and K^+/Na^+ were further evaluated at 100 mM NaCl.

Physiological parameters under salt stress. A high K^+:Na^+ value indicates a high level of salt tolerance, because it shows that plant have greater ability to exclude Na^+ and accumulate K^+ at high NaCl concentrations. To accumulate more K^+ compared to Na^+ under saline conditions is a character that determines salinity tolerance of plant at the seedling stage.

In the ten selected lines at 75 mM NaCl, the highest K^+:Na^+ was observed in SH-16 (3.83), followed by SH-85 (3.49), SH-78 (3.04), and SH-42 (3.01) and the lowest was found in SH-4 (1.0). The salt-tolerant genotype Shorawaki had a K^+:Na^+ ratio of 4.96 and the salt-susceptible PDW 34 was 0.84 (Table 102). The highest chlorophyll content was found in SH-16 (1.42 mg/g), followed by SH-78 (1.20 mg/g), SH-85 (1.16 mg/g), and SH-82 (1.15 mg/g), and the lowest was observed in SH-43 (0.62 mg/g). The salt-tolerant genotype Shorawaki had a 2.75 mg/g chlorophyll content and in the salt-susceptible genotype PDW 34, it was reduced to 0.32 mg/g. Sugar content did not increase much compared to that at the control level, but in the salt-sensitive genotype PDW 34, sugar content was increased from 1.47 mg/g under control condition to 2.93 mg/g under stress conditions.

Table 102. Means of physiological parameters of ten selected wheat lines at control and stress level (75 mM NaCl).

	Chlorophyll content (mg/g)		Sugar (mg/g)		K^+:Na^+ discrimination
Genotype	0 mM	75 mM	0 mM	75 mM	75mM
SH-4	1.11	1.08	1.14	1.27	1.00
SH-16	1.55	1.42	1.17	1.34	3.83
SH-40	0.63	0.51	0.83	1.04	2.53
SH-42	0.85	0.62	0.89	1.07	3.01
SH-43	0.85	0.67	0.95	1.22	3.00
SH-44	1.10	0.52	0.82	1.20	1.32
SH-53	1.17	1.10	0.96	1.10	2.00
SH-78	1.23	1.20	0.81	1.20	3.04
SH-82	1.24	1.15	0.72	1.09	2.14
SH-85	1.34	1.16	1.16	1.24	3.49
Shorawaki	2.94	2.75	1.47	1.52	4.96
Pbw343	1.10	0.32	1.47	2.93	0.84

Morphological parameters under salt stress. The ten lines also performed well regarding their agronomical parameters and showed less reduction in biomass as compared to the controls. At 75 mM in ten tolerant lines, the maximum shoot length was observed in SH-42 (30 cm). Shoot length was 40 cm in Shorawaki and 26 cm in PDW 34 under salt stress (Table 103, p. 235).

The highest shoot fresh weight in the selected lines at 75 $molm^{-3}$ was found in SH-16 (0.42 g), followed by SH-82 (0.37 g) and SH-4 (0.32 g). In the salt-tolerant check Shorawaki, shoot fresh weight was 0.47 g, whereas in PDW 34, shoot fresh weight decreased from 0.39 (control) to 0.25 (stress). SH-4 and SH-16 (0.13 g) performed well with respect to root fresh weight, whereas in Shorawaki, root fresh weight was 0.11 g and in PDW 34 0.06 g (Table 103, p. 235).

Genotypes with high K^+:Na^+ value have a high shoot dry weight, indicating that this trait is associated with performance of plant under stress. The highest shoot dry weight was found in SH-16 (0.06 g) among the ten lines under saline conditions. The shoot dry weight of Shorawaki was 0.11 g and that of PDW 34 was 0.025 g at this stress level. Among the selected lines, the highest root dry weight was observed in SH-40 and SH-42 (0.01 g) (Table 103, p. 235).

Screening for salt tolerance at 100 mM NaCl stress. The ten best lines identified at 75 mM and the salt-tolerant check Kharchia 65 were subjected to proline, protein, and superoxide dismutase (SOD) analysis at 100 mM NaCl stress to further fine tune the identification of tolerant lines for future use. The shoot dry weight and K^+:Na^+ also also recorded to check salt tolerance at 100 mM (Table 104, p. 235).

Table 103. Mean values of morphological parameters of ten selected wheat lines at control and stress level (75 mM NaCl)

Genotype	Shoot length (cm)		Root length (cm)		Shoot fresh weight (g)		Root fresh weight (g)		Shoot dry weight (g)		Root dry weight (g)	
	0 mM	75 mM	0 mM	75 mM	0 mM	75 mM	0 mM	75 mM	0 mM	75 mM	0 mM	75 mM
SH-4	29	26	6.0	7.7	0.49	0.32	0.15	0.13	0.042	0.040	0.009	0.008
SH-16	28	27	7.0	9.1	0.50	0.42	0.15	0.13	0.070	0.060	0.006	0.008
SH-40	23	22	6.3	9.0	0.24	0.22	0.03	0.03	0.042	0.040	0.010	0.010
SH-42	31	30	6.4	7.0	0.23	0.19	0.03	0.02	0.040	0.035	0.010	0.010
SH-43	30	23	6.2	7.0	0.26	0.23	0.05	0.04	0.030	0.030	0.010	0.009
SH-44	27	26	6.0	8.0	0.18	0.15	0.05	0.04	0.030	0.030	0.010	0.009
SH-53	27	26	5.0	7.0	0.20	0.18	0.01	0.01	0.031	0.030	0.005	0.004
SH-78	23	23	5.0	6.0	0.30	0.28	0.10	0.06	0.032	0.030	0.008	0.007
SH-82	29	27	5.0	7.0	0.39	0.37	0.10	0.08	0.040	0.040	0.009	0.007
SH-85	26	25	4.0	6.0	0.40	0.32	0.08	0.07	0.040	0.040	0.010	0.009
Shorawaki	42	40	13.0	14.0	0.55	0.47	0.13	0.11	0.160	0.110	0.014	0.011
PDW34	35	26	6.0	9.0	0.39	0.25	0.11	0.06	0.034	0.025	0.020	0.006

Among the selected lines at 100 mM salt stress, the SOD value ranged from 25.8 μg/mL to 74.3μg/mL. SH-42 (74.3 units/g) was found to have highest SOD content, and the lowest was observed in SH-85 (25.8 units/g). The check Kharchia 65 had 51.2 units/g SOD content (Table 104).

The highest protein content accumulated in SH-16 (1,291.8 μg/g) and the lowest was observed in SH-78 (1,134.8 μg/g). Kharchia 65 showed a protein content of 1,256.7 μg/g at 100mM NaCl stress (Table 104).

Table 104. Mean evaluation superoxide dismutase (SOD), protein, proline, shoot dry weight, and K^+:Na^+ of ten selected wheat synthetic lines at 100mM NaCl stress.

Genotype	SOD (units/g)	Protein (μg/g)	Proline (μg/mL)	Shoot dry weight (g)	K^+:Na^+ discrimination
SH-4	49.9	1,291.4	338.8	0.023	0.850
SH-16	66.0	1,291.8	328.8	0.040	2.310
SH-40	71.7	1,224.6	257.4	0.030	2.050
SH-42	74.3	1,141.7	259.1	0.024	1.251
SH-43	53.5	1,181.8	195.9	0.033	2.540
SH-44	64.2	1,206.4	300.6	0.024	0.870
SH-53	30.1	1,163.9	134.5	0.025	0.941
SH-78	50.8	1,134.8	209.2	0.036	2.650
SH-82	46.6	1,259.1	554.7	0.028	1.140
SH-85	25.8	1,206.7	368.6	0.032	2.740
Kharchia 65	51.2	1,256.7	702.5	0.072	3.450

Synthetic SH-53 showed the lowest proline content of 134.5 μg/mL and the highest value of 554.7 μg/mL was found in SH-82. Kharchia 65 accumulated proline content of 702.4 μg/mL at 100 mM saline condition (Table 104).

The highest shoot dry weight among ten SHs was in SH-16 (0.040 g) and the lowest in SH-4 (0.023 g). Kharchia 65 was found to have a 0.072 g shoot dry weight at 100 mM stress.

