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ITEMS FROM PAKISTAN

NATIONAL AGRICULTURAL RESEARCH CENTER (NARC), ISLAMABAD
Wheat Wide Crosses, NARC, Islamabad, Pakistan.

The development of a wide-cross program in wheat in Pakistan.

A. Mujeeb-Kazi, Alvina G. Kazi, and Iqbal Ayub Khan.

The unequivocal status of wheat importance as a food cereal is paramount and the need to be on secure production grounds a national priority. A national coördination program exists that has alliances with all professionals involved in wheat improvement across the country with international linkages. However, the changing international scenarios

around wheat production in light of productivity constraints and new, sophisticated technologies necessitate that Pakistani researchers move with time and be proactive. This involves a swift research program re-structuring that generates outputs efficiently and execution of which demonstrates optimum use of top-class professional and economic factors.

National wheat yields are 2.6 t/ha and annual productivity around 21.6 x 10^6 tons as of mid-2008; a decrease from the 23.5 x 10^6 tons in mid-2007. An increase in productivity is necessary in the coming years to keep pace with population increases and food necessities. Global figures show that from the current 6.3 billion people around 8.2 billion will inhabit the planet by 2025, requiring a substantial annual increase in order to cope with this elevated population need. In Pakistan the short-term goals set for yield increases are to reach 2.9 t/ha, thus increasing the total yield to 26.41 x 10^6 t by mid-2010. This necessitates a consistent per annum growth and is an uphill task that requires some astute pro-active measures across several wheat research and developmental scenarios. These measures will encompass a wide range of factors that would integrate several disciplines within Pakistan and across our country boundaries. The strengthening emphasis will stringently focus upon time bound multifaceted integrated activities where the pre-requisite factors to determine such goals will impinge upon policy setting, partitioning of basic, strategic, applied research scenarios and timely facilitating budgetary allocations. The area under wheat cultivation has progressively increased and may have reached its maximum of 8.29 million hectares as of the 2004–05 crop cycle. Increasing planted area and not enhance yield levels per unit area is not a valid production strategy. Thus, the need to adapt to this situation and other production aspects requires a vision that recognizes change and addresses it through integrative technologies harnessing selective national and international expertise. Furthermore, the pressures of set cropping systems and international pathogenic variations pose a grave threat to our national production levels of wheat. A concerted effort is crucial to combat these looming constraints and give varietal outputs that will provide national security around durable resistance levels that can only be realized if we have the genetic strength in place via gene and varietal deployment coupled with changed outlook for wheat research.

Ideally, strengthening the wheat program should not relate to financial inputs alone, but should translate into 'scientific' strengthening structured across quality scientific scenarios that require a completely different operational mode in order to bring quantum productivity increases that Pakistan urgently needs.

Production is a realization contribution that is essentially controlled by environment, genetics of the crop, plus management. All parameters have to be in unison to provide maximum impact. Over the years we have seen shifts in stress factors that control biotic and abiotic stresses, seen an emergence of new management technologies, emphasis directed towards diverse cropping systems, prevailing dominance of mono-culture of a few varieties, seed supply and extension avenues being addressed or remaining elusive, and more emphatically attention being placed upon budgetary constraints. Thus, many facets are known to govern a crops performance with all being vital for delivering the end output measured by t/ha and the resulting annual national yield levels. Some clear priorities can be set and, if these are the major biotic or abiotic stresses that limit wheat production, then around these stress constraints will be embodied several supporting multiple objectives crucial for the crops performance where key abiotic or biotic stresses will also warrant research attention. If the genetic resource is scarce to provide the relevant genes then one has to rely on internationally acquired materials that fit the category of conventional and novel resources obtained from all contributors of the wheat families relatives.

Our reliance has accordingly been heavy on international nurseries and these to date have played a crucial role in Pakistan agriculture. The trend will continue to flourish but we are now seeing the dawn of new methodologies where hard to obtain resources can be assembled and harnessed. The future ahead for deriving maximum benefit from all types of genetic materials, utilize all applicable technologies that cover the three domains of basic, strategic and applied activities is well apparent to research professionals but not fully operational in Pakistani wheat research endeavors. On the applied front, major limitations that surround wheat productivity are combating stress constraints that encompass drought, heat, salinity, all three rusts, increase in aphid populations, observations for powdery mildew, barley yellow dwarf virus, Karnal bunt, and grain quality for starters. Other constraints are prevalent but to a lesser degree (eg., *Bipolaris sorokiniana* being one) and require attention for which a comfortable situation would be to rigorously monitor progress and development of all stresses even to the extent that we need to be cognizant of the situation beyond our own national boundaries. The danger of stem rust around Ug99 plus its variants from Kenya via Iran. Despite the constraint, priority production can only occur and be sustainable if multiple stress factors are well targeted around gene pyramiding strategies. These strategies have their roots within the explanation that follows.

Two other phases of crop improvement programs revolve around basic and strategic research. These are the

home grounds of quality scientific innovations and accordingly are complex to manage for applied goal pursuits. Over the last two decades however, the backbone structure has been well explained and a superior comfort zone for achieving success on a projected time scale has become visible. These two areas will tap on unique genes from hard to combine wheat relatives, place them in their best location within our top cultivars, and provide adequate genetic structure diversity associated with durable resistance potent to offer a sustainable production system.

Interlinked with the above three phases of research/production activities will be new efficiency enhancing technologies with their focus to be set by the priority goals of a wheat improvement program. To make such techniques viable it is imperative that capable groups in excellent structural surroundings and compatible minds are combined for collaborating and giving osmotically superior outputs around synergism. The current focus would elucidate the DNA polymorphism status of our wheat germ plasm so we can better use such germ plasm for our practical benefits and will be the basis of cementing genetic diversity in our wheat cultivars.

A mere understanding of diversity will not resolve the situation. There is a dire need to put this knowledge into applied domains in order to unravel the contributions of each wheat genome as well as its constituting chromosomes, thus allowing for finer data generation based upon which genes could be tagged and molecular mapping conducted. Hence, if the focus is rust resistance our strategy should be to develop such wheat varieties with multiple resistances aided by all top class tools of fungal diagnostics and screening sophistication with the ultimate correlation to be made through structured mapping populations that would unravel the genetic elements that contribute to this biotic stress trait. Supplementary to such biotechnological links would also be the doubled haploid technology that not only can assist the molecular mapping area but can also significantly reduce the time for variety stabilization by several generations.

In order to further the objectives of the program breeding initiatives will digress from the current prevalent approaches within the country to focus actively on accessing diversity that has either been scarcely used or not utilized at all. These sources are as follows:

1. use the mammoth diversity of the accessions of each of the three progenitor genomes of wheat;
2. exploit the AB-genome tetraploids suca as *T. turgidum* subsps. *dicoccum*, *dicoccoides*, and *carthlicum* and the ABD diversity of *T. aestivum* subsp. *spelta*;
3. exploit the following germ plasm for yield enhancement:
   a) the multiple-ovary trait that sets three seed/floret,
   b) synthetics with high 1,000-kernel weight, i.e., 60–65 g versus the normal 40–44 g,
   c) the large-spike character present in Buitre-type T7DS·7DL-7AG wheats called super wheats,
   d) the heterotic vigor of F_1 derivatives from quality wheat/wheat cross combinations that structure a hybrid wheat program using the F_1-based, doubled-haploid strategy,
   e) wheat/alien chromosome translocations other than the famous T1BL·1RS, and
   f) target genes for addressing stress constraints mediated by molecular markers for breeding efficiency;
4. in addition to 'adaptation' breeding, develop a volatile recombinant-breeding program that uses the elite, older cultivars not in present day use due to some stress susceptibility, land races, and novel genetic diversity by a limited backcrossing approach coupled by F_3-based doubled haploidy input.

A modified structure was initiated by the Federal Ministry of Agriculture under the leadership role given to a former CIMMYT wide cross expert who started activities in Pakistan from the end of 2004 from allocation in NWFP, Peshawar. The location changed to Islamabad in mid-2005 and since then the process of wheat research has taken up around the focus mentioned above. The basic theme of activities is to establish a infra-structure, generate a integrated research team and embark on a research program targeted to provide practical outputs. The *modus operandi* has been to maintain strong alliances with CIMMYT, Mexico, harness national linkages, have a multidisciplinary research team at base in Islamabad, extend further international alliances and generate lucrative funding through national and international sources.

After four regular and a few summer crop cycles the status of the wide cross and conventional outputs are highlighted in the *Newsletter* for the global community of colleagues to be informed of our building and initial wheat improvement efforts in Pakistan.

Evaluation of wheat germ plasm for resistance against Karnal bunt in Pakistan.

Muhammad Zakria, Javed Iqbal Mirza, Alvina G. Kazi, and Abdul Mujeeb-Kazi.

Karnal bunt or partial bunt of wheat, caused by *T. indica*, is a disease of concern globally and is also a serious quarantine issue. Thus, cultivar release requirements include resistance to Karnal bunt as mandatory in Pakistan. Strict quarantine measures have been adopted in several countries that affect not only wheat grain trade but also germ plasm exchange.

Because the pathogen is seed, soil, and air borne, limited control is achieved through the application of fungicides (Singh et al. 1985). Crop rotation, seed certification, and different fungicide treatments can be used to manage the disease. However, these methods may not eliminate the disease. The preferred method of control is by developing resistant cultivars through screening against *T. indica*. Breeding for Karnal bunt-resistant cultivars requires a reliable screening method that facilitates the selection of segregating plants. Screening is by creating artificial epiphytotic conditions at boot leaf stage.

The combination of resistance from *Ae. tauschii* with field resistance of durum through synthetic hexaploids wheats can exploit the combined resistance of A, B, and D genomes for wheat improvement. This involves identifying synthetic wheats with resistance to Karnal bunt and then incorporating these synthetics in the breeding effort. Synthetics can be successfully crossed with commercial wheat cultivars (Mujeeb-Kazi and Rajaram 2002).

Germ plasm of 1,500 entries comprising of conventional wheat cultivars, synthetics, and their advanced derivates from crosses with bread wheats were screened for resistance against Karnal bunt. Screening protocols were similar to those reported by Mujeeb-Kazi et al. 2006. Thirty-three percent of the germ plasm was free of Karnal bunt. The limited conventional bread wheat lines included were predominantly susceptible. The advanced test lines derived from conventional wheat cultivars crossed with resistant synthetic hexaploids possessed a high frequency of derivatives that were Karnal bunt free. Disease ratings were from 0% to 65.0% and this screening is being repeated in the current cycle of 2008-2009. The lines after the 2008 May harvest were planted in Kaghan over the summer cycle and analyzed for powdery mildew resistance and this year yellow rust evaluations also are being conducted along with the KB screening. The cumulative screening will allow selection of entries with multiple biotic stress resistances coupled with superior phenological attributes required for varietal outputs. Only those entries that have scores of less than 3.0% infection across all spikes tested per entry will be advanced as potential varietal candidates.

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Virulence pattern of leaf rust in Pakistan.

Muhammad Fayyaz, Atiq-ur-Rehman Rattu, Muhammad Afzal Akhtar, Muhammad Shahzad, Saima Irem Farooq, and Abdul Mujeeb-Kazi.

The occurrence of rust diseases in cultivated cereals has significantly influenced the development of human civilization (Rolf et al. 1992). Wheat rusts have historically been one of the major biotic stress production constraints in Asia and globally (Singh and Rajaram 1991). Leaf rust is a serious wheat production hazard (Mclntosh et al. 1995) and the most destructive and devastating disease due to its time of appearance, nature of attack, regular occurrence, and prolonged growing season that is prevalent for its development in the wheat growing areas of the world (Khan et al. 1997). Leaf rust incurs significant yield losses (Khan et al. 1987; Hussain et al. 1980).

In order to determine the presence and virulence of leaf rust distribution in Pakistan, a trap nursery comprising of 39 isogenic lines and 10 commercial bread cultivars with different *Lr* genes were planted and evaluated at four locations over two consecutive years. Morocco was the susceptible spreader ion and around the test plots. The four locations across two provinces were Karachi and Nawabshah (in SINDH), Bahawalpur, and Faisalabad (in PUNJAB). The study objective was to identify the naturally prevailing leaf rust virulences.

Entries with leaf rust resistance genes *Lr9, Lr19*, and *Lr28* were resistant at all locations. Leaf rust resistance genes *Lr1, Lr2a, Lr2b Lr2c, Lr3,Lr3ka, Lr3bg, Lr10,Lr11, Lr12, Lr14a, Lr14b, Lr16, Lr18, Lr20,Lr23, Lr24, Lr25, Lr26, Lr10, 27+31, Lr29, Lr30, Lr32, Lr33, Lrb*, and *Lr23+* indicated presence of virulence at most of the locations. The genes *Lr13, Lr22a, Lr34*, and *Lr35* possessed virulence at Karachi and Nawabshah. Partial virulence was observed on genes *Lr36* and *Lr37* at three locations. A majority of the commercial cultivars in Sindh showed susceptibility against leaf rust

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New stem rust virulence detected in Pakistan: a potential threat to adopted bread wheat breeding strategy against Ug99.

Javed I. Mirza, Alvina G. Kazi, and A. Mujeeb-Kazi.

Historically stem rust of wheat incurred serious yield losses in Indo-Pak region. The disease had been controlled successfully since introduction of semidwarf stem rust resistant wheats during the Green Revolution of 1960s and 70s. Resistance in the majority of the wheat cultivars currently sown in the region is mainly based on gene *Sr31* that is present in 80% of the developing countries leading wheat cultivars. The evolution of the *pgt* race Ug99 to be capable of overcoming resistance imparted by *Sr31* in Uganda during 1999 created an alarming situation throughout the world (Pretorius 2000). Pakistani wheat cultivars tested under Kenyan field conditions were highly susceptible to Ug99 (Anonymous 1995). The new race is expected to follow the path of *Yr9* virulence that began in eastern Africa and spread to this part of the world (Singh et al. 2004). Efforts to identify and incorporate genes resistant to Ug99 have been intensified to develop and distribute resistant germ plasm through centralized breeding programs.

The evolution of the *Sr31*-virulent stem rust race Ug99 created an alarming global situation, because stem rust resistance in the world's leading wheat cultivars is based on *Sr31*. Among genes found resistant to Ug99, *Sr13, Sr22, Sr24, Sr26, Sr29, Sr35*, and *Sr36* were thought to have some immediate value. Detection of Ug99 strains (variants) virulent to *Sr24, Sr32, Sr33, Sr35*, and *Sr36* already have negated the potential use of these important genes. In 2006, stem rust infection in the commercial wheat cultivar Sarsabz in our Sindh province created a concern. The symptoms of stem rust prevalence in Sindh reoccurred in 2007 and again in 2008. During this time period across the national terrain, stem rust followed a rapid migration path and was reported from Iran. Thus, concern emerged whether or not it also had entered Pakistan.

Pathogen samples collected in 2008 from Sindh were analyzed on a stem rust differential set and specifically checked on the T1BL·1RS translocated wheat cultivars with *Sr31*. Presence of Ug99 was nullified based upon the symptoms seen on the test set, and the *Sr31*-based stock that remained disease free under Pakistan test conditions. Our concern, however, is that the local race affects some of the genes that are reported to be of value for resistance to Ug99. Hence, our choice of genes to be deployed in our breeding programs for imparting stem rust resistance to both the local plus Ug99 race and its variants are narrowed.

Single-pustule isolates were analyzed from stem rust diseased samples collected from Sindh (farmers' fields) of Juddo and Mirpurkhas. Urediospores from pustules inocula were multiplied on the susceptible cultivar Morocco as described by Knott (1989). Single-pustule inocula were tested on ten-day-old seedlings of the tester host set consisting of 40 NILs, including three sets of the North American stem rust differentials (Roelfs and Martens 2007). Morocco and the commercial cultivar Sarsabz were included as checks (Table 1). After inoculation, the plant trays were transferred to a growth room set at conditions mentioned earlier. After 24 hrs incubation, seedling trays were transferred to the glasshouse set at 18-20°C. Stem rust data for seedling infection types, described by Stakman et al. (1962), was recorded on the 10th day after inoculation or when pustules on susceptible cultivar Morocco were sporulating. The Pgt race was designated following the international system of nomenclature (Roelfs and Martens 2007).

All the isolates were designated race *TRT-Sr/13, Sr25, Sr33, Sr*37 on the basis of seedling reaction (Stakman et al. 1962) on the three North American sets of differentials (Roelfs and Martens 2007). The seedling reaction of all NILs tested remained high, except for those with genes *Sr8a, Sr22, Sr24, Sr26+Sr9g, Sr27, Sr31*, and *Sr32* (Table 1). Lines with genes *Sr39, Sr40, Sr43, Sr44*, and *SrTmp* were not available and, thus, were not included in the test.

Seedlings with genes *Sr5, Sr6, Sr7a, Sr7b, Sr8b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9f, Sr9g, Sr11, Sr12, Sr13, Sr14, Sr15, Sr16, Sr17, Sr18, Sr19, Sr20, Sr21, Sr23, Sr25, Sr28, Sr29, Sr30, Sr33, Sr34, Sr35, Sr36, Sr37*, and *Srwld* all had high infection types and were considered ineffective to the local race.

Table 1. Response of stem rust differentials to race *TRT-Sr13, Sr25, Sr33,* and *Sr37* in Pakistan.

Isogenic lines	*Sr* gene	High / low response to isolates			
		2007	2008		
		Juddo	Juddo	Matli	Badin
ISR5RA	*Sr5*	H	H	H	H
T. monococcum derivative	*Sr21*	H	H	H	H
Vernsten	*Sr9e*	H	H	H	H
ISR7BRA	*Sr7b*	H	H	H	H
ISR11RA	*Sr11*	H	H	H	H
W2691SR6	*Sr6*	H	H	H	H
ISR8ARA	*Sr8a*	L	L	L	L
CNS(TC2B)/LINE E	*Sr9g*	H	H	H	H
W2691SRTT1	*Sr36*	H	H	H	H
W2691SR9B	*Sr9b*	H	H	H	H
BTSR30WST	*Sr30*	H	H	H	H
LC/Kenya Hunter	*Sr17*	H	H	H	H
ISR9ARA	*Sr9a*	H	H	H	H
ISR9DRA	*Sr9d*	H	H	H	H
W2691SR10	*Sr10*	H	H	H	H
LINE G	*Sr7a*	H	H	H	H
Barleta Benvenuto	*Sr8b*	H	H	H	H
ISR5SB	*Sr9f*	H	H	H	H
CH.SP.(TC3B)	*Sr12*	H	H	H	H
W2691SR13	*Sr13*	H	H	H	H
Line Aseln	*Sr14*	H	H	H	H
W2691SR15NK	*Sr15*	H	H	H	H
ISR16RA	*Sr16*	H	H	H	H
LCSR19MG	*Sr19*	H	H	H	H
LCSR20MG	*Sr20*	H	H	H	H
SWSR22T.B.	*Sr22*	L	L	L	L
EXCHANGE	*Sr23*	H	H	H	H
BT SR24 A9	*Sr24*	L	L	L	L
LC SR25 ARS	*Sr25*	H	H	H	H
EAGLE	*Sr26+Sr9g*	L	L	L	L
Coorong triticale	*Sr27*	L	L	L	L
W2691SR28KT	*Sr28*	H	H	H	H
PUSA/EDCH	*Sr29*	H	H	H	H
LINE E/KVZ	*Sr31*	L	L	L	L
C77.19	*Sr32*	L	L	L	L
Tetra-Canthatch/*Ae. tauschii* RL5045)	*Sr33*	H	H	H	H
COMPARE	*Sr34*	H	H	H	H
W3763	*Sr35*	H	H	H	H
W2691 SRTT2	*Sr37*	H	H	H	H
BT/WLD	*SrWLD*	H	H	H	H
LCSR18PL	*Sr18*	H	H	H	H
SARSABZ (Check)	*Sr* 23	H	H	H	H
MOROCCO (Check)		H	H	H	H

The local races were virulent to *Sr5, Sr6, Sr7b, Sr9a, Sr9b, Sr9d, Sr9e, Sr9f, Sr9g, Sr10, Sr11, Sr12, Sr15, Sr16, Sr17, Sr18, Sr19, Sr20, S21, Sr23, Sr30, Sr34,* and *Srwld*, which was similar to that of Ug99. However, unlike Ug99, the local race is avirulent to *Sr8a* and *Sr31* and virulent to *Sr7a, Sr13, Sr14, Sr25, Sr28, Sr29, Sr33, Sr35, Sr36,* and *Sr37*. Stem rust resistance genes *Sr13, Sr14* from *T. turgidum*; *Sr28, Sr29* from *T. aestivum*; *Sr33* from *Ae. tauschii*, *Sr36, Sr37* from *T. turgidum* subsp. *timopheevii*, and *Sr35* from *T. monococum* were of special interest to breeders after the evolution of Ug99 (Singh et al., 2006). The capability of race TRT to infect these genes negates their usage and further limits the availability of stem rust resistance genes resistant to stem rust races TTKS and TRT.

Our strategy for addressing the imminent entering of Ug99 in Pakistan will be as follows.

a) Exploit the EBWYT trials that are distributed by CIMMYT, select the best performers, and deploy them in regions of concern. Accordingly, the three best performing lines have been acquired from CIMMYT based upon 2EBWYT yield performance data and these have been targeted for deployment to farmers' fields after rapid increase assistance from private growers.

b) Add the eight best performing lines across the Pakistani provinces where the 3EBWYT was tested and include another two lines that performed well in India. All 10 entries were increased in Kaghan in the summer of 2008 and are being further increased during the 2008–09 cycle after which they shall be deployed to progressive farmers in selected provinces focusing on lower Punjab and Sindh.

c) Utilize a volatile recombination breeding effort with the leading Pakistani high-yielding cultivars in crosses with the Elite II and 3EBWYT entries. In addition, the SRSN nursery obtained from CIMMYT has several desirable entries that possess derivatives from D-genome synthetic hexaploid wheats and tertiary gene pool genetic resources (e.g., *L. racemosus* and *Th. curvifolium*) that are also being exploited as donor sources in our recombination breeding efforts.

d) Focus on additional crosses utilizes the genes of interest that have value for Ug99 resistance and are avirulent to the local stem rust race. In addition, a major effort is in place to screen all available wheat genomic genetic stocks and their free-threshing advanced derivatives that are of superior agronomic types. The bulk of this diversity is D-genome based. The screening is done locally, and a limited set also is targeted for screening in Kenya.

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Screening of mapping population against powdery mildew under field and glass house conditions in Pakistan.

Shahzad Asad, Alvina G. Kazi, Yahya Rauf, and Abdul Mujeeb-Kazi.

Powdery mildew of wheat is becoming an important disease of wheat in Pakistan. The disease is mostly prevalent in cooler places where temperature ranges are between 15–22ºC with humidity up to 75% (Khan 2006). Due to the obligate parasitic and airborne nature of the organism it is very difficult to combat the disease. The best economical and the most effective mean to control the disease is to screen and identify genetic resistance. In Pakistan, data are scarce regarding this disease but its presence, however, has been observed to be on the rise and consequently artificial and natural screening in a hot spot will aid in identifying resistance thus promoting germ plasm security efforts.

Three DH-based combinations (Filin/Kariega comprising of 47 entries, Filin/Saar of 24 entries, and Kariega/Saar of 81 entries) were screened for seedling resistance under glass house conditions in Murree (artificial inoculation with a bulk collection from a rice-wheat field area in the Punjab province of Pakistan). The same materials also were screened for adult-plant resistance under field conditions in a natural hot spot at Kaghan. Both studies were conducted in the summer of 2008 during late May and mid-October.

Table 2. Disease reaction of the diverse germ plasm against powdery mildew seedling and adult-plant screening (S = susceptible, MS = moderately susceptible, MR = moderately resistant, and R = resistant).