The K^+:Na^+ ratio ranged from 0.85 (SH-4) to 2.74 (SH-85) and the check Kharchia 65 was 3.450 (Table 104).

Conclusion. In addition to achieving quantity and quality, wheat improvement programs focus on two major problems, biotic and abiotic production constraints. In Pakistan, rusts are the key biotic constraint to production and the abiotic factors of drought, salinity, and heat play significant roles. For each objective to be achieved, the appropriate genetic diversity is essential to have in a cultivar to give a useful product. Focusing on salinity, which is an abiotic constraint mainly in irrigated agriculture, the available wheat cultivars do not have an abundance of diversity. Limited conventional cultivars are available, and these have been underexploited and poorly characterized for their molecular profiles. However, new diversity around parental genomes (particularly *Ae. tauschii*) has become available and is a potent re-

source for wheat improvement. Resistance against many of the biotic and abiotic factors has been incorporated from *Ae. tauschii* to synthetic wheats. We focused on screening for salinity tolerant lines on the basis of morphological characters and physiological/biochemical attributes.

Based on overall performance of 54 synthetic wheat lines, SH-16, SH-82, and SH-78 performed better with respect to morphological, physiological, and biochemical attributes and were found to be tolerant at 75 mM NaCl stress. Lines SH-13, SH-10, and SH-77 had poor performance with respect to biomass production and physiological and biochemical traits and were susceptible at 75 mM NaCl stress.

The highest chlorophyll content was found in SH-16 and also the minimum (1.55–1.42 mg/g) reduction of chlorophyll under salt stress. Among the genotypes screened at 75 mM NaCl, SH-16 was found to have the highest K^+:Na^+ ratio; the lowest was observed in SH-13. Tolerant lines also performed better with respect to their morphological traits.

We found that synthetic wheat lines provide a good source of tolerance to salinity and are morphologically and physiologically good with a high level of genetic diversity, which is a prerequisite of any crop improvement program. We conclude that these SHs are a valuable source for genetic improvement of wheat for salinity tolerancefor plant breeders to use in breeding for saline soils.

Phenological and molecular characterization of the International Triticeae Mapping Initiative Wheat Population for salinity tolerance.

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Salinity is a worldwide problem causing reduction in growth and yield of many crops including wheat. In Pakistan, rusts are is the key biotic constraint to production and in the abiotic category, drought, salinity, and heat play a significant role. The feasible way to address this problem is development of wheat cultivars that can thrive and give reasonable yield, when grown in such problem soils. To achieve this, existence of appropriate amount of genetic variability and physiological and biochemical mechanisms of salinity tolerance are prerequisite. New diversity around the parental genomes (particularly D-genome diploid) has become available and is a potent resource for wheat improvement. Resistance against many of the biotic and abiotic factors has been incorporated from this diploid wheat progenitor to synthetic wheats. This study focused on determining genetic diversity of some salinity tolerant germ plasm for its utilization in crop improvement programs.

The germ plasm selected for this study has been characterized as the International Triticeae Mapping Initiative (ITMI) population produced in the USA using two wheat parents from CIMMYT, Mexico; Opata and a D-genome synthetic hexaploid wheat. The F_1s derived from the cross between the above two parents have been advanced by single seed descent (SSD) up to F_6 and have 149 recombinant inbred lines. The whole ITMI population was subjected to chlorophyll analysis, sugar, shoot fresh weight, root fresh weight, and their biomass were taken at 0 and 75mM stress, K^+:Na^+ discrimination was taken only at 75 mM salt stress, and the top ten tolerant lines were subjected to SOD, protein, and proline analyses at 100 mM salt stress.

Table 105. Analysis of variance summaries (mean squares) of total chlorophyll content (mg/g), sugar content (mg/g), and K^+:Na^+ ratio of 97 wheat lines grown at two salinity levels, 0 mM NaCl and 75 mM NaCl (* = significant).

Source of variation	Degrees of freedom	Total chlorophyll (mg/g)	Degrees of freedom	Sugar content (mg/g)	Degrees of freedom	K^+:Na^+
Treatment	1	22.39*	1	8.646*		
Variety	96	1.416*	96	1.979*	96	0.850*
Treatment x Variety	96	0.200*	96	0.185		
Error	777	0.397	386	0.355	388	0.124

The analyses of variance of total chlorophyll content, sugar content, and K^+:Na^+ ratio of 97 RILs grown under control and stress conditions are presented (Table 105) shows that stress (treatment) has significant ($P \leq 0.05$) adverse effect on total chlorophyll content while sugar content increase significantly ($P \leq 0.05$) under stress (75 mM) conditions.

Lines also differed significantly from each other but the 'variety x treatment' interactions were not significant in both the above parameters. There was significant ($P \leq 0.05$) difference in varieties for K^+:Na^+ ratio.

The total chlorophyll decreased due to salinity stress, ranging from 0.09 mg/g to 4.02 mg/g in the control and 0.09–3.75 mg/g at saline conditions. The population had a higher skewness value for total chlorophyll in saline conditions (1.46) than at control conditions (1.45). A positive value of the skewness showed that these values were equal or greater than the mean value. Heritability for total chlorophyll was 0.80 and 0.82 at control and salt conditions, respectively (Table 106).

Sugar content increased in stressed conditions; the range was 0.17–4.11 mg/g in the control and 0.16–7.39

Table 106. Physiological and morphological characterization of the ITMI mapping population at 0 mM and 75 mM NaCl (Shoot fresh weight = SFW, shoot dry weight = SDW, root fresh weight = RFW, and root dry weight = RDW)..

	Mean	Standard deviation	Minimum value	Maximum value	Skewness	Kurtosis	Heritibility
0 mM NaCl							
Cholophyll content	1.12	0.60	0.09	3.75	1.45	2.95	0.80
Shoot length (cm)	23.3	4.56	12.8	36.5	0.14	-0.35	0.83
Root length (cm)	4.38	2.05	1.00	14.5	1.04	1.64	0.78
Shoot fresh weight (g)	0.21	0.10	0.09	0.79	1.57	3.08	0.89
Root fresh weight (g)	0.07	0.06	0.01	0.67	3.52	23.6	0.33
Shoot dry weight (g)	0.02	0.009	0.01	0.06	1.19	1.88	0.27
Root dry weight (g)	0.008	0.004	0.01	0.02	0.57	-0.38	0.82
SDW/RDW	3.61	2.78	0.83	29	3.58	19.9	0.73
RDW/SDW	0.38	0.19	0.03	1.2	1.14	2.07	0.82
Sugar (mg/g)	1.03	0.67	0.17	4.11	1.97	4.55	0.80
75 mM NaCl							
Cholophyll	0.91	0.55	0.09	4.02	1.46	3.65	0.82
K	3.37	1.30	0.87	7.41	0.43	0.01	0.86
Na	5.57	2.95	0.63	15.7	0.96	0.66	0.93
K^+:Na^+	0.77	0.56	0.15	6.00	3.58	22.5	0.84
Shoot length (cm)	21.2	4.42	10.2	34.0	0.29	0.06	0.84
Root length (cm)	3.72	1.84	1.0	12.9	1.36	2.47	0.79
Shoot fresh weight (g)	0.19	0.07	0.1	0.50	1.29	2.09	0.88
Root fresh weight (g)	0.05	0.04	0.01	0.26	1.70	3.41	-0.25
Shoot dry weight (g)	0.02	0.008	0.01	0.06	1.74	5.23	0.20
Root dry weight (g)	0.007	0.004	0.001	0.01	0.70	–0.20	0.27
SDW/RDW	3.84	3.54	0.5	25.7	2.99	11.12	0.74
RDW/SDW	0.41	0.28	0.04	2.00	1.87	5.44	0.83
Sugar (mg/g)	1.27	0.85	0.16	7.39	2.35	9.60	0.70

mg/g in salt strell, a significant increase of from 4.11 mg/g in the control. Skewness in the population was 2.35 in saline and 1.97 in nonsaline conditions. Sugar showed a positive value of skewness and was much less in saline conditions. Heritability for sugar content in the control was 0.80, whereas in salt conditions was 0.70 (Table 106). The K^+:Na^+ ratio ranged from 0.15–6.00 with a skewness value of 3.58.

Total chlorophyll content, sugar content, and K^+:Na^+ discrimination were measured for the 97 RILs (Table 107, pp. 238-239). The data gives a clear picture that salt stress caused significant reductions in total chlorophyll content in the RILs. The greatest total chlorophyll content was found in ITMI-6 (2.01 mg/g), followed by ITMI-97 (1.93 mg/g) and ITMI-29 (1.92 mg/g). The lowest value of total chlorophyll content was in ITMI-14 (0.14 mg/g), ITMI-96 (0.19 mg/g), and ITMI-75 (0.48 mg/g).

Sugar content increased under salt stress. The maximum sugar content was observed in ITMI-34 (3.27 mg/g), followed by ITMI-3 (3.11 mg/g) and ITMI-12 (2.83 mg/g), and the minimum was in ITMI-77 (0.43 mg/g), ITMI-4 (0.48 mg/g), and ITMI-61 (0.49 mg/g).

K^+:Na^+ increased under salt stress. The maximum K^+:Na^+ was observed in ITMI-24 (2.69), followed by ITMI-23 (2.60) and ITMI-20 (1.77) and the minimum was found in ITMI-73 (0.27), ITMI-84 (0.28 mg/g), and ITMI-81 (0.31).

The analysis of variance (Table 108, p. 239) for shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight indicate that the treatment (stress) had a significant ($P \leq 0.05$) adverse effect on all these parameters. A significant ($P \leq 0.05$) difference was found between varieties for all these traits and the 'treatment x variety' interaction was not significant for shoot length and root length and significant ($P \leq 0.05$) for the remaining parameters.

The shoot length ranged from 10.2 –34.0 cm in populations under saline conditions, whereas in the control conditions, it was 12.8–36.5 cm, a decrease under salinity. The skewness of the shoot length was 0.29 at saline and 0.14 at control conditions, positive from the mean values. Heritability was 0.83 at control and 0.84 at salt stress conditions (Table 109, pp. 240-241).

Morphological traits also decreased under salt stress. Shoot length decreased under salt stress. RILs ITMI-32 (28.7 cm), ITMI-86 (28.1 cm), and ITMI-17 (26 cm) had the highest shoot length and the lowest was in ITMI-81 (12.7 cm), ITMI-60 (12.8 cm), and ITMI-79 (13.7 cm). Root length increased under salt stress (Table 109, pp. 242-243). The maximum root length was recorded in ITMI-3 (9.4 cm), followed by ITMI-8 (7.6 cm) and ITMI-7 (7.5 cm) and the minimum was found in ITMI-122 (1.3 cm), ITMI-144 (1.2 cm) and ITMI-95 (1.1 cm).

Table 107. Mean values for chlorophyll, sugar, and K^+:Na^+ discrimination 97 lines of ITMI population

	Chlorophyll (mg/g)		Sugar (mg/g)		K^+:Na^+
Genotype	0 mM	75 mM	0 mM	75 mM	75 mM
ITMI-6	2.10	2.01	0.58	0.60	1.35
ITMI-8	2.11	1.47	0.37	1.85	1.23
ITMI-12	1.27	1.31	2.74	2.98	0.65
ITMI-13	1.00	0.95	0.93	1.45	0.65
ITMI-3	1.20	0.99	3.10	3.12	1.61
ITMI-4	1.50	0.31	0.48	0.49	0.69
ITMI-7	1.30	0.53	1.30	1.40	0.79
ITMI-11	1.00	0.71	1.29	1.50	1.00
ITMI-17	1.50	1.10	0.98	1.21	0.73
ITMI-18	1.50	1.50	1.09	1.82	0.53
ITMI-21	1.54	1.10	1.32	1.40	1.03
ITMI-25	1.46	1.13	0.86	0.93	0.78
ITMI-27	1.30	1.13	0.94	1.16	0.83
ITMI-28	1.50	1.13	0.90	0.97	0.64
ITMI-32	1.50	1.47	1.84	2.41	0.64
ITMI-36	1.50	1.45	0.67	0.76	0.94
ITMI-39	0.87	0.84	1.12	1.45	0.35
ITMI-40	1.50	1.36	0.80	0.87	0.72
ITMI-41	2.00	1.36	0.51	0.71	1.28
ITMI-44	0.70	0.73	0.50	0.51	1.77
ITMI-45	1.30	1.54	0.84	0.80	1.01
ITMI-46	1.70	1.00	0.93	1.45	0.57
ITMI-50	1.20	1.07	1.12	1.69	2.60
ITMI-52	1.20	1.20	0.94	0.97	2.69
ITMI-66	0.72	0.49	1.07	1.14	0.60
ITMI-67	0.70	0.39	0.46	0.55	0.59
ITMI-72	0.50	0.57	0.84	1.23	0.93
ITMI-75	0.52	0.45	0.66	1.06	1.37
ITMI-77	0.69	0.63	0.60	0.26	1.04
ITMI-78	1.98	1.84	0.71	0.75	0.72
ITMI-82	3.06	1.62	0.72	0.74	0.76
ITMI-84	2.38	0.75	1.14	1.22	0.28
ITMI-85	1.66	0.56	0.79	0.91	0.34
ITMI-86	0.98	0.95	0.53	1.10	0.56
ITMI-88	1.32	1.20	0.98	1.11	0.41
ITMI-91	0.91	0.63	1.28	1.39	0.33
ITMI-92	1.30	0.75	0.86	1.61	0.38
ITMI-94	1.48	1.90	0.96	0.99	0.54
ITMI-96	1.24	1.09	0.62	0.64	0.71
ITMI-97	2.11	1.93	0.93	1.08	0.48
ITMI-98	1.49	0.56	0.80	1.01	0.45
ITMI-99	0.77	0.72	1.00	1.20	0.46
ITMI-101	0.68	0.60	0.74	0.86	0.36
ITMI-79	1.05	0.85	1.22	0.93	1.02
ITMI-81	0.78	0.55	0.47	0.63	0.72
ITMI-116	0.70	0.59	1.22	1.24	0.64
ITMI-85	0.78	0.67	0.45	0.53	1.49
ITMI-14	0.35	0.14	0.48	3.03	0.37
ITMI-16	0.84	0.25	0.28	0.97	0.39
ITMI-47	0.74	0.33	0.32	0.85	0.36
ITMI-49	0.74	0.19	2.11	2.31	0.42
ITMI-54	0.78	0.29	0.97	1.14	0.33
ITMI-57	0.71	0.33	0.97	0.99	0.54
ITMI-58	0.71	0.27	0.95	0.97	0.36
ITMI-59	0.89	0.29	0.85	1.03	0.35
ITMI-60	0.83	0.42	1.46	1.63	0.53

Shoot fresh weight also decreased under salt stress. The largest shoot fresh weight was observed in ITMI-66 (0.32 g), followed by ITMI-11 (0.30 g) and ITMI-3 (0.28 g). ITMI-96 (0.12 g), ITMI-50 (0.14 g) and ITMI-29 (0.15 g) had the lowest shoot fresh weight values. Shoot fresh weight was higher in the control condition (0.09–0.70 g) and reduced in saline (0.1–0.5 g). The skewness of shoot fresh weight in the RIL population was 1.29 in saline and 1.57 in nonsaline conditions. Skewness at saline conditions was higher than the mean with a positive value and smaller in the controlled condition. Heritability for shoot fresh weight in the control was 0.89 and 0.88 at salt stress (Table 109, pp. 240-241).

Root fresh weight was the maximum in ITMI-67 (0.14 g), ITMI-28 (0.13 g), and ITMI-14 (0.08 g) and the minimum in ITMI-80 (0.02 g) and ITMI-20 (0.01 g). Shoot dry weight and root dry weight also decreased under stress.

Root fresh weight was higher in the control (0.01–0.67 g) and reduced in saline conditions (0.01–0.26 g). The skewness of root fresh weight in the RIL population was 1.70 in saline and 3.52 in nonsaline conditions. The skewness for shoot fresh weight in saline conditions was low with a positive value and higher in the control. Heritability for shoot fresh weight in the control was 0.33 and at salt stress was –0.25 (Table 109, pp. 240-241).