Group	No. of Entries	Disease reaction							
		Field				Glass house			
		S	MS	MR	R	S	MS	MR	R
Filin / Kariega	47	2	6	4	35	17	24	3	3
Filin / Saar	24	0	0	0	24	3	19	2	0
Kariega / Saar	81	0	3	5	73	21	51	5	4

Under glass house conditions in the Murree research station of CDRP, a majority of the entries of all the three populations exhibited a susceptible to moderately susceptible reaction at the seedling stage. In contrast, under the adult-plant field screening majority of the same entries exhibited a resistant reaction (Table 2). Plant symptoms were recorded on the infection type scale starting from 0 to 9, where 0 is no visible fungal growth and 9 is abundant growth and sporulation (Hiura 1978). Double-digit scoring was utilized.

The above results are indicative of action of more than one gene in the respective entries where varied degrees of susceptibilities are observed at the seedling stage and resistance is present at the adult plant stage. These results are consistent with those of Khan (2006) who screened wheat plants of 'Poros-monos/M30' both at seedling and adult plant stage against powdery mildew. Such entries are desirable materials from which potential minor gene effects can be incorporated into breeding materials in order to achieve durable resistance.

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In-vitro screening of synthetic hexaploid wheat lines against Cochliobolus sativus in Pakistan.

Shamim Iftikhar, Shahzad Asad, Alvina G. Kazi, and A. Mujeeb-Kazi.

C*ochliobolus sativus* leaf blight is a world-wide, economically important foliar disease of wheat. Leaf blight or spot blotch mainly occurs in warm, humid wheat growing areas. In Pakistan, spot blotch has been observed in different agro-ecological wheat production zones especially where winter temperatures are warmer. Spot blotch was identified as a predominant pathogen of leaf spotting in the national wheat growing areas during 2003–06. Out of 87 isolates collected from different agro-ecological zones of wheat production, the most aggressive isolate P2-9 was used to screen the synthetic hexaploids wheat subsets Elite I and Elite II plus their durum female parents under in vitro conditions. None of the Elite I gave

Table 3. Screening of synthetic hexaploids (CIMMYT Elite II) against *Cochliobolus sativus* in 2005 and 2006 (scale: 0 = resistant, 1–2 = moderately resistant, 3–4 = moderately susceptible, and 5 = susceptible).

Entry #	Genotype	2005	2006
3	DVERD_2 / *Ae. tauschii* 214)	3	3
4	ARLIN_1 / *Ae. tauschii* (218)	2	2
9	STYUS / CELTA // PALS. /3/ SRM_5 /4/ *Ae. tauschii* (431)	3	3
10	LCK59.61 / *Ae. tauschii* (693)\	2	2
11	CETA / *Ae. tauschii* (1025)\	2	2
16	CPI / GEDIZ /3/ GOO / JO / CRA /4/ *Ae. tauschii* (1018)	2	2
18	CETA / *Ae. tauschii* (1038)	2	2
22	CETA / *Ae. tauschii* (368)	2	2
26	GAN / *Ae. tauschii* (206)	2	2
27	ARLIN_1 / *Ae. tauschii* (335)	2	2
28	GAN / *Ae. squar tauschii rosa* (335)	2	2

any indication of resistance. Nine Elite II entries (Table 3, p. 156) and three durum wheats were found to be moderately resistant across 2 years of *in vitro* studies. Additionally, 16 synthetic hexaploids of the Elite II subset and 12 durum wheats (Table 4) were moderately resistant and moderately susceptible, respectively, over the 2-year test. These entries classified as moderate may further be exploited in wheat-breeding programs to enhance allelic diversity across the three wheat genomes via the A and B genomes of durum wheats and the D genome of the synthetic entries. Where durum wheat shows desirable ratings and a synthetic is not identified in the same category, that entry also could be a candidate for breeding via the pentaploid route of recombination breeding.

Table 4. Screening of durum wheat parents against *Cochliobolus sativus* in 2005 and 2006 (scale: 0 = resistant, 1–2 = moderately resistant, 3–4 = moderately susceptible, and 5 = susceptible).

Entry #	Genotype	2005	2006
6	LARU	3	3
11	CPI / GEDIZ /3/ GOO // J0 / CRA	2	3
18	SNIPE / YAV79 / DACK / TEAL	3	3
19	TKSN1081	2	3
20	YAV-2 / TEZ	1	3
25	ARAOS	2	2
26	GAN	3	3
28	STY-US / CELTA // PALS /3/ SRN-5	2	2
29	AGAMI	3	3
30	YAV-3 / SCOT // J069 / CRA /3/ YAV79	3	2
43	FALCIN-1	2	2
46	KAPUDE-1	3	3

The in vitro screening methodology. The inoculum of single spore culture of the most aggressive isolate (P2-9) was multiplied on potato dextrose agar and was selected after aggressiveness analysis of 87 isolates, collected from different agro-ecological, wheat-production zones. Test tubes (20 cm x 3 cm) were filled a quarter from the bottom with cotton and distilled water (20 mL) was added in each tube. The prepared tubes were covered with aluminum foil, autoclaved, and were ready for experimental usage.

Screening. The test synthetic hexaploid wheat germ plasm with the durum parent cultivars is maintained as a working collection in the Wheat Wide Crossing program at NARC, Islamabad. This resource was produced in CIMMYT by their Wheat Wide Crosses program (Mujeeb-Kazi 2003). The materials were screened against the most aggressive isolate (P2-9) of *C. sativus*. The hexaploids wheat cultivar Wafaq was the check. Three seeds/tube were surface disinfected with a 1% Clorox solution for 1 min and placed on the moist cotton swab within each test tube. One 5-mm disc of the fungal isolate was placed adjacent to the seeds with the help of a cork borer. The tubes were placed in randomized manner in steel racks. After inoculation, tubes were recovered with aluminum foil and placed in the growth chamber at 25^0C for incubation. Data was recorded upon the appearance of spots on leaves on a 0–5 scale where 0 = no spotting symptoms, 1 = 1–5% spots, 2 = 6–20% spots, 3 = 21–40% spots, 4 = 41–60%, and 5 = more than 60% (IRRI 1996). The above scale is considered as 0 = resistant, 1–2 = moderately resistant, 3–4 = moderately susceptible and 5 = susceptible.

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Current scenario of yellow rust of wheat in Pakistan.

Atiq-ur-Rehman Rattu, Muhammad Fayyaz, Muhammad Afzal Akhtar, Muhammad Shahzad, Saima Aamir, and Abdul Mujeeb-Kazi.

Wheat in Pakistan is the main staple food and covers 8.2 x 10^6 ha. Of the many diseases that attack the wheat crop, rusts are by far the most important and have continued to ravage this crop since ancient times. The rust of wheat has historically been one of the major biotic production constraints both in Asia and rest of the world (Singh and Rajaram 1991). Yellow rust is one of the most important disease of the wheat in world (Roelfs et al. 1992). The annual yield losses due to wheat yellow rust have been estimated up to 8–75% (Elahinia 2000). Severe epidemics of the disease may result

in losses of up to 70% in commercial fields (Mclntosh et al. 1995). Severe epidemics of the disease have reported in central and west Asia (Braun and Saari 1992; Torabi et al. 1995). Ahmad et al. (1991) reported an estimated US$ 8 x 10^6 revenue loss just in three districts of Baluchistan in Pakistan. Our objective was to identify the prevailing virulences of yellow rust in nature by planting yellow rust trap nurseries at different hot spots of the country. The principal and practical purpose for studying the rust population is to identify the effective genes.

The trap nursery specially designed for yellow rust comprised of 24 wheat isogenic lines and commercial cultivars were planted at four locations; Faisalabad, Peshawar, Nowshera, and Islamabad. This nursery was evaluated for 3 years. The locations represented different agro-ecological zones and hot spots where the conditions were mostly favorable for yellow rust development. Each entry was planted in single meter rows 30 cm apart. Two rows of rust susceptible spreaders (Morocco) were planted around the nursery. The observations were recorded on natural occurrence and first appearance of rust infection on susceptible check. The observation at all the locations on response of leaf rust was recorded according to the modified Cobb's Scale (Peterson et al. 1948).

Our results revealed that virulence factors for *Yr2, Yr6, Yr7, Yr9, Yr17, Yr27, Yr28*, Avocet *Yr-A*, Avocet+*YrA*, and Jupateco-S were present at all test locations. Similarly, *Yr1*, *Yr8, Yr18, Yr29*, and Jupateco-R had virulence at all the locations except Faisalabad. Yellow rust resistance genes *Yr24*, *Yr26*, and *YrCV* showed partial virulence. No virulence was observed on the yellow rust resistance genes *Yr5, Yr10, Yr15*, and *YrSP.* Tatara was the only cultivar that was found resistant during the study period. Tatara is widely grown in the northern area of the country and showed resistance most probably due to *Yr3* resistant gene against all the prevailing races of the yellow rust.

Yr6, Yr7, and *Yr9* are the most dominant genes that are postulated in our commercial cultivars, which suggests that the virulence for these genes are prevailing in the country as most of the wheat cultivars possessing these genes are continuously cultivated regularly in the most part of the country.

Following the initiation of use of genetic resources in wheat improvement, our integrated group has embarked on a strategy that will diversify the genetic composition of our varietal production efforts by utilizing conventional minor genes for durable resistance, focusing on targeted gene transfers that will promote gene and cultivar deployment aspects across the nations provinces creating internal barriers as an obstacle to rapid disease spread, incorporate intervention on use of molecular markers for adding to breeding efficiency, and capitalize on the vast novel genetic stocks that are available through our wide cross program that encompasses the genetic richness of each of the wheats three genomes and also taps on the allelic values of selected genomes from genera belonging to the secondary and tertiary gene pools.

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Evaluation of the Elite II synthetic hexaploid wheats to barley yellow dwarf virus and their molecular diversity.

Huma Saffdar, Alvina G. Kazi, Shahid Hameed, Attiq-ur-Rehman, and A. Mujeeb-Kazi.

Wheat, one of the most important cereals in the world, has its production affected by various biotic and abiotic stresses. Of these stresses, barley yellow dwarf virus is an important and widespread viral disease. This disease can cause yield losses up to 75% in severe cases. Diverse genetic resistance sources provide a potent means that offer environmentally safe control measures. The resistant sources can be harnessed from about 325 perennial and annual species that are distributed within three gene pools of the tribe Triticeae. Currently, *Ae. tauschii,* which possesses diversity for BYDV is the prime source. This diploid, D-genome donor after crossing with elite durum wheat cultivars has generated a unique source of user-friendly germ plasm for wheat improvement. This process has produced over 1,000 synthetic hexaploid wheats, and an Elite II subset based upon multiple stress resistance/tolerance was established. In this study, the Elite II subset was screened against BYDV *in vitro* and *in vivo*, using DAC-ELISA (Table 5 and Table 6, p. 160). *In vitro* screening results showed that out of 33 genotypes, seven (3, 4, 5, 6, 14, 19, and 33) were resistant, and three (22, 28, and 31) tolerant. *In vivo* screening results showed that out of 33 genotypes, 19 (3, 4, 5, 9, 13, 14, 17, 18, 20, 22, 23, 25, 26, 27, 28, 29, 31, 32, and 33) were tolerant. From the cumulative *in vitro* and *in vivo* testing, 15 genotypes were stringently selected for assessing their diversity levels.

Table 5. Number of Elite II genotypes with barley yellow dwarf virus symptoms and positive to ELISA.