The largest root dry weight was found in ITMI-52 (0.02 g) and ITMI-77 (0.011 g) and lowest in ITMI-95 (0.002 g) and ITMI-34 (0.003 g). All other RILs had intermediate values for each attribute. Root dry weight decreased significantly in stressed conditions. In the controlled condition, root dry weight was 0.004–0.014 g and 0.004–0.001 g in stress conditions. Skewness in population was 0.70 in saline and 0.57 in non-saline conditions. The skewness value was higher in saline conditions (0.70) than in the control (0.57). Heritability was 0.82

Table 107. Mean values for chlorophyll, sugar, and K^+:Na^+ discrimination 97 lines of ITMI population

Genotype	Chlorophyll (mg/g)		Sugar (mg/g)		K^+:Na^+
	0 mM	75 mM	0 mM	75 mM	75 mM
ITMI-62	0.57	0.52	0.77	0.87	0.41
ITMI-70	0.71	0.69	0.63	0.86	0.33
ITMI-73	0.84	0.82	1.99	2.06	0.27
ITMI-74	0.87	0.87	1.14	1.24	0.42
ITMI-81	0.78	0.76	2.15	2.39	0.31
ITMI-87	1.21	0.94	1.22	1.29	0.43
ITMI-15	1.14	1.06	2.16	2.20	0.53
ITMI-103	1.10	1.06	0.63	0.80	0.39
ITMI-95	1.03	0.96	1.02	1.54	0.35
ITMI-31	1.35	1.24	0.79	1.44	0.52
ITMI-35	0.94	0.89	0.47	1.02	0.47
ITMI-9	0.87	0.84	0.61	0.68	0.63
ITMI-20	0.93	0.86	0.53	0.61	0.69
ITMI-110	1.10	1.02	0.72	0.92	1.00
ITMI-105	1.48	1.18	0.66	0.97	0.38
ITMI-26	1.33	1.25	0.89	0.92	0.57
ITMI-24	0.88	0.65	0.54	0.57	0.73
ITMI-55	1.00	0.87	0.86	1.50	0.74
ITMI-61	0.89	0.94	0.48	0.49	0.46
ITMI-63	1.29	0.66	0.59	0.70	0.39
ITMI-68	1.57	0.83	0.58	0.59	1.02
ITMI-90	1.11	0.87	0.73	0.78	0.57
ITMI-93	1.76	0.87	0.77	0.78	0.69
ITMI-33	1.02	0.67	0.58	0.62	0.79
ITMI-1	1.32	0.73	1.13	1.17	0.57
ITMI-89	1.15	1.05	1.64	1.67	0.81
ITMI-34	1.52	1.25	2.92	3.61	0.82
ITMI-144	1.49	0.50	1.49	2.08	0.77
ITMI-104	2.07	0.56	1.83	2.12	0.86
ITMI-2	1.34	0.62	0.80	0.83	1.18
ITMI-121	1.16	0.83	0.99	1.04	0.80
ITMI-55	1.48	0.90	0.71	1.37	1.37
ITMI-51	2.33	0.62	1.68	1.97	1.35
ITMI-122	1.11	1.35	1.69	1.96	1.11
ITMI-56	0.99	1.03	0.87	1.42	1.49
ITMI-69	1.53	1.38	0.8	0.92	0.91
ITMI-29	1.97	1.92	1.09	1.71	0.7
ITMI-19	1.85	1.10	1.95	2.06	1.37
ITMI-76	1.96	0.82	2.00	2.25	1.51
ITMI-26	1.23	1.17	1.88	2.23	1.13

Table 108. Analysis of variance summaries (mean squares) of shoot length (cm), root length (cm), shoot fresh weight (g), root fresh weight (g), shoot dry weight (g), and root dry weight (g) of the 97 RILs fo the ITMI mapping population grown at two salinity levels, 0 mM and 75 mM NaCl (* = significant).

Source of variation	Degrees of freedom	Shoot length (g)	Root length (g)	Shoot fresh weight (g)	Root fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Treatment	1	1,006.63*	108.022*	0.192*	0.061*	0.002*	1.614*
Variety	96	107.91*	16.9071*	0.043*	0.016*	2.593*	6.307*
Treatment x Variety	96	7.44	1.929	0.007*	0.004*	1.803*	4.4869*
Error	777	10.78	2.416	0.003	0.001	4.297	1.039

Table 109. Mean values for the 97 RILs of the ITMI population for shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight.

	Shoot length (cm)		Root length (cm)		Shoot fresh weight (g)		Root fresh weight (g)		Shoot dry weight (g)		Root dry weight (g)	
Genotype	0 mM	75 mM	0 mM	75 mM	0 mM	75 mM	0 mM	75 mM	0 mM	75 mM	0 mM	75 mM
ITMI-6	24.6	22.9	3.7	4.2	0.28	0.25	0.11	0.07	0.025	0.01	0.09	0.01
ITMI-8	26.1	22.7	5.8	7.6	0.25	0.21	0.11	0.06	0.020	0.01	0.01	0.01
ITMI-12	30.0	25.5	5.7	6.4	0.30	0.20	0.04	0.03	0.03	0.02	0.01	0.01
ITMI-13	28.1	26.7	4.6	4.7	0.26	0.24	0.04	0.03	0.02	0.01	0.004	0.01
ITMI-3	29.9	28.0	5.9	9.4	0.50	0.41	0.11	0.07	0.04	0.03	0.01	0.009
ITMI-4	27.8	25.1	3.7	4.7	0.32	0.23	0.06	0.07	0.02	0.01	0.007	0.003
ITMI-7	28.5	26.1	3.2	7.5	0.41	0.28	0.10	0.09	0.04	0.03	0.01	0.005
ITMI-11	25.5	24.4	5.9	6.5	0.40	0.30	0.11	0.06	0.03	0.02	0.01	0.01
ITMI-17	29.0	26.6	5.6	6.0	0.27	0.26	0.11	0.09	0.03	0.02	0.01	0.008
ITMI-18	29.0	26.6	5.4	6.0	0.33	0.27	0.10	0.09	0.03	0.02	0.01	0.005
ITMI-21	24.0	23.4	5.2	5.0	0.29	0.25	0.11	0.10	0.02	0.01	0.01	0.01
ITMI-25	27.0	24.2	7.0	7.0	0.39	0.30	0.15	0.13	0.04	0.02	0.01	0.01
ITMI-27	29.0	25.7	3.7	4.0	0.33	0.25	0.18	0.09	0.03	0.01	0.01	0.01
ITMI-28	25.8	21.8	5.2	5.2	0.43	0.30	0.18	0.13	0.02	0.01	0.01	0.01
ITMI-32	30.0	28.7	4.6	6.0	0.34	0.28	0.18	0.16	0.02	0.01	0.01	0.008
ITMI-36	24.0	20.8	6.0	6.0	0.37	0.26	0.19	0.12	0.02	0.01	0.01	0.01
ITMI-39	25.9	25.8	5.4	6.0	0.28	0.18	0.11	0.04	0.02	0.01	0.008	0.01
ITMI-40	27.9	25.6	4.7	5.1	0.34	0.19	0.17	0.04	0.03	0.02	0.01	0.004
ITMI-41	22.5	21.5	5.3	5.8	0.18	0.15	0.13	0.02	0.03	0.02	0.008	0.001
ITMI-44	25.2	22.9	4.0	4.2	0.21	0.17	0.03	0.01	0.03	0.02	0.01	0.01
ITMI-45	24.6	23.0	3.0	3.4	0.18	0.12	0.02	0.02	0.03	0.01	0.01	0.02
ITMI-46	27.2	18.6	5.2	4.8	0.20	0.23	0.02	0.01	0.03	0.01	0.009	0.02
ITMI-50	19.8	18.4	4.3	4.4	0.18	0.14	0.02	0.01	0.02	0.01	0.01	0.004
ITMI-52	24.7	17.0	4.3	4.4	0.17	0.22	0.04	0.03	0.03	0.02	0.02	0.02
ITMI-66	20.2	19.0	6.3	6.6	0.53	0.32	0.19	0.09	0.03	0.01	0.01	0.004
ITMI-67	26.8	25.1	4.3	6.0	0.25	0.27	0.20	0.14	0.02	0.01	0.01	0.01
ITMI-72	26.2	24.4	4.3	4.7	0.23	0.18	0.18	0.08	0.02	0.01	0.01	0.01
ITMI-75	22.0	21.0	2.8	3.8	0.18	0.15	0.09	0.06	0.02	0.01	0.006	0.01
ITMI-77	22.8	19.0	4.6	5.2	0.17	0.15	0.11	0.05	0.02	0.02	0.02	0.01
ITMI-78	28.7	26.5	3.6	4.0	0.29	0.25	0.02	0.01	0.03	0.02	0.02	0.01
ITMI-80	24.6	23.6	2.6	3.6	0.20	0.16	0.02	0.01	0.03	0.02	0.01	0.01
ITMI-82	24.3	22.2	4.6	4.6	0.16	0.17	0.02	0.01	0.02	0.02	0.01	0.004
ITMI-84	25.5	22.9	5.2	5.6	0.19	0.14	0.02	0.01	0.02	0.02	0.01	0.01
ITMI-85	26.1	26.0	3.3	3.9	0.26	0.19	0.03	0.01	0.03	0.01	0.01	0.005
ITMI-86	28.4	28.1	4.4	4.8	0.28	0.23	0.03	0.02	0.03	0.02	0.01	0.01
ITMI-88	24.3	20.0	4.5	5.3	0.23	0.17	0.04	0.03	0.03	0.02	0.01	0.01
ITMI-91	22.7	20.2	5.3	5.9	0.20	0.14	0.04	0.03	0.02	0.01	0.01	0.01
ITMI-92	19.1	17.6	2.8	3.1	0.14	0.13	0.01	0.02	0.021	0.01	0.004	0.01
ITMI-94	23.7	22.1	3.0	3.6	0.20	0.19	0.04	0.03	0.02	0.02	0.01	0.005
ITMI-96	20.0	19.5	3.2	2.5	0.12	0.12	0.02	0.01	0.01	0.02	0.01	0.005
ITMI-97	20.7	19.9	2.7	4.3	0.20	0.14	0.04	0.02	0.02	0.03	0.01	0.004
ITMI-98	27.2	26.5	2.5	4.8	0.20	0.15	0.02	0.03	0.02	0.02	0.01	0.01
ITMI-99	26.6	24.0	5.1	5.4	0.17	0.17	0.03	0.02	0.03	0.02	0.01	0.005
ITMI-101	22.3	22.2	2.4	2.6	0.16	0.13	0.02	0.01	0.01	0.02	0.01	0.01
ITMI-79	16.2	13.7	2.3	2.8	0.14	0.22	0.06	0.04	0.02	0.02	0.01	0.01
ITMI-81	13.7	12.7	3.02	3.9	0.16	0.11	0.04	0.03	0.02	0.02	0.01	0.01
ITMI-116	16.5	14.9	2.0	2.7	0.16	0.16	0.03	0.02	0.01	0.02	0.004	0.01
ITMI-85	16.6	16.4	3.3	3.6	0.17	0.14	0.04	0.03	0.02	0.03	0.01	0.01
ITMI-14	21.4	18.8	4.8	4.3	0.16	0.14	0.03	0.02	0.03	0.01	0.01	0.01
ITMI-16	20.9	19.7	4.5	5.0	0.13	0.12	0.04	0.03	0.02	0.02	0.01	0.01
ITMI-47	23.0	18.4	4.5	4.3	0.14	0.12	0.04	0.02	0.03	0.02	0.01	0.01
ITMI-49	21.4	19.2	5.0	5.5	0.15	0.12	0.10	0.09	0.02	0.01	0.01	0.01