No.	Symptoms +ve	% plants showing symtoms	ELISA +ve	% Plants infected
1	—	—	—	—
2	4/5	80	0/5	0
3	0/5	0	4/5	80
4	0/5	0	4/5	80
5	0/5	0	0/5	0
6	5/5	100	5/5	100
7	3/5	60	4/5	80
8	4/5	80	5/5	100
9	0/5	0	5/5	100
10	4/5	80	5/5	100
11	2/5	40	5/5	100
12	3/5	60	5/5	100
13	0/5	0	5/5	100
14	0/5	0	5/5	100
15	1/5	20	5/5	100
16	3/5	60	5/5	100
17	0/5	0	5/5	100
18	0/5	0	5/5	100
19	2/5	40	5/5	100
20	0/5	0	4/5	80
21	2/5	40	5/5	100
22	0/5	0	5/5	100
23	2/5	40	5/5	100
24	—	—	—	—
25	0/5	0	5/5	100
26	0/5	0	5/5	100
27	0/5	0	5/5	100
28	0/5	0	5/5	100
29	0/5	0	4/5	80
30	2/5	40	5/5	100
31	0/5	0	5/5	100
32	0/5	0	5/5	100
33	0/5	0	5/5	100

Ten, decamer RAPD primers (OPG-1, OPG-2, OPG-3, OPG-4, OPG-5, OPA-3, OPA-4, OPA-5, OPA-8, and OPA-15) evaluated the diversity profile of the selected 15 SH entries. Out of these ten primers, five primers (OPG-2, OPG-3, OPG-5, OPA-4, and OPA-15) showed amplification with these genotypes, whereas another five did not amplify any genotype. Out of the positive five, OPG-2 amplified seven genotypes out of 15. All 15 genotypes were monomorphic for the 1,000-bp band. The primer OPA-4 amplified six genotypes out of 15. Genotypes 26, 28, and 29 were monomorphic for the 750-bp band. Genotype 22 was monomorphic for the band between 1,000 and 1,500 bp, whereas genotype 6 was polymorphic for the 750- and 1,500-bp bands. Genotype 18 was polymorphic for bands at 750 bp and between 1,000 and 1,500 bp. OPG-5 amplified four out of 15 genotypes, all of them monomorphic for the 750-bp band. OPA-15 amplified three out of 15 genotypes, whereas OPG-3 amplified only one genotype out of 15.

The most genetically similar lines were 1, 20, and 30. The value of similarity matrix ranged from 54–100% in which minimum similarity was manifested by genotypes 9 and 13; maximum similarity of 100% was shown between genotypes 1 and 20, 1 and 30, and 20 and 30. The dendrogram divided the genotypes into two major clusters without genotype 17, which remained independent of both clusters. Cluster A comprised of 11 (1, 20, 30, 6, 26, 28, 9, 29, 21, 22, and 7) and cluster B of three genotypes (13, 18, and 23).

We recommend that the allelic variation of the SH resistance germ plasm is a potent mean to enrich and improve bread wheat cultivars where BYDV is a production threat. Phenological data (Table 7, p. 160-161) provides an additional descriptor resource and sieve for targeting the best BYDV-tolerant synthetics for use in wheat breeding. An

Table 6. ELISA values and *in vitro* and *in vivo* scoring (0–9 scale; R = resistant, S = susceptible, and SLC = symptom-less carrier) for barley yellow dwarf virus in Elite II lines.

No.	*In vitro*			*In vivo*		
	Symptoms	ELISA	Comment	Symptoms	ELISA	Comment
1	0.50/2	2.393/2	S	—	—	—
2	2.00/2	2.812/2	S	0.80/5	0.6848/5	S
3	0.00/1	0.568/1	R	0.00/5	0.794/5	SLC/T
4	0.50/2	0.608/2	R	0.00/5	0.8036/5	SLC/T
5	1.00/2	0.547/2	R	0.00/5	0.6608/5	SLC/T
6	0.00/2	0.576/2	R	1.60/5	1.0514/5	HS
7	0.50/2	2.417/2	S	0.80/5	1.0414/5	HS
8	3.00/3	2.521/3	S	1.80/5	1.0044/5	S
9	0.50/3	2.684/3	S	0.00/5	0.8204/5	SLC/T
10	0.66/3	2.527/3	S	0.80/5	1.0394/5	S
11	2.00/1	1.803/1	S	2.40/5	0.914/5	S
12	1.00/5	2.649/5	S	0.60/5	0.9992/5	HS
13	0.25/4	2.628/4	S	0.00/5	0.8686/5	SLC/T
14	0.00/2	0.919/2	R	0.00/5	0.9028/5	SLC/T
15	1.25/4	2.012/4	S	0.20/5	0.9226/5	HS
16	1.00/2	2.638/2	S	1.20/5	1.114/5	HS
17	0.33/3	2.515/3	S	0.00/5	0.9626/5	SLC/T
18	0.25/4	2.185/4	S	0.00/5	1.1218/5	SLC/T
19	1.00/4	0.572/4	R	0.80/5	0.8528/5	S
20	0.50/2	2.168/2	S	0.00/5	0.8900/5	SLC/T
21	0.50/2	2.112/2	S	0.60/5	0.8508/5	S
22	0.00/2	2.806/2	SLC/T	0.00/5	0.791/5	SLC/T
23	0.50/2	2.10/2	S	0.00/5	0.7802/5	SLC/T
24	1.00/1	1.802/1	S	—	—	—
25	1.00/2	1.497/2	S	0.00/5	0.8078/5	SLC/T
26	0.50/4	2.542/4	S	0.00/5	0.8698/5	SLC/T
27	1.00/2	1.60/2	S	0.00/5	0.8836/5	SLC/T
28	0.00/3	1.321/3	SLC/T	0.00/5	0.9688/5	SLC/T
29	0.50/2	2.289/2	S	0.00/5	0.8524/5	SLC/T
30	0.50/2	2.72/2	S	0.00/5	0.9372/5	S
31	0.00/3	2.177/3	SLC/T	0.00/5	0.9690/5	SLC/T
32	1.00/2	1.889/2	S	0.00/5	1.0198/5	SLC/T
33	0.00/1	0.811/1	R	0.00/5	0.8872/5	SLC/T

additional, stringent round of seasonal screening is mandatory prior to embarking on an applied program of genetic recombination and having elite germ plasm included that possesses *bdv1* and *bdv2* genes around germ plasm such as Anza, TC14, Kivu, and *Agrotricum*.

Table 7. Some phenological parameters of Elite II synthetic hexaploid entries (PIG = tiller pigmentation; PUB = Pubescence; FLOW = days-to-flowering; HT = plant height at maturity (cm); AWN = awn color (B = brown, LB = light brown, DB = dark brown, and W = whitish); PMA = days-to-physiological maturity; and TKW = 1,000-kernel weight (g))

Pedigree	PIG	PUB	FLOW	HT	AWN	PMA	TKW
SORA/*Ae. tauschii*(192)	—	—	—	—	—	—	—
CROC-1/*Ae. tauschii* (210)	+	—	117	115	B	152	30
DVERD2/*Ae. tauschii* (214)	—	—	128	120	LB	152	33
DVERD2/*Ae. tauschii* (218)	—	—	117	100	DB	145	33
TKSN1081/*Ae. tauschii* (222)	+	+	128	100	W	148	36
CAN/*Ae. tauschii* (236)	—	+	133	95	W	152	31
SORA/*Ae. tauschii* (323)	+	—	128	100	DB	145	33
D67.2/P66.270//*Ae. tauschii* (308)	+	—	117	60	LB	152	12
STY-US/CELTA//PALS/3/SRN5/4/*Ae. tauschii* (431)	+	—	126	90	LB	152	37
LCK59.61/*Ae. tauschii* (693)	+	—	117	115	LB	152	32
SKARV2/*Ae. tauschii* (304)	+	+	133	65	B	152	30
CETA/*Ae. tauschii* (1025)	+	+	112	95	LB	148	40
DOY-1/*Ae. tauschii* (1027)	+	—	133	100	LB	148	36

Table 7. Some phenological parameters of Elite II synthetic hexaploid entries (PIG = tiller pigmentation; PUB = Pubescence; FLOW = days-to-flowering; HT = plant height at maturity (cm); AWN = awn color (B = brown, LB = light brown, DB = dark brown, and W = whitish); PMA = days to physiological maturity; and TKW = 1,000-kernel weight (g))

Pedigree	PIG	PUB	FLOW	HT	AWN	PMA	TKW
CETA/*Ae. tauschii* (386)	+	+	133	90	LB	149	34
CETA/*Ae. tauschii* (392)	+	+	133	80	LB	149	27
CETA/*Ae. tauschii* (533)	—	—	112	85	LB	152	25
CPI/GEDIZ/3/GOO//JO/CRA/4/*Ae. tauschii* (1018)	+	—	133	110	B	152	46
CETA/*Ae. tauschii* (1031)	+	—	143	100	LB	145	44
CETA/*Ae. tauschii* (1038)	+	—	117	115	DB	145	35
CETA/*Ae. tauschii* (1046)	+	—	122	100	DB	145	38
CETA/*Ae. tauschii* (1053)	+	+	112	85	B	151	36
CROC-1/*Ae. tauschii* (212)	+	+	133	90	LB	148	36
CETA/*Ae. tauschii* (368)	+	+	117	95	LB	145	32
ARLIN-1/*Ae. tauschii* (430)	—	—	—	—	—	—	—
D67.2/P66.270//*Ae. tauschii* (497)	+	+	122	100	DB	145	32
D67.2/P66.270//*Ae. tauschii* (1015)	+	+	117	85	B	152	34
GAN/*Ae. tauschii* (206)	+	—	117	100	DB	139	33
ARLIN-1/*Ae. tauschii* (335)	+	—	126	100	DB	152	35
GAN/*Ae. tauschii* (335)	+	—	117	95	DB	145	33
68.111/RGB-U//WARD RESEL/3/STIL/4/*Ae. tauschii* (385)	+	—	133	100	DB	149	38
CETA/*Ae. tauschii i*(417)	+	+	133	105	B	152	34
68.111/RGB—U//WARD RESEL/3/STIL/4/*Ae. tauschii* (431)	+	—	117	100	DB	145	35
DOY1/*Ae. tauschii* (534)	+	—	133	100	LB	152	47

Molecular and phenological identification of diversity in some durum resources.

Mah Jabeen Tariq, Alvina G. Kazi, Hafiz Asim Ayaz, Muhammad Faheem, and A. Mujeeb-Kazi.

Triticum turgidum is a unique tetraploid that has the potency to contribute allelic genetic diversity from its A and B genomes for both durum and bread wheat improvement and has been significantly used in the production of synthetic hexaploid wheats (*T. turgidum*/*Ae. tauschii*, 2n = 6x = 42; AABBDD). These synthetics have been a rich source of genetic diversity with a high potential for global wheat improvement that is associated with the D genome of *Ae. tauschii*. When SH wheats are used in breeding, the diversity of D-genome is exploited. However, the durum A and B genomes also play a significant role since they form a variation pool of allelic richness that in earlier decades was exploited via pentaploid breeding (*T. aestivum*/*T. turgidum*).

In the SH wheat production at CIMMYT, 51 durum wheat genotypes have been used and over 1,000 synthetics produced. The parameters analyzed in this study involving the 51 durums were related to phenological traits and molecular diversity as differentiated by RAPD and SSR primers.

According to phenological data, D-1, D-7, D-14, D-25, D-28, and D-48 performed very well in the field specially with reference to their yield-enhancing characters. Cluster analysis of RAPD primers revealed maximum genetic diversity in D-47 followed by D-12, D-26, and D-10, whereas SSR analysis showed D-24, D-25, D-33, and D-36 as the most genetically diverse lines.

Out of 22 RAPD primers used (OPA10, OPC7, OPE1, OPE2, OPE3, OPE4, OPE5, OPE6, OPE7, OPE8, OPE9, OPE13, OPH15, OPH16, OPL1, OPL8, OPL9, OPL10, OPL11, OPN19, OPN20, and OPS17), 12 primers showed clear and polymorphic amplification patterns in terms of band numbers. The total number of loci traced by these primers were 129, 75 of which were polymorphic according to population genetic analysis. The percentage of polymorphism among these genotypes was 58.13%, and the size of amplification ranged from 250 to 10,000 bp. The highest number of scorable bands was obtained with primer OPE1 and the lowest was with OPA10. Maximum genotypes (16) were amplified by primer OPE3 and minimum (1) by OPE4 and OPL1. RAPD primers OPE1 and OPE3 showed the highest polymorphism

and primer OPL8 the lowest. Genotype D-12 was amplified by maximum number of primers (7), whereas genotypes D-3 and D-13 were not amplified by any primer. Genotypes D-47 and D-12 showed maximum polymorphism. The efficiency of these primers to amplify the genotypes ranged from 16 genotypes by primer OPE13, 12 genotypes by OPE1 to eight genotypes by OPN19.