Table 109. Mean values for the 97 RILs of the ITMI population for shoot length, root length, shoot fresh weight, root fresh weight, shoot dry weight, and root dry weight.

Genotype	Shoot length (cm)		Root length (cm)		Shoot fresh weight (g)		Root fresh weight (g)		Shoot dry weight (g)		Root dry weight (g)	
	0 mM	75 mM	0 mM	75 mM	0 mM	75 mM	0 mM	75 mM	0 mM	75 mM	0 mM	75 mM
ITMI-54	21.1	20.5	2.0	2.4	0.14	0.11	0.02	0.01	0.02	0.02	0.01	0.01
ITMI-57	25.5	23.8	2.9	3.4	0.12	0.20	0.05	0.04	0.03	0.02	0.01	0.01
ITMI-58	24.1	19.9	2.2	2.7	0.14	0.14	0.03	0.02	0.02	0.02	0.01	0.01
ITMI-59	19.3	18.9	2.1	3.1	0.15	0.13	0.02	0.01	0.01	0.03	0.01	0.01
ITMI-60	18.3	12.8	3.6	4.6	0.15	0.15	0.04	0.03	0.02	0.02	0.01	0.01
ITMI-62	22.1	18.1	4.2	5.2	0.16	0.17	0.07	0.05	0.03	0.02	0.01	0.01
ITMI-70	23.4	21.1	4.0	5.1	0.22	0.16	0.06	0.05	0.02	0.02	0.01	0.01
ITMI-73	21.1	20.3	3.8	4.0	0.16	0.13	0.04	0.03	0.01	0.03	0.004	0.01
ITMI-74	21.8	21.3	2.9	3.9	0.15	0.22	0.05	0.04	0.01	0.03	0.005	0.01
ITMI-81	21.5	20.5	3.6	4.4	0.13	0.13	0.08	0.06	0.01	0.02	0.01	0.01
ITMI-87	24.3	23.1	4.6	6.2	0.22	0.23	0.06	0.04	0.05	0.01	0.01	0.01
ITMI-15	19.8	18.0	3.0	3.1	0.32	0.29	0.11	0.08	0.05	0.03	0.02	0.01
ITMI-103	19.9	18.7	2.6	2.9	0.15	0.15	0.04	0.02	0.01	0.01	0.005	0.01
ITMI-95	18.2	15.1	1.2	1.4	0.15	0.18	0.03	0.01	0.03	0.02	0.01	0.002
ITMI-31	23.6	21.1	3.1	5.2	0.24	0.22	0.07	0.06	0.02	0.04	0.01	0.01
ITMI-35	22.8	20.8	2.8	3.8	0.29	0.25	0.10	0.08	0.02	0.02	0.01	0.01
ITMI-9	22.4	21.4	3.6	4.0	0.18	0.28	0.05	0.04	0.02	0.02	0.01	0.02
ITMI-20	21.2	19.3	3.7	4.1	0.15	0.12	0.05	0.04	0.02	0.02	0.004	0.01
ITMI-110	20.2	18.0	1.3	4.2	0.17	0.15	0.04	0.03	0.02	0.02	0.01	0.05
ITMI-105	19.3	15.8	2.7	4.1	0.14	0.19	0.07	0.03	0.02	0.03	0.005	0.01
ITMI-26	20.0	18.7	2.8	3.2	0.16	0.15	0.06	0.05	0.02	0.01	0.01	0.01
ITMI-24	20.9	19.8	2.8	3.2	0.17	0.13	0.06	0.05	0.01	0.02	0.007	0.01
ITMI-55	23.6	21.8	4.1	4.0	0.23	0.20	0.06	0.04	0.02	0.02	0.005	0.01
ITMI-61	23.7	22	3.9	4.4	0.21	0.24	0.08	0.07	0.02	0.02	0.005	0.01
ITMI-63	19.8	18.8	3.1	3.7	0.19	0.16	0.06	0.04	0.02	0.01	0.006	0.01
ITMI-68	25.2	23.6	3.3	3.8	0.23	0.22	0.07	0.06	0.04	0.04	0.005	0.01
ITMI-90	22.5	21.7	2.8	4.3	0.21	0.12	0.10	0.04	0.02	0.01	0.01	0.005
ITMI-93	23.0	22.2	2.8	6.1	0.23	0.22	0.06	0.05	0.02	0.02	0.08	0.01
ITMI-33	20.4	19.4	4.2	5.5	0.13	0.21	0.17	0.03	0.02	0.02	0.01	0.01
ITMI-1	24.0	23.0	2.0	3.4	0.14	0.13	0.05	0.06	0.02	0.03	0.01	0.01
ITMI-89	20.4	17.6	2.9	2.8	0.14	0.14	0.05	0.03	0.02	0.01	0.01	0.01
ITMI-34	21.8	17.4	2.7	2.3	0.16	0.12	0.07	0.04	0.02	0.02	0.01	0.003
ITMI-144	19.0	18.2	1.5	1.2	0.13	0.13	0.04	0.02	0.02	0.01	0.005	0.004
ITMI-104	26.6	23.6	2.3	4.9	0.13	0.17	0.02	0.02	0.02	0.02	0.01	0.004
ITMI-2	19.7	18.7	2.4	2.9	0.21	0.15	0.07	0.05	0.01	0.02	0.003	0.01
ITMI-121	23.5	20.2	2.5	3.6	0.24	0.15	0.07	0.04	0.02	0.02	0.004	0.01
ITMI-55	23.0	20.1	3.8	4.5	0.16	0.15	0.02	0.01	0.03	0.02	0.01	0.004
ITMI-51	18.8	17.3	1.6	3.3	0.13	0.12	0.03	0.02	0.02	0.01	0.005	0.004
ITMI-122	23.3	22.2	2.2	1.3	0.22	0.16	0.05	0.08	0.02	0.02	0.01	0.01
ITMI-56	27.9	19.4	3.0	3.2	0.17	0.14	0.06	0.09	0.02	0.01	0.01	0.01
ITMI-69	26.9	22.8	2.1	2.3	0.31	0.29	0.12	0.10	0.02	0.02	0.01	0.01
ITMI-29	21.2	21.5	2.3	2.7	0.24	0.28	0.07	0.06	0.02	0.02	0.01	0.01
ITMI-19	20.2	19.8	1.7	2.9	0.16	0.24	0.07	0.06	0.02	0.01	0.01	0.005
ITMI-76	22.6	22.4	5.2	4.1	0.23	0.19	0.06	0.05	0.02	0.02	0.01	0.01
ITMI-26	25.2	25.7	2.0	2.4	0.19	0.17	0.06	0.08	0.02	0.02	0.01	0.01

in control and 0.27 at salt stress condition (Table 109, pp. 240-241).

Of the 97 RILs, ITMI-3, ITMI-17, and ITMI-32 performed better with respect to morphological, physiological, and biochemical attributes. Lines ITMI-85, ITMI-90, and ITMI-66 did not perform with good results with respect to biomass production, morphological, physiological, and biochemical attributes, thus, they were not salt tolerant.

Total chlorophyll also decreased in the population. Line ITMI-6 has the highest chlorophyll content and ITMI-3 the lowest. The reduction in chlorophyll content is to be expected under stress. Being membrane bound, chlorophyll stability dependends on membrane stability, which under saline condition seldom remains intact. In this study, the minimum reduction in chlorophyll content was observed in ITMI-3, ITMI-32, and ITMI-17 and, because of this positive response, these lines are tolerant.

The ITMI population was subjected to salt stress of 75mM NaCl and highly significant differences among the RILs were found for all the morpho-physiological traits studied. At salt stress conditions, the K^+:Na^+ ratio decreases. Salinity tolerance in wheat is associated with the accumulation of K^+ and exclusion of Na^+ under saline conditions. In our study, a similar percent decrease in the K^+:Na^+ ratio was found. Genotype ITMI-1 has the highest K^+:Na^+ ratio and ITMI-21 has the lowest.

During salt stress, an increase in sugar concentration has been reported in many species. Sugar might contribute to salt stress tolerance either by serving as osmotic or as respiratory substrate. High sugar under salt stress prevents plants from oxidative damage and maintains the structure of different proteins and membranes. Similar behavior was observed in tje population with an increase of sugar content in ITMI-17 and ITMI-32, thus showing their tolerant behavior, whereas ITMI-3 showed less sugar content under salt stress.

Table 110. Mean values for plant height, spike length, number of grains/spike, number of spikelets/spike, and 1,000–kernel weight for 97 RILs of the ITMI mapping population (not pubescent (–) or pubescent (+)).

Entry (ITMI–)	Pubes-cence	Plant height (cm)	Spike length (cm)	Grains/ spike	Spikelets/ spike	1,000-kernel weight (g)
1	–	110.0	11.2	26	20	34.2
2	–	97.0	11.9	32	22	28.7
3	+	103.9	12.0	54	18	35.5
4	+	103.9	11.3	32	21	35.4
6	–	102.8	11.2	43	18	29.9
7	+	99.4	9.8	21	17	27.7
8	+	86.9	10.1	22	19	20.1
9	+	90.9	10.4	26	18	30.9
11	+	93.1	10.6	45	17	30.5
12	–	115.1	11.35	42	17	36.7
13	+	89.6	10.0	37	18	36.9
14	+	97.6	10.1	26	19	32.6
16	–	86.9	8.1	29	17	29.5
17	–	84.5	8.0	52	17	34.9
18	–	97.0	10.5	50	16	37.0
19	+	97.5	10.3	48	18	35.9
20	+	97.0	9.86	27	17	36.0
21	+	104.0	9.75	38	19	35.9
22	+	101.8	9.8	50	21	41.0
24	+	103.7	10.0	26	18	40.8
25	+	104.9	10.9	27	19	44.6
26	+	98.0	9.3	26	16	39.7
27	+	96.8	10.1	34	17	35.8
28	–	75.0	9.1	43	17	33.9
29	+	77.6	9.6	47	19	37.0
31	+	97.5	12.9	38	22	40.0
32	–	92.3	11.1	42	23	42.3
33	+	88.1	10.1	45	23	36.8
34	+	93.4	9.9	19	23	37.4
35	+	95.1	10.7	34	18	36.5
36	+	86.6	10.5	27	18	35.3
39	–	77.3	10.3	36	18	44.6
40	–	94.3	9.3	45	18	40.5
41	–	89.6	10.8	52	18	33.2
44	+	84.5	10.6	50	20	33.3
45	–	78.0	10.1	49	17	30.6
46	+	116.1	9.6	39	17	43.0
47	+	113.7	9.7	20	18	41.6
49	+	85.3	9.4	29	18	32.7
50	+	81.9	8.3	43	17	34.9
51	+	94.0	10.6	43	17	31.4
52	–	92.3	9.3	52	16	43.6
53	+	93.1	9.6	50	17	43.2
54	+	90.0	8.3	26	18	29.6
55	+	81.4	9.1	25	18	39.3

Salinity reduced the dry weight of roots and shoots by 52.5% and 60.6%, respectively, for plant grown on sand soil compared with those grown in clay soil. On the other hand, the dry weight of roots and shoots decreased by about 24% and 21%, respectively, due to salinity compared with the control treatment. ITMI-3 and ITMI-32 showed the least dry biomass and, on this basis, were considered tolerant. Salinity inhibits the growth of many plants.

Shoot fresh weight and root fresh weight are generally retarded with elevated salinity in wheat. ITMI-3, ITMI-17 and ITMI-32 showed a minimal reduction in their fresh biomass. Increasing the concentration of NaCl significantly reduces plant growth. This reduction in root and shoot fresh weight is due to less uptake of essential nutrients.

in vitro studies indicate that ITMI-77, ITMI-8, ITMI-6, ITMI-50, ITMI-51. ITMI-117, ITMI-21, ITMI-75, ITMI-44, and ITMI-3 performed well, especially with reference to K^+:Na^+ discrimination.

Phenotypic evaluation. Morphological characters of selected ITMI lines were assessed phenotypically (Table 110, pp. 242-243).

Plant height (cm). Moderate plant height is good for a crop because it makes the mechanical operations easy and also reduces the chances of loss from lodging. The value for plant height ranged from 55.1 cm to 116.1 cm. Maximum height was observed ITMI-46 and the minimum was in ITMI-120.

Pubescence. Data for pubescence were taken using hand lens by observing hairs on the base of spikes, i.e., the peduncle. Pubescence was scored as absent (–) or present (+). Most lines were pubescent (+).

Table 110. Mean values for plant height, spike length, number of grains/spike, number of spikelets/spike, and 1,000–kernel weight for 97 RILs of the ITMI mapping population (not pubescent (–) or pubescent (+)).