The value of the similarity coefficient of selected durum wheat lines ranged from 0.6133 (61.33%) to 0.100 (100%). Minimum similarity of 61.33% was shown by D-1 with D-26. The genotypes, which showed a value of maximum similarity of 100%, are D-22 with D-23 and D-34 with D-35 and D-44. The similarity of the remaining genotypes was between 83.89 to 100%. The dendrogram was divided into three main clusters. Cluster A included two genotypes D-1 and D-3 with a genetic distance of 0.1278 (12.78%). Cluster B was further divided into three subclusters. Subcluster B1 included a total of eight genotypes; D-2, D-6, D-11, D-22, D-23, D-24, D-13, and D-14. The genotypes D-22 and D-23 were genetically identical. D-2 showed a genetic distance of 0.2744 (27.44%). The genetic distance of the remaining genotypes of this subcluster ranged between 0 and 27%. Subcluster B2 included a total of 18 genotypes (D-29, D-48, D-34, D-35, D-44, D-37, D-43, D-45, D-38, D-41, D-49, D-50, D-51, D-32, D-36, D-42, D-25, and D-19) with a minimum genetic distance of 0 between D-34, D-35, and D-44. D-19 showed the maximum genetic distance of 0.3285 (32.85%). The genetic distance of the remaining genotypes ranged between 0 to 32.85%. Subcluster B3 included 13 genotypes (D-46, D-17, D-28, D-33, D-31, D-9, D-39, D-4, D-20, D-27, D-30, D-40, and D-47). The minimum genetic distance of 0.1128 (11.28%) was present between D-28 and D-38, and the maximum genetic distance of 0.4673 (46.73%) was exhibited by D-47. Cluster C included ten genotypes (D-5, D-7, D-8, D-15, D-16, D-18, D-10, D-12, D-21, and D-26. In this cluster, the minimum genetic distance of 0.0548 (5.48%) was present between D-5 and D-7 and the maximum genetic distance of 0.4257 (42.57%) was shown by D-12. The genetic distance of the rest of the genotypes in this cluster ranged between 5.48 to 42.57%.

A RAPD-based, cluster analysis of dendrogram depicted that subcluster B3 has the maximum diverse lines followed by cluster C. Genotype D-47 of subcluster B3 is the most diverse line among 51 selected durum wheat lines with genetic distance of 46.73%. D-12, D-21, D-26, and D-10 of cluster C also are considered as genetically diverse lines with genetic distances of 42.57, 40.0, 40.0, and 36.62% respectively. A total of 12 SSR primers (GWM47, GWM55.1, GWM120, GWM191, GWM333, GWM382, GWM388, GWM410, GWM493, GWM501, GWM526, and GWM674) were used to analyze the genetic diversity of 51 durum wheat genotypes. All 12 showed clear and polymorphic patterns. Population genetic analysis showed a total of 125 loci out of which 75 were polymorphic. The percentage of polymorphism is 60%. The size of amplification products ranged from 50 to 800 bp.

The highest number of scorable bands were obtained with primer GWM55.1 and the lowest number of bands were obtained with primer gwm410. The maximum number of genotypes (47) were amplified by primer gwm493 and the minimum (4) by GWM388. Primer gwm55.1 showed the highest polymorphism and primer gwm191 the lowest. Genotype D-30 was amplified by maximum number of primers (12), whereas genotype D-19 was amplified by only two primers. Genotype D-24 and D-25 showed maximum polymorphism. The value of similarity coefficient of selected durum wheat lines ranged from 0.5467 (54.67%) to 0.9867 (98.67%). Minimum similarity of 54.67% was shown by D-5 with D-33, whereas genotypes showing maximum similarity of 98.67% were D-19 with D-23.

The dendrogram of SSR-based, genetic diversity evaluation clearly indicated five main clusters A, B, C, D, and E. Cluster A included four genotypes D-1, D-9, D-10, and D-11. Among these genotypes, D-10 and D-11 were genetically less diverse showing a genetic distance of 0.1431 (14.31%) with the remaining genotypes, whereas genotypes D-1 and D-9 showed maximum genetic diversity of 0.1744 (17.44%). Cluster B included a total of 11 genotypes; D-7, D-19, D-23, D-27, D-37, D-51, D-28, D-36, D-45, D-39, and D-26. Among these genotypes, D-19 and D-23 showed the minimum genetic distance of 0.0134 (1.34%) with rest of the genotypes. D-26 showed the maximum genetic distance of 0.4673 (46.73%). The genetic distance of the remaining genotypes of this cluster remained between 0 to 46.73%. Cluster C included a total of 12 genotypes; D-12, D-20, D-49, D-50, D-18, D-35, D-29, D-47, D-48, D-43, D-44, and D-46. In this cluster, D-43 and D-44 were genetically similar with genetic distance of 0.098 (9.98%) with the rest of the genotypes. The maximum genetic distance of 0.4252 (42.52%) was shown between D-18 and D-35. The genetic distance of the remaining genotypes of this cluster fell in the range of 9.98% to 42.52%. Cluster D included total twelve genotypes; D-38, D-34, D-42, D-41, D-30, D-40, D-8, D-13, D-21, D-22, D-16, and D-17. A minimum genetic distance of 0.0270 (2.70%) was shown by D-16 and D-17, whereas D-38 showed maximum genetic distance of 0.4888 (48.88%). The genetic distance of remaining genotypes of this cluster stayed between 2.70 and 48.8%. Cluster E includes 12 genotypes; D-25, D-24, D-31, D-32, D-33, D-2, D-3, D-4, D-5, D-6, D-14, and D-15. Genotypes D-2 and D-3 showed a minimum genetic distance of 0.0408 (4.08%). The maximum genetic distance of 0.5333 (53.33%) was shown by D-24

followed by D-25 with genetic distance of 0.5108 (51.08%). The genetic distance of remaining genotypes of this cluster was between 4.08 and 53.33%.

An SSR-based cluster analysis of dendrogram depicted the same level of genetic diversity in cluster A and cluster C, whereas the minimum genetic diversity was shown by the genotypes of cluster B. Genotypes of cluster E showed the maximum genetic diversity in comparison to all the clusters of the dendrogram. Genotype D-24 of cluster E is the most diverse line among the 51 durum wheat lines with a maximum genetic distance of 53.33. D-25, D-33, and D-38 also are considered as genetically diverse lines.

The percentage of polymorphism among durum wheat lines in RAPD and SSR is 58.6 and 60, respectively, indicating the genetic diversity of durum wheat lines. RAPD primers showed an average of six polymorphic loci per primer amplified, whereas SSR primers showed an average of 6.25. The percentage of these polymorphic loci per primer is 8 and 8.33 % for RAPD and SSR primers, respectively.

RAPD and SSR analysis demonstrated that these durum genotypes can be recommended for targeted use of synthetic wheats with the selected durums and also can be good candidates for direct hybridization with national breadwheat cultivars forming the pentaploid breeding strategy to capture good genes from A and B genomes of these durum wheat lines. Rare has been the use of durums for breadwheat improvement, but the cultivar AS-2002 has a tetraploid parent in its pedigree. Although SHs in breeding bring in the durum genomic component, direct utilization also may be looked at that could encompass other tetraploids such as *T. turgidum* subsps. *dicoccum*, *dicoccoides*, and *carthlicum*. From the phenological descriptors of the 51 durum cultivars (Table 8, p. 163-164), selective usage of a few can be made, e.g., the

Table 8. Some phenological descriptors of the 51 durum cultivars used in D-genome-based synthetic hexaploid production. PUB = pubescence; FLOW = days-to-flowering; HT = plant height at maturity (cm); AWN = awn color (B = brown, DB = dark brown, LB = light brown, and W = whitish); PMA = days-to-physiological maturity; and TKW = 1,000-kernel weight (g).

No.	Pedigree	PUB	FLOW	HT	AWN	PMA	TKW	Nodes /spike	Grains /spike	Spike length (cm)
1	Croc-1	—	87	86	LB	101	45.0	8	45	9
2	Arlin-1	—	86	86	LB	105	18.5	10	16	9
3	Rok/Kml	—	81	105	LB	95	45.0	9	42	9
4	Altar84	—	89	78	LB	108	33.0	8	26	6
5	Dverd_2	—	87	76	LB	112	37.6	7	18	6
6	Laru	—	95	80	LB	110	34.4	8	35	8
7	68.111/RGB-U//Ward Resel/3/Stil	—	92	97	LB	108	51.4	9	46	10
8	68.111/RGB-U//Ward	—	95	103	LB	108	32.2	11	30	9
9	68.111/RGB-U//Ward/3/FGO/4/Rabi	—	88	103	LB	103	41.6	11	38	8
10	6973/Ward.7463//74110	—	90	99	LB	105	31.1	8	30	10
11	CPI/Gediz/3/Goo//Jo/Cra	—	85	102	LB	99	46.0	9	28	6
12	D67.2/P66.270	—	98	96	LB	110	37.0	11	41	10
13	Cerceta	—	88	102	LB	100	41.1	7	28	7
14	Sterna-DW	—	87	85	LB	106	46.5	8	31	7
15	Rabi//GS/Cra	—	99	86	LB	115	40.0	7	34	8
16	Sora	—	88	82	LB	102	38.4	10	31	9
17	Scaup	—	92	83	LB	112	32.0	9	42	11
18	Snipe/Yav79//Dack/Teal	—	87	76	LB	108	44.4	10	36	11
19	TK SN1081	—	82	72	LB	106	39.0	9	31	9
20	Yav_2/Tez	—	89	85	LB	114	38.4	10	47	10
21	Yarmuk	—	87	90	LB	102	35.1	9	34	9
22	Decoy 1	—	89	103	LB	115	34.8	10	48	9
23	Garza/Boy	—	100	68	LB	115	12.5	8	9	8
24	68.111/RGB-U//Ward	—	98	105	LB	108	27.1	19	18	9
25	Araos	—	89	75	LB	100	42.5	13	36	8
26	Gan	—	82	104	LB	98	35.5	11	41	9
27	Scoop_1	—	82	90	LB	95	41.2	9	45	8
28	Sty-Us/Celta//Pals/3/Srn_5	—	88	92	LB	93	44.2	10	28	8
29	Agami	—	87	93	LB	98	41.7	9	38	8
30	Yav_3/Scot//JO69/Cra/3/Yav79	—	92	88	LB	109	45.1	9	34	6
31	YAR	—	92	97	LB	109	45.1	9	32	7
32	68112/Ward	—	101	95	LB	118	39.9	8	33	8

Table 8. Some phenological descriptors of the 51 durum cultivars used in D-genome-based synthetic hexaploid production. PUB = pubescence; FLOW = days-to-flowering; HT = plant height at maturity (cm); AWN = awn color (B = brown, DB = dark brown, LB = light brown, and W = whitish); PMA = days-to-physiological maturity; and TKW = 1,000-kernel weight (g).

No.	Pedigree	PUB	FLOW	HT	AWN	PMA	TKW	Nodes /spike	Grains /spike	Spike length (cm)
33	FGO/USA2111	—	91	93	LB	105	37.5	8	30	5
34	ALG86/4/FGO/Pales//Mexi_1/3/ Ruff/FGO/5/ENTE	—	87	104	LB	98	39.9	10	40	5
35	BOTNO	—	102	97	LB	116	32.4	11	23	7
36	CIT71/CPI	—	103	90	LB	116	—	—	—	8
37	LCK59.61	—	100	96	LB	112	29.7	7	9	9
38	Trinakria	—	87	86	LB	99	37.1	7	15	8
39	Rascon_37	—	84	92	LB	95	32.3	9	44	7
40	Ajaia_9	—	100	78	LB	118	34.2	9	30	9
41	Cerceta	—	98	86	LB	108	43.4	8	17	10
42	Scot/Mexi_1	—	89	103	LB	104	37.7	10	43	8
43	Falcin_1	—	89	95	LB	105	37.6	8	37	7
44	Green-3	—	89	95	LB	104	47.3	7	37	7
45	Shag_22	—	89	87	LB	103	42.7	7	15	7
46	Kapude_1	—	100	85	LB	115	30.7	10	4	8
47	Arlequin	—	88	84	LB	100	39.9	10	62	7
48	Chen_7	—	87	88	LB	95	46.2	9	28	8
49	Aconchi 89	—	90	78	LB	106	36.5	10	48	7
50	Alcatraz_3	—	98	85	LB	120	30.9	8	48	9
51	Local Red	—	82	74	LB	95	25.0	8	13	8

1,000-kernel weight category permits targeting those durums that have a 1,000-kernel weight higher than 42g, which could be exploited for enhancing yield.