Entry (ITMI–)	Pubes-cence	Plant height (cm)	Spike length (cm)	Grains/ spike	Spikelets/ spike	1,000-kernel weight (g)
56	+	94.8	11.0	27	18	41.7
57	–	107.9	11.4	29	18	41.6
58	–	110.1	11.6	30	19	39.9
59	+	104.4	13.8	35	20	38.8
60	+	101.0	13.1	32	20	42.4
61	+	97.0	9.0	22	16	41.1
62	+	90.4	7.8	29	17	32.5
63	+	91.4	9.3	28	17	41.9
66	+	95.4	10.4	27	15	42.6
67	+	103.8	11.8	18	21	48.8
68	+	98.6	12.1	46	21	49.1
69	+	98.8	11.5	33	20	47.3
70	+	99.4	12.1	36	18	44.8
72	+	87.3	10.5	32	17	38.1
73	+	108.6	9.6	19	17	38.5
74	+	99.4	11.4	40	18	39.0
75	+	103.6	11.1	42	17	47.5
76	+	94.3	8.3	39	17	45.4
77	–	107.9	8.8	36	15	38.8
78	+	97.9	10.2	29	20	39.5
79	+	93.3	11.5	35	19	36.9
80	–	96.5	11.5	44	20	40.9
81	+	91.5	11.3	51	19	48.3
82	+	93.2	10.4	50	20	37.5
83	+	85.8	8.3	52	20	33.8
84	–	80.0	9.0	38	18	34.2
85	+	82.7	11.4	33	21	35.3
86	+	82.4	10.9	27	18	40.2
87	+	88.6	7.4	47	17	35.7
88	+	103.5	11.8	32	18	40.3
89	+	83.0	7.0	28	18	39.3
90	+	83.6	8.4	44	17	42.4
91	+	70.9	8.4	46	20	31.5
92	–	97.4	12.4	36	19	37.1
93	–	96.7	11.9	39	18	40.4
94	+	65.2	8.2	45	18	29.3
95	+	70.6	12.4	32	18	22.5
96	+	68.6	10.5	36	14	23.3
97	+	66.3	8.8	27	14	33.2
98	+	85.1	10.5	20	23	34.1
99	+	86.4	11.0	34	21	35.9
101	+	85.3	8.4	50	19	36.8
103	+	90.8	8.3	29	19	38.7
104	+	93.1	11.1	26	19	34.0
105	–	92.0	10.8	38	20	31.9
110	+	87.8	11.0	30	19	32.0
117	–	80.7	10.3	29	23	38.6
120	+	55.1	10.7	32	20	40.6
121	+	64.0	10.1	44	20	35.6
122	+	85.3	12.9	39	19	38.3
144	–	85.5	11.5	30	21	36.6

Spike length. Spike length was measured in centimeters and ranged from 7.0 cm to 13.8 cm. Lines ITMI-17, ITMI-62, and ITMI-87 had the shortest and ITMI-59 (13.8 cm) had the longest spikes.

Number of grains/spike. The data for number of grains/spike ranged from 15 to 54. Line ITMI-3 had the maximum number and line ITMI-18 the least; this was a great range of variation.

Number of spikelets/spike. The number of spikelets/spike in ITMI RIL population ranged from 15 to 23. Genotypes with long spikes had a greater number. Lines ITMI-32, ITMI-33, ITMI-98, and ITMI-117 had the maximum number.

1,000-kernel weight. Grain weight is major component of seed yield. For each entry, 1,000-kernel weight was calculated, and it ranged from 20.1 to 49.1 g. Line ITMI-68 was very good and useful in wheat breeding. The above mentioned genotypes are suggested as agronomically good with high yield potential. This morphological analysis showed that there is maximum diversity among the ITMI population.

Physiological characterization of ten selected lines. The top ten lines, ITMI-77, ITMI-8, ITMI-6, ITMI-50, ITMI-51, ITMI-117, ITMI-21, ITMI-75, ITMI-44, and ITMI-3, were selected on the basis of their performance on K^+:Na^+ discrimination levels (Table 111, p. 246). The maximum shoot length was in ITMI-3 (25.06 cm) and the minimum was in ITMI-117 (14.94 cm). The greatest root length was found in ITMI-3 (5.9 cm), followed by ITMI-8, ITMI-50, and ITMI-6. The largest shoot fresh weight was 0.28 g in ITMI-3 and lowest, at 0.12 g, was found in ITMI-50 and ITMI-51.

Root fresh weight under salt stresss among the top 10 lines was found highest in ITMI-6 (0.07 g) and 0.01 g at control conditions, indicating that root fresh weight decreased at high salinity levels (Table 111). Chlorophyll content ranged from 2.01 to 0.42. The highest amount of chlorophyll was found in ITMI-6 (2.01) and the lowest in ITMI-33 (0.42). Sugar content increased at 75 mM NaCl in the top 10 tolerant lines, ranging from 0.51 to 3.72. The lowest sugar content was detected in ITMI-44 and the highest in ITMI-3, followed by ITMI-51, ITMI-8, ITMI-50, ITMI-21, and ITMI-117. Among top ten lines, the K+/Na+ ratio ranged from 2.69 to 1.03. The highest range of K^+:Na^+ was found to be 2.69 (ITMI-51) followed by ITMI-50, ITMI-44, and ITMI-75.

Table 111. Mean evaluation of morphological parameters of top 10 tolerant RILs in the ITMI Mapping Population at 0 mM and 75 mM NaCl.

	Shoot length (cm)		Root length (cm)		Shoot fresh weight (g)		Root fresh weight (g)		
Genotype	**0 mM**	**75 mM**	**0 mM**	**75 mM**	**0 mM**	**75 mM**	**0 mM**	**75 mM**	
ITMI-77	19.82	18.82	3.72	3.12	0.19	0.16	0.06	0.04	
ITMI-8	26.10	22.78	7.60	5.84	0.25	0.21	0.11	0.06	
ITMI-6	24.600	22.92	4.20	3.70	0.28	0.25	0.11	0.07	
ITMI-50	20.92	19.78	5.08	4.50	0.13	0.12	0.04	0.03	
ITMI-51	23.04	18.40	4.38	3.20	0.15	0.12	0.04	0.03	
ITMI-117	16.52	14.94	2.70	2.02	0.16	0.15	0.03	0.02	
ITMI-21	24.62	23.08	3.44	3.06	0.18	0.12	0.02	0.01	
ITMI-75	23.62	21.84	4.08	3.14	0.23	0.20	0.06	0.04	
ITMI-44	22.34	20.20	2.66	2.42	0.16	0.13	0.02	0.01	
ITMI-3	29.96	25.06	9.42	5.90	0.50	0.28	0.11	0.60	
	Shoot dry weight (g)		**Root dry weight (g)**		**Chlorophyll (mg/g)**		**Sugar (g)**		
Genotype	**0 mM**	**75 mM**	**0 mM**	**75 mM**	**0 mM**	**75 mM**	**0 mM**	**75 mM**	**K^+:Na^+**
ITMI-77	0.02	0.01	0.01	0.008	0.69	0.63	0.60	0.76	1.04
ITMI-8	0.02	0.10	0.01	0.009	2.11	1.48	0.37	1.85	1.23
ITMI-6	0.02	0.01	0.01	0.008	2.11	2.01	0.50	0.68	1.35
ITMI-50	0.02	0.02	0.01	0.009	1.23	1.07	1.12	1.69	2.60
ITMI-51	0.03	0.02	0.01	0.010	2.33	0.62	1.68	1.97	2.69
ITMI-117	0.02	0.01	0.01	0.008	0.78	0.67	1.22	1.24	1.61
ITMI-21	0.03	0.20	0.01	0.017	1.54	1.19	1.32	1.40	1.03
ITMI-75	0.02	0.10	0.01	0.008	0.52	0.45	0.66	1.06	1.70
ITMI-44	0.02	0.10	0.01	0.007	0.73	0.73	0.50	0.51	1.77

The best top lines were then subjected to higher salinity level, 100 mM NaCl, and tested for protein, proline, and sodium oxide dismutase (SOD) levels (Table 112). Superoxide dismutase increased under stress condition, the value ranging from 80.47 to 28.86, in ITMI-8 and ITMI-77, respectively. The highest SOD value was in ITMI-8, followed by ITMI-51, ITMI-50, ITMI-6, ITMI-117, ITMI-3, ITMI-75, ITMI-21, ITMI-44, and ITMI-77. Similarly the proline and protein content also increased under stress conditions. Among all the lines, the best proline content was found in ITMI-3 (1,164.1) and the highest protein content was recorded in ITMI-8. K^+:Na^+ ranged from 1.00 to 1.69, where the highest value was observed in ITMI-51, followed by ITMI-50, ITMI-3, ITMI-75, ITMI-44, and ITMI-6. The performance of top ten selected lines was checked at both 75 mM and 100 mM salinity levels. The overall performance of the ten lines was found to ITMI-3, ITMI-22, ITMI-25, ITMI-31, ITMI-52, ITMI-59, ITMI-68, and ITMI-81.