Screening of a synthetic hexaploid wheat subset to spot blotch.

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Wheat is the leading food grain of Pakistan and, being a staple cereal in the diet, occupies a central position in agriculture. In the southern province of Sind, where winter temperatures are warmer, leaf spot (spot blotch) has been noted and presence of *C. sativus* reported. The pathogen is considered aggressive and a cause of severe yield loss, even in the Punjab province.

A set of synthetic hexaploid wheats was grouped into different subsets. An enlarged set, comprised of 42 synthetic-based entries plus five Mayoor sister lines in conventional global use for wheat breeding and resistant to *C. sativus*, exhibited stress diversity upon screening under Pakistan conditions and also showed molecular diversity. The germ plasm was screened under in vitro and in vivo conditions. From the 47 lines screened, three were moderately resistant and 12 moderately susceptible under *in vitro* conditions.

Screening under field conditions revealed that 36 lines out of 47 showed moderate resistance, 10 lines showed moderate susceptibility, and one was resistant. From this germ plasm, 15 lines were selected (2, 4, 8, 19, 20, 23, 10, 16, 29, 31, 15, 18, 33, 32, 37) and subjected to molecular diagnostics to unravel their DNA polymorphism profile using RAPD primers. Out of the seven RAPD primers utilized, scorable bands were obtained with four RAPD primers (OPG-2, OPG-9, OPC-8, and OPG-13).

Based on the screening results, molecular diagnostics, and other phenotypic characterization (Tables 9, p. 165-166, and 10, p. 166), two promising moderately resistant lines (19 and 20) are recommended for incorporation of genetic diversity for spot blotch resistance by introducing its allelic resistance into Pakistani cultivars using a limited backcrossing method mediated by wheat/maize double-haploid production technique to accelerate the germ plasm output process.

Table 9. Phenotypic evaluation of 47 synthetic hexaploid wheat lines under study (Height = plant height at maturity; awn color; B = brown, LB = light brown, DB = dark brown, and W = white) and PMA = days-to-physiological maturity.

Line No.	Pedigree	Height (cm)	Awn color	PMA	Spike length (cm)	Grain weight (g)
1	GAN/*Ae. tauschii* (236)//CETA/*Ae. tauschii* (895)/3/MAIZ/4/ INQ91	100	DB	128	13	42
2	GAN/*Ae. tauschii* (236)//CETA/*Ae. tauschii* ((895)/3/MAIZ/4/ INQ91	97	DB	128	14	38
3	GAN/*Ae. tauschii* (236)//CETA/*Ae. tauschii* (895)/3/MAIZ/4/ INQ91	93	DB	128	13	38
4	GAN/*Ae. tauschii* (236)//CETA/*Ae. tauschii* (895)/3/MAIZ/4/ INQ91	101	LB	129	12	35
5	GAN/*Ae. tauschii* (236)//CETA/*Ae. tauschii* (895)/3/MAIZ/4/ INQ91	96	LB	129	12	36
6	GAN/*Ae. tauschii* (236)//CETA/*Ae. tauschii* (895)/3/MAIZ/4/ INQ91	93	LB	129	12	40
7	GAN/*Ae. tauschii* (236)//CETA/*Ae. tauschii* (895)/3/MAIZ/4/ INQ91	105	LB	129	15	42
8	GAN/*Ae. tauschii* (236)//CETA/*Ae. tauschii* (895)/3/MAIZ/4/ INQ91	97	LB	129	12	41
9	GAN/*Ae. tauschii* (236)//CETA/*Ae. tauschii* (895)/3/MAIZ/4/ INQ91	99	LB	129	14	40
10	DOY1/*Ae. tauschii* (447)//CETA/*Ae. tauschii* (895)3/MAIZ/4/ INQ91	94	LB	129	13	50
11	BCN//CETA/*Ae. tauschii* (895)	100	LB	129	9	40
12	ALTAR84/*Ae. tauschii* (219)//2*SERI	98	LB	129	12	40
13	ALTAR84/*Ae. tauschii* (219)//OPATA	107	LB	129	12	40
14	SABUF/7/ALTAR84/*Ae. tauschii* (224)//YACO/6 /CROC-1/*Ae. tauschii* (205)/5/BRI2*3/4/…	97	LB	129	9	50
15	BCN/4/68.111/RGB-U//WARD/3/*Ae. tauschii* (325)	96	LB	129	10	49
16	SABUF/7/ALTAR84/*Ae. tauschii* (224)//YACO/6/CROC-1/*Ae. tauschii* (205)/5/BRI2*3/4	95	LB	129	10	41
17	SABUF/7/ALTAR84/*Ae. tauschii* (224)//YACO/6/CROC-1/*Ae. tauschii* (205)/5/BRI2*3/4	98	LB	129	13	40
18	ALTAR84/*Ae. tauschii* (191)//OPATA/3/ALTAR84/*Ae. tauschii* (224)//YACO	96	LB	129	12	50
19	GAN/*Ae. tauschii* (236)//CETA/*Ae. tauschii* (895)/3/MAIZ	101	LB	129	12	46
20	DOY1/*Ae. tauschii* (447)//CETA/*Ae. tauschii* (895)/3/MAIZ	96	B	129	13	48
21	68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae. tauschii* (629)	106	LB	136	13	48
22	FGO/USA2111//*Ae. tauschii* (658)	98	LB	136	12	46
23	68.111/RGB-U//WARD/3/FGO/4/RABI/5/*Ae.tauschii* (878)	100	W	136	12	36
24	SCA/*Ae. tauschii* (518)	98	LB	136	12	38
25	GAN/*Ae. tauschii* (897)	95	LB	136	12	41
26	YAV-2/TEZ/*Ae. tauschii* (895)	106	LB	136	14	48
27	GREEN/*Ae. tauschii* (458)	88	LB	136	13	46
28	SCA/*Ae. tauschii* (409)	88	LB	136	13	45
29	CPI/GEDIZ/3/GOO//JO60/CRA/4/*Ae. tauschii* (409)	104	LB	136	13	39
30	ALTAR84/*Ae. tauschii* (502)	106	LB	136	12	40
31	GAN/*Ae. tauschii* (236)//DOY1/*Ae. tauschii* (447)	90	LB	128	12	54
32	GAN/*Ae. tauschii* (236)//CETA/*Ae. tauschii* (895)	90	LB	128	12	41
33	SCOOP1/*Ae. tauschii* (434)//CETA/*Ae. tauschii* (895)	89	LB	128	13	47
34	DOY1/*Ae. tauschii* (447)//CETA/*Ae. tauschii* (895)	90	DB	128	9	50

Table 9. Phenotypic evaluation of 47 synthetic hexaploid wheat lines under study (Height = plant height at maturity; awn color; B = brown, LB = light brown, DB = dark brown, and W = white) and PMA = days-to-physiological maturity.

Line No.	Pedigree	Height (cm)	Awn color	PMA	Spike length (cm)	Grain weight (g)
35	68.111/RGB-U/WARD/3/FGO/4/*Ae. tauschii* (629)/5/CETA/ *Ae. tauschii* (895)	86	LB	128	12	46
36	ALTAR84/*Ae. tauschii* (224)/2*YACO	88	LB	128	13	51
37	SABUF/ALTAR84/*Ae. tauschii* (224)/3/YACO/CRO-1/*Ae. tauschii* (205)	88	LB	128	13	51
38	BCN//SORA/*Ae. tauschii* (323)	95	DB	128	12	50
39	OPATA/3/SORA//*Ae. tauschii* (323)	91	DB	128	11	46
40	BCN/4/68.111/RGB-U//WARD/3/*Ae. tauschii* (325)	88	DB	128	12	44
41	BCN//DOY 1/*Ae. tauschii* (447)	108	DB	128	12	53
42	BCN/4/RABI//GS/CRA/3/*Ae. tauschii* (895)	101	DB	128	13	45
43	MAYOOR	97	LB	127	15	41
44	MAYOOR	97	LB	127	13	38

Table 10. Details of entires with moderate resistance and susceptibility to the spot blotck fungus *C. sativus* in *in vivo* and *in vitro* conditions.

In vivo			*In vitro*		
Scale	**Entry Detail**	**Reaction**	**Scale**	**Entry Detail**	**Reaction**
1–2	2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 31, 32, 33, 35, 37, 38, 41, 42, 43, 46, 47	MR	1–2	8, 23, 19	MR
3–4	1, 8, 14, 29, 30, 34, 36, 39, 44, 45	MS	3–4	2, 4, 10, 29, 15, 16, 18, 20, 31, 32, 33, 37	MS
0	40	R	0	—	—

Phenotypic evaluation and molecular characterization of selected wheat landraces of Pakistan.

Rabia Amir, Alvina G. Kazi, Aziz-ur-Rehman, Rumana Keyani, Attiq-ur-Rehman, Farrukh Bashir, and A. Mujeeb-Kazi.

Landrace cultivars undoubtedly represent an important source of genetic variation in wheat. Although modern cultivars are derived from traditional land races, significant, unexploited variation remains among and between landraces held in gene banks. Landraces have been used successfully to improve the stress adaptations in modern cultivars. This study determined the genetic diversity of selected landraces of Pakistan by RAPD and SSR primers. Some phenological traits including plant height, spike length, awn color, 1,000-kernel weight, grains/spike, nodes/spike, days-to-flowering, physiological maturity, and pubescence also were investigated (Table 11, p. 167).

Of the 12 RAPD primers used, six (OPG9, OPG11, OPG15, OPF18, OPO20, and OPS5) gave no amplification and the remaining six (OPA10, OPC8, OPG2, OPG6, OPG12, and OPG13) amplified the polymorphic pattern. The size of the amplification products ranged from 500 to 10,000 bp. The highest number of scorable bands was obtained with primers OPG-12 and OPG-6 and the lowest with primer OPA-10. The maximum number of genotypes (21) were amplified by primer OPG-2 and minimum (6) by OPG-13. Different primers showed variation in their ability to detect polymorphism. Primers OPA-10 and OPG-6 showed the highest polymorphism and primer OPC-8 the lowest. Wheat genotypes T12 and T18 were amplified by a maximum number of primers (5). Genotypes T15 and 8A were not amplified by any primer. Genotypes C-258 and T3 showed maximum polymorphism. The RAPD amplification data was used to obtain a similarity matrix and for dendrogram generation. The value of similarity coefficient ranged from 41 to 100%.

Table 11. Phenotypic evaluation of traits of 28 wheat landraces (FLOW = days-to-flowering; HT = plant height at maturity (cm); AWN = awn color (B = brown, LB = light brown, DB = dark brown, and W = whitish); PMA = days-to-physiological maturity; and TKW = 1,000-kernel weight).