Table 112. Mean evaluation of physiological parameters of top 10 tolerant lines at 100 mM NaCl (SOD = superoxide dismutase).

Genotype	SOD	Protein	Proline	SDW	K^+:Na^+
ITMI-77	28.863	951.562	951.562	0.019	1.000
ITMI-8	80.471	820.369	820.369	0.026	1.060
ITMI-6	60.510	166.067	166.067	0.016	1.110
ITMI-50	63.275	1,150.842	1,150.842	0.022	1.500
ITMI-51	64.314	345.419	345.419	0.016	1.690
ITMI-117	56.176	255.743	255.743	0.028	1.090
ITMI-21	32.392	114.586	114.586	0.019	1.000
ITMI-75	40.000	1,081.094	1,081.094	0.022	1.210
ITMI-44	29.235	242.457	242.457	0.020	1.120
ITMI-3	49.392	1,164.127	1,164.127	0.017	1.220

Estimating genetic diversity in the ITIM Mapping Population. SSR markers (*Xwmc-304*, *Xwmc-179*, *Xwmc-18*, *Xwmc-134*, *Xwmc-134*, *Xwmc-141*, *Xwmc-154*, *Xwmc-160*, *Xwmc-11*, *Xwmc-116*, and *Xwmc-84*) were used to assess the genetic diversity among the ten tolerant genotypes of the ITMI Mapping Population. All the SSR markers gave clear and polymorphic bands. The range of the bands was 100–800 bp. Genetic diversity of top 10 salinity-tolerant genotypes was estimated from a dendrogram and similarity matrix. Dendrogram and similarity matrix were constructed using amplification (bivariate 1–0) data generated by SSR primers.

Dendrogram. In the dendrogram, genotypes are grouped on the basis of their genetic similarities and differences, using UPGMA. Based on their genetic distance, the clustering pattern of the 10 wheat genotypes is divided into three main clusters A, B and C (Fig. 42). The dendrogram reveals that these ten genotypes are genetically diverse from each other. Among these genotypes, ITMI-21 is most diverse, not forming any cluster with any other genotype. Cluster A had four genotypes, ITMI-3, ITMI-51, ITMI-8, and ITMI-77. Out of these four, ITMI-77 is the more diverse and ITMI-3 and ITMI-51 had about 80% genetic similarity or 20% genetic diversity. The other main cluster (cluster B) contained five genotypes, ITMI-6, ITMI-44, ITMI-75, ITMI-50, and ITMI-17. Among these five genotypes, ITMI-6 is more diverse than other four genotypes. Although ITMI-44/ITMI-75 and ITMI-50/ITMI-17 showed the maximum genetic similarity of 80% or 20% genetic diversity.

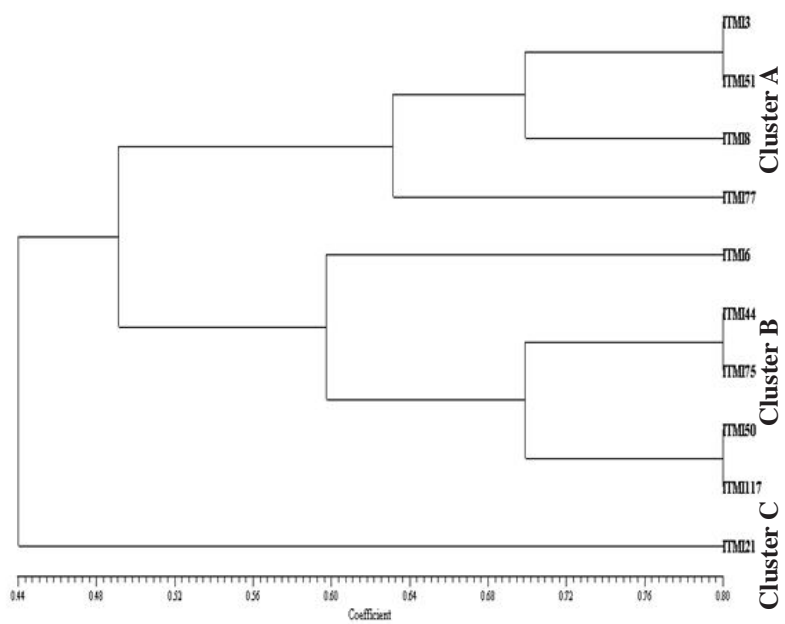

Fig 42. A dendrogram of 10 tolerant genotypes from the ITMI Mapping Population based on SSR markers.

Similarity matrix. A similarity matrix of top 10 lines based upon Nei and Li's similarity coefficient gives the degree of similarity among the genotypes (Table 113). Values range from 1–0, for which 1 represents 100% similarity and 0 represents 100% genetic diversity/distance. Ten microsatellite loci were used for this purpose. The range of genetic similarity was 20–80% among these genotypes. Maximum similarity was observed between ITMI-3 and ITMI-51, ITMI-44 and ITMI-75, and ITMI-50 and ITMI-17, and the minimum similarity and maximum genetic diversity was observed between ITMI-21 and ITMI-77.

Table 113. Similarity matrix among ten tolerant genotypes from the ITMI Mapping Population based on SSR markers

	3	6	8	21	44	50	51	75	77	117
3	1.00									
6	0.40	1.00								
8	0.60	0.40	1.00							
21	0.70	0.50	0.30	1.00						
44	0.60	0.60	0.60	0.30	1.00					
50	0.50	0.50	0.50	0.40	0.70	1.00				
51	0.80	0.40	0.80	0.50	0.60	0.50	1.00			
75	0.60	0.60	0.60	0.50	0.80	0.70	0.60	1.00		
77	0.50	0.30	0.70	0.20	0.70	0.60	0.70	0.50	1.00	
117	0.50	0.70	0.30	0.60	0.70	0.80	0.30	0.70	0.40	1.00

Conclusion. The salt tolerance potential of germ plasm was determined using the in vitro evaluation parameter K^+:Na^+ discrimination. The entire ITMI Mapping Population of 97 lines was subjected to hydroponic tests at 75 mol/m^3 NaCl, phenotypic characterization, and a molecular diversity estimation. For salinity tolerance, genotypes ITMI-51, ITMI-50, ITMI-75, and ITMI-44 were found to be most tolerant to salinity with high K^+:Na^+ values. In addition, ITMI-3, ITMI-8, and ITMI-6 were found to be tolerant because they show better performance for chlorophyll, sugar, shoot length, shoot fresh weight, root fresh weight, and dry mass.

A phenotypic study of the material revealed that ITMI-3, ITMI-22, ITMI-25, ITMI-31, ITMI-52, ITMI-59, ITMI-68, and ITMI-81 had good performance for plant height, grains/spike, spikelets/spike, spike length, and grain weight (Table 114). On the other hand, genotype ITMI-68 showed the highest grain weight (Table 114).

Table 114. Evaluation of phenological parameters of the top eight lines (pubescence; – (absent) or + (present)).

Entry	Pubescence	Plant height (cm)	Spike length (cm)	Grains/spike	Spikelets/spike	1,000-kernel weight (g)
ITMI-3	+	103.9	12.0	54	18	35.5
ITMI-22	+	101.8	9.8	50	21	41.0
ITMI-25	+	104.9	10.9	27	19	44.6
ITMI-31	+	97.5	12.9	38	22	40.0
ITMI-52	–	92.3	9.3	52	16	43.6
ITMI-59	+	104.4	13.8	35	20	38.8
ITMI-68	+	98.6	12.1	46	21	49.1
ITMI-81	+	91.5	11.3	51	19	48.3

From present study, we found that this experimental material provides a good source of tolerance to salinity and is agronomically good with a high level of genetic diversity, which is a prerequisite of any crop improvement program. We conclude, therefore, that this ITMI Mapping Population is a valuable source for genetic improvement of wheat for salinity tolerance.