Pedigree	PUB	FLOW	HT (cm)	AWN	PMA	TKW (g)	Nodes /spike	Grains /spike	Spike length (cm)
T1 (*T. durum* subsp. *durum*)	—	130	106.0	B	145	32.2	12	47	8.0
T2 (*T. durum* subsp. *durum*)	—	130	112.0	W	144	31.2	12	36	8.7
T3 (*T. durum* subsp. *durum*)	—	129	108.0	LB	142	30.3	12	25	10.2
T7 (*T. aestivum* subsp. *sphaerococcum*)	—	129	94.3	—	137	21.6	10	42	7.3
T8 (*T. aestivum* subsp. *aestivum*)	—	128	106.0	DB	137	30.5	8	34	8.2
T9 (*T. aestivum* subsp. *aestivum*)	—	127	104.3	DB	137	24.7	8	49	9.3
T12 (*T. aestivum* subsp. *aestivum*)	—	126	103.0	DB	145	25.2	7	79	9.6
T14 (*T. aestivum* subsp. *aestivum*)	—	123	99.0	LB	144	24.5	10	58	11.0
T15 (*T. aestivum* subsp. *aestivum*)	—	122	98.0	LB	145	26.6	10	34	11.0
T16 (*T. aestivum* subsp. *aestivum*)	—	128	104.0	LB	133	23.7	8	51	9.7
T17 (*T. aestivum* subsp. *aestivum*)	—	121	104.0	—	137	20.3	11	40	12.3
T18 (*T. aestivum* subsp. *aestivum*)	—	122	111.0	—	137	23.8	9	47	9.4
T20 (*T. aestivum* subsp. *aestivum*)	—	127	100.0	—	137	34.5	10	70	11.8
T24 (*T. aestivum* subsp. *aestivum*)	—	126	99.3	—	137	28.0	9	52	8.5
8A (Selection)	—	121	90.0	DB	144	29.4	11	31	8.8
D-9 (Barani)	—	122	105.0	LB	140	29.5	9	47	10.2
C-217 (C-516 / C-591)	—	125	106.0	B	140	36.6	8	48	8.7
C-228 (Hard Federation/9D)	—	119	111.3	B	144	31.2	9	45	9.8
C-245	—	120	108.0	LB	144	30.0	9	45	8.7
C-247	—	119	114.3	DB	144	30.5	10	55	8.0
C-248	—	126	104.0	B	144	27.8	11	40	9.8
C-250 (Hard Federation/9D)	—	128	102.0	LB	145	30.8	8	26	12.0
C-256	—	124	100.3	LB	133	21.7	8	43	9.3
C-258	—	125	85.0	—	133	37.2	7	29	8.8
C-269	—	124	94.0	—	137	32.6	10	51	10.0
C-271 (C-220 / IP165)	—	119	113.0	B	137	41.2	10	55	10.4
C-288	—	126	101.0	LB	137	33.1	10	46	7.5
C-518 (T9 / 8A)	—	119	103.0	B	140	28.0	8	43	7.7

Genotypes showing the least similarity were 2 with 11 (41%) and 6 with 21 (43%). Similarly, genotypes showing 100% similarity were 4 with 8, 16 with 17, and 26 with 27. The similarity of remaining genotypes was between 41 to 100%.

The dendrogram showed three clusters. Cluster A included two genotypes (T1 and C-247) with a maximum genetic distance of 0.1032 (10%). Cluster B had 21 genotypes (T2, T8, T15, 8A, D-9, C-217, C-228, C-288, C-518, C-271, C-245, C-248, T16, T24, T14, C-250, C-269, C-256, C-258, T17, and T20) with genetically similar genotypes being T8 and T15, C-217 and C-518, and C-288 and C-518. In this cluster, most genetically dissimilar genotypes were C-256 and C-258 with C-256, showing a genetic distance of 0.5996 (59%), and C-258, being the maximum genetically

diverse genotype of this cluster with genetic distance of 0.7129 (71%). The remaining genotypes of this cluster were in the range of 0.00 to 59%. Cluster C included five genotypes in which genotypes T7 and T9 showed the least genetic distance of 0.1252 (12%) and genotype T18 with maximum genetic distance of 0.8873 (88%). The remaining genotypes of this cluster had a genetic distance in the range of 12 to 75%. Thus, cluster analysis indicated genotypes C-258 and T-20 (71%) and genotypes T18 and T2 (88%) as being genetically the most distinct genotypes.

The SSR amplification data was used to obtain a similarity matrix and for generation of dendrogram using 15 SSR primers (GWM33, GWM106, GWM232, GWM337, GWM458, GWM642, GWM165, GWM194, GWM608, GWM609, GWM624, GWM11, GWM18, GWM550, and GWM582) of chromosomes 1D, 4D, and 1B. The value of similarity coefficient ranged from 40 to 95%. Genotypes with the least similarity were 4 with 27 and 9 with 27. The genotypes with 95% similarity are 17 with 18 and 18 with 19. The similarity of remaining genotypes ranged between 40 to 95%.

The dendrogram is divided into three main clusters; A, B, and C. Cluster A included three genotypes (T1, T2, and C-217). T1 and T2 being genetically identical showed a genetic distance of 0.0855 (8%) with the remaining genotypes, whereas C-217 had a genetic distance of 0.3267 (32%). Cluster B included 23 genotypes (T3, T8, C-228, C-245, C-247, C-269, C-271, T24, C-248, C-250, C-256, C-258, C-288, C-518, T9, T20, T18, T14, D-9, T7, T15, T16, and T17). This cluster showed minimum genetic distance between genotypes T3 and T8 (0.0855), C-228 and C-245 (0.0504), C-269 and C-271 (0.0678), C-258 and C-256 (0.1035), C-288 and C-518 (0.2191), T18 and T20 (0.0504), T14 and D-9 (0.1035), and T15 and T16 (0.0855). The maximum genetic distance of 0.4473 (44%) was shown by T7 in this cluster. The genetic distance of remaining genotypes of this cluster remained in the range of 5 to 70%. Cluster C included only two genotypes (T12 and C-258) with T12 showing a genetic distance of 0.8920 (89%) and C-258 of 0.645 (64%). Analysis of dendrogram revealed T12 and T7 (89%) and C-258 and T7 (64%) as most genetically distinct genotypes.

The six RAPD primers yielded on the average 17 bands/primer, whereas 15 SSR primers amplified on the average eight bands/primer. The average number of polymorphic bands/primer was higher in case of RAPDs (11.1) than SSRs (4.5). The percentage of polymorphism among wheat land races in RAPDs and SSRs was 66.6 and 68%, respectively, revealing that wheat land races are highly diverse and can be used for improvement of local Pakistani cultivars.

After morphological examination, genotypes T2, T3, T7, T18, C-217, and C-258 were found to be diverse. In case of the RAPDs, the amplification products of 28 landraces with six primers yielded a total of 102 scorable bands, 68 of which were polymorphic. Thus, the percentage of polymorphism among these genotypes was 66.6%. Primers OPA-10 and OPG-8 showed highest polymorphism. In the SSRs, the amplification product yielded a total of 112 scorable bands of which 83 were polymorphic. The percentage of polymorphism was 68%. Primers GWM337 and GWM194 showed highest polymorphism. The RAPD study indicated genotypes T3, T18, and C-258 as genetically most diverse, whereas the SSR study indicated genotypes T7, T12, and C-258 as most diverse. Thus, genotype C-258 is indicated as the most genetically diverse genotype.

Phenotypic evaluation and molecular characterization of selected wheat land races suggests that the allelic variation of this germ plasm can be used in improving new wheat cultivars for high yield, resistance to rusts, and desirable quality traits. The germ plasm is maintained as a working collection in Ayub Agricultural Research Institute, Faisalabad, and the gene bank storage in PGRI, National Agricultural Research Center, Islamabad.

In vitro screening of a double-haploid mapping population developed for spot blotch resistance with selective molecular characterization.

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Wheat is the most important staple food crop of Pakistan that occupies more farmland than any other crop and is grown under irrigated and rain-fed conditions in Pakistan. Among fungal diseases, foliar pathogens other than rusts contribute significantly to low average yields of cereal crops. Spot blotch/leaf blight is the most severe constraint of wheat production in the countries of Southeast Asia where climates are warm and moist. Currently, this biotic constraint requires investigative research in our country.

The best method for controlling the disease is through use of resistant materials that can be used in breeding programs to obtain durable resistance to *C. sativus*. One such germ plasm form is from the primary gene pool that harnesses the D-genome accessional diversity of *Ae. tauschii* in the form of synthetic-hexaploid wheats. To gain more insight into genetic control, molecular mapping populations were developed previously at CIMMYT using the DH methodology. One population of 171 DH individuals is 'Mayoor//TKSN1081/*Ae. tauschii* (222)/3/Flycatcher'. This population was phenotyped by a stringent *in vitro* screening test. Selective commercial cultivars also were assessed for resistance or susceptibility. The screening results showed that out of the 171 DH entries, 12 lines (107, 112, 114, 116, 120, 122, 125, 128, 138, 144, 152, and 156) and three commercial cultivars (Chakwal-86, Kirin-95, and Bakhtawar-93) possessed moderate resistance to *C. sativus* (Table 12). These resistant lines were subjected to molecular evaluation for assessing their diversity levels. Five RAPD primers (OPG-2, OPG-5, OPG-8, OPG-10, and OPG-12) were evaluated for their diversity profiles, out of which OPG-12 amplified 12 out of 13 samples and two cultivars. The primer OPG-5 amplified nine double haploids out of 13 and one cultivar out of three. OPG-10 amplified a total of ten samples including eight double haploids and two cultivars. One hundred-one DNA fragments were amplified with four RAPD primers, with an average of 25 bands/primer. The number and size of the amplified fragments also varied with different primers. A maximum of 36 bands were amplified with primer OPG-12 and a minimum of seven fragments with primer OPG-8. The amplified products ranged from 500–2,500 bp. The genetic similarity between the population ranged between 0.4118 and 0.9412. The maximum coefficient (0.9412) was observed for pairs 1–2, 6–7, and 5–10, whereas the lowest coefficient (0.4118) was observed for pairs 5–7, 3–16, and 9–16. The remaining population had similarity coefficients between 0.4706 and 0.8824. Data from the RAPD primers indicated that DH entry 125 was the most genetically diverse and showed a maximum genetic distance with Kirin-95. Among the commercial cultivars, Chakwal-86 exhibited the maximum genetic distance with M.FCT-125. Hence, we recommend introducing its allelic resistance into commercial cultivars for sustainable production and making their moderate resistance more stable and durable. Phenology estimates provided an additional selective sieve for the preferential use of other moderately resistant lines in wheat breeding (Table 13).

Table 12. *In vitro* screening of commercial cultivars against *Cochliobolus sativus* (progressive 1–5 scoring scale where 1 = resistant and 5 = susceptible).

Cultivar	Leaf score	Response
Bakhtawar-93	2	MR
Inqilab-91	3	MS
Kirin-95	2	MR
Tandojam-83	5	S
SH-2002	5	S
Bhakkar-2002	4	MS
Fakhr-e-Sarhad	5	S
Marvi-2000	5	S
Tatara	5	S
Takbeer	5	S
AS-2002	4	MS
Iqbal-2000	5	S
Auqab-2000	5	S
Zarlashta	5	S
Wafaq-2001	5	S
Margalla-99	5	S
Chakwal-86	2	MR
Nowshera-96	3	MS
GA-2002	5	S
Manthar-3	4	MS

Table 13. Some phenological traits of 12 moderately resistant doubled-haploid entries in the mapping population (Mayoor//TKSN1081/*Ae. tauschii* (222)/3/Flycatcher) studied.

Line No.	Plant height (cm)	Awn color	Spike length (cm)	Grain weight (g)
107	97	light brown	11	32.3
112	104	light brown	10	43.4
114	100	light brown	12	38.0
116	109	light brown	12	38.8
120	104	light brown	10	33.0
122	93	light brown	10	37.6
125	98	light brown	12	47.8
128	103	light brown	9	43.1
138	107	light brown	12	35.8
144	107	light brown	10	32.0
152	118	light brown	10	39.2
156	113	light brown	12	34.7

Molecular fingerprinting of some advanced bread wheat-breeding lines resistant to stem rust (Ug99) and their utilization in wheat production.

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Stem rust of wheat is one of the three rusts that are a major production constraint for the cereal globally. Recently, an alarming situation has arisen due to Ug99 race in Kenya where susceptibility of Pakistani cultivars has been observed. Furthermore, the new race has reached Yemen and chances of it entering Pakistan are a few years away. Because our wheat germ plasm is vulnerable, internationally identified materials have been introduced for assessing adaptation, diversity, and release of suitable introduced germ plasm in stem rust affected areas. Our focus is on Sindh and lower Punjab, either directly or after being bred into the high yielding cultivars of these locations. We analyzed the 29 entries of the International Elite Bread Wheat Yield Trials (2EBWYT) for adaptation, phenology, and RAPD- and SSR-based molecular diversity.

Twenty-nine RAPD primers (OPC8, OPG11, OPG13, OPO20, OPG5, OPS6, OPF18, OPA10, OPA15, OPA2, OPA4, OPA11, OPU13, OPU14, OPU15, OPA1, OPA6, OPA9, OPO6, OPR6, OPR15, OPE1, OPE2, OPE3, OPE4, OPE5, OPE6, OPJ20, and OPN13) were used to detect genetic polymorphism at DNA level in the 29 2EBWYT lines. After screening the 29 primers, seven showed amplification. The efficiency of the primers to amplify the genotypes ranged from six genotypes by OPJ20 to five by OPU3 and two by OPN13. Population genetic analysis showed that the total number of loci is 140, out of which 36 are polymorphic with a percentage of 25.71. Scorable bands ranged from 500 to 2,000 bp. The maximum scorable bands (five) were shown in 2EBWYT-21 and the minimum number of bands (one) was in 2EBWYT-3, 2EBWYT-6, and 2EBWYT-13. The value of the similarity matrix ranged from 88.57% (minimum) between genotypes 2EBWYT-3 and 2EBWYT-20, 2EBWYT-20, and 2EBWYT-27. The maximum (100%) similarity between genotypes was observed between 2EBWYT-1 and 2EBWYT-4, -5, -10, -11, -16, -22, -23, -24, -26, -28, and -29; 2EBWYT-2 and 2EBWYT-8; 2EBWYT-4 and 2EBWYT-5, -10, -11, -16, -22, -23, -24, -26, -28, and -29; 2EBWYT-5 and 2EBWYT-10, -11, -16, -22, -23, -24, -26, -28, and -29; 2EBWYT-10 and 2EBWYT-11, -16, -22, -23, -24, -26, -28, and -29; 2EBWYT-11 and 2EBWYT-16, -22, -23, -24, -26, -28, and -29; 2EBWYT-16 and 2EBWYT-22, -23, -24, -26, -28, and -29; 2EBWYT-22 and 2EBWYT-23, -24, -26, -28, and -29; 2EBWYT-23 and 2EBWYT-24, -26, -28, and -29; 2EBWYT-24 and 2EBWYT-26, -28, and -29; 2EBWYT-26 and 2EBWYT-28 and -29; and 2EBWYT-28 and 2EBWYT-29.

The genetic distances among the 29 genotypes were used to construct a dendrogram by UPGMA analysis for determining grouping of 2EBWYT lines on the basis of similarities and differences. The dendrogram generated was divided into three clusters. Cluster A included a total of 12 genotypes (2EBWYT-1, -4, -5, -10, -11, -16, -22, -23, -24, -26, -27, and -29) all being genetically identical. Cluster B included nine genotypes (2EBWYT-14, -17, -2, -8, -9, -20, -12, -18, and -15) with a minimum genetic distance of 0 present between 2EBWYT-2 and -8, and 2EBWYT-15 showed the maximum genetic distance of 0.448. Cluster C included eight genotypes (2EBWYT-7, -20, -19, -25, -28, -21, -3, and -13) with a minimum genetic distance of 0.402 present between 2EBWYT-7 and 2EBWYT-20 and between 2EBWYT-25 and 2EBWYT-27. The maximum genetic distance of 0.587 was present in 2EBWYT-21 followed by 0.539 in 2EBWYT-18, and 0.492 in 2EBWYT-3 and 2EWBYT-13. A RAPD-based cluster analysis depicted that 2EBWYT-21 is genetically the most diverse line with maximum genetic distance of 0.587. Furthermore, 2EBWYT-3, -13, and -18 also were considered as diverse lines.

Twenty SSR primers (GWM33, GWM106, GWM232, GWM337, GWM458, GWM642, GWM165, GWM194, GWM608, GWM609, GWM624, GWM37, GWM44, GWM111, GWM121, GWM295, GWM350, GWM428, GWM437, and GWM635) specific to chromosomes 1D, 4D, and 7D, were used to screen the germ plasm and resulted in the identification of 12 primers showing the amplification. The efficiency of the SSR primers to amplify the genotypes ranged from six genotypes by primer GWM33 and two genotypes by primer GWM165 to six genotypes by primers GWM608, GWM44, and GWM635. Population genetic analysis showed that the total number of loci is 204 out of which 36 are polymorphic and their percentage is 17.65.

The value of the similarity matrix of the SSR primers ranged from 91.18% (minimum) between genotypes 2EBWYT-15 and 2EBWYT-9 and 2EBWYT-6 and 2EBWYT-21 and was 100% between genotypes 2EBWYT-22 and 2EBWYT-25. The dendrogram showed four clusters. Cluster A included ten genotypes (2EBWYT-1, -2, -3, -9, -10, -24, -26, -27, -7, and -8) with a minimum genetic distance of 0.087 present between 2EBWYT-24 and 2EBWYT-26 and maximum genetic distance of 0.448 present between 2EBWYT-9 and 2EBWYT-10. Cluster B included seven

Table 14. Phenological evaluation of 29 lines from the Second International Elite Bread Wheat Yield Trials sown at National Agricultural Research Center, Islamabad, Pakistan, in 2007–08 (Items for awn color; DB = dark brown, B = brown, LB = light brown, and W = whitish).

Entry No.	Pedigree	Pubescence	Days-to-flower	Height at maturity (cm)	Awn color	Days-to-physical marurity	1,000-kernel weight (g)	Nodes /spike	Grains /spike	Spike length (cm)
1	WBLL1*2/TUKURU	–	130	98	LB	135	20.9	12	46.0	13
2	FRET2/TUKURU//FRET2	+	130	96	LB	134	51.8	12	30.0	14
3	MILAN/S87230//BABAX	+	129	102	LB	132	29.8	12	53.0	13
4	ATTILA/3*BCN//BAV92/3/TILHI	+	129	98	LB	132	51.9	10	46.0	12
5	TILHI/PASTOR	–	128	95	LB	136	29.7	8	47.0	13
6	WAXWING*2/TUKURU	+	127	97	LB	132	34.8	8	60.0	19
7	FRET2*2/BRAMBLING	+	126	97	LB	132	38.1	7	54.6	13
8	WBLL1*2/BRAMBLING	+	123	96	LB	135	30.6	10	47.0	10
9	WBLL1*2/KIRITATI	–	122	99	LB	132	39.3	10	42.0	11
10	VORB/FISCAL	+	128	98	LB	132	49.2	8	34.0	13
11	CHIBIA//PRLII/CM65531/3/FISCAL	+	120	96	LB	132	48.2	11	48.0	14
12	BL2064//SW89-5124*2/FASAN/3/TILHI	+	121	96	LB	135	49.2	9	44.0	9
13	OASIS/SKAUZ//4*BCN/3/2*PASTOR	–	127	91	LB	132	34.4	10	40.0	9
14	KIRITATI/WBLL1	+	126	97	LB	133	44.9	9	61.0	11
15	PFAU/SERI.1B//AMAD/3/WAXWING	–	121	96	LB	132	29.1	11	56.0	10
16	WBLL1*2/BRAMBLING	–	122	100	LB	132	40.7	9	61.0	13
17	MUNIA/CHTO/3/PFAU/BOW//VEE#9/4/CHEN/….	+	125	94	LB	132	29.8	8	52.0	11
18	BABAX/LR24//BABAX*2/3/VIVITSI	–	119	97	LB	132	30.8	9	53.0	12
19	BABAX/LR24//BABAX*2/3/VIVITSI	–	120	97	LB	132	26.2	9	40.0	11
20	WAXWING*2/KIRITATI	–	119	98	LB	132	21.4	10	22.0	12
21	WBLL1*2/BRAMBLING	+	126	92	LB	132	28.6	11	47.0	13
22	PFAU/WEAVER*2//TUKURU	+	128	94	LB	132	31.4	8	36.0	10
23	KIRITATI//PRL/2*PASTOR	+	124	85	LB	132	37.6	8	30.0	10
24	WORRAKATTA/PASTOR	+	125	101	LB	136	39.1	7	58.0	12
25	TAM200/PASTOR//TOBA97	–	124	96	LB	132	26.4	10	48.0	10
26	HPO/TAN//VEE/3/2*PGO/4/MILAN/5/SSERI1	–	119	90	LB	133	35.1	10	38.0	11
27	PFAU/WEAVER*2//KIRITATI	+	126	95	LB	133	41.7	10	65.0	12
28	PFAU/WEAVER*2//KIRITATI	–	119	92	LB	133	51.2	8	41.0	11
29	SKAUZ/BAV92	+	126	103	LB	136	31.7	10	45.0	13

genotypes (2EBWYT-16, -23, -11, -20, -13, -28, and -29) with minimum genetic distance of 0.448 present between 2EBWYT-27 and 2EBWYT-29; 2EBWYT-13 showed the maximum genetic distance of 0.585 followed by 0.492 between 2EBWYT-11 and 2EBWYT-20. Cluster C included six genotypes (2EBWYT-8, -22, -25, -17, -18, and -19) with a minimum genetic distance of 0 between 2EBWYT-22 and 2EBWYT-25. The maximum genetic distance of 0.539 was manifested between genotypes 2EBWYT-18 and 2EBWYT-19. Cluster D included six genotypes (2EBWYT-12, -4, -5, -6, -14, and -15) with minimum genetic distance of 0.448 present between 2EBWYT-4 and 2EBWYT-5 and a maximum genetic distance of 0.693 for 2EBWYT-12, 0.639 between 2EBWYT-14 and 2EBWYT-15, and 0.587 in 2EBWYT-6.

An SSR-based cluster analysis of dendrogram depicted that 2EBWYT-12 is the most diverse line among the 29 2EBWYT lines with maximum genetic distance of 0.693. Furthermore, 2EBWYT-14 and 2EBWYT-15 also are considered as genetically diverse lines with genetic distance of 0.639.

Phenotypic data depicted that 2EBWYT-2, 2EBWYT-4, 2EBWYT-10, 2EBWYT-11, 2EBWYT-12, and 2EBWYT-28 are the best lines on the basis of yield enhancing characters (Table 14, p. 171). Cluster analysis of RAPD and SSR primers revealed that genotypes 2EBWYT-21 showed maximum diversity of 0.587 followed by 2EBWYT-18 and 2EBWYT-13 in case of RAPD primers, whereas SSR analysis depicted 2EBWYT-12, 2EBWYT-14, 2EBWYT-15, and 2EBWYT-6 as diverse genotypes. These results form the basis of direct line selection for wheat production security for stem rust and also provide the guideline for targeted breeding goals against the pathogen.

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ITEMS FROM THE RUSSIAN FEDERATION

AGRICULTURAL RESEARCH INSTITUTE OF THE CENTRAL REGION OF NON-CHENOZEM ZONE
143026, Moscow region, Nemchinovka, Kalinina 1, Russian Federation.

Soft wheat hybrids showing no segregation for resistance to leaf rust.

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The soft winter wheat cultivar Nemchinovskaya 24 has demonstrated absolute resistance to leaf rust since the time of its releaset 20 years ago. In order to understand the genetic basis of the resistance, we crossed Nemchinovskaya 24 with tester lines of spring wheat with genes *Lr9*, *Lr24*, *Lr24 + Sr24*, *Lr27 + Lr31*, *Lr28*, *Lr29*, *Lr38*, and *LrTr*). The susceptible soft spring wheat Khakasskaya was used as a check.

The F_1 hybrids and their parental lines were not susceptible to leaf rust and that the resistance genes of their parental lines appeared to be dominant. The F_2 hybrid progeny of the cross 'Nemchinovskaya 24 / Khakasskaya' segregated according to a trihybrid pattern, 43 resistant plants : 21 susceptible plants (Table 1).

Table 1. Segregation patterns in the F_2 hybrids of crosses with Nemchinovskaya 24 (N24) and lines carrying *Lr* genes for resistance to leaf rust. Critical $\chi^2 = 3.84$.

Cross	Number of resistant plants	Number of susceptible plants	Ratio of resistant to susceptible plants		χ^2
			Observed	Expected	
N24 / Khakasskaya	120	64	42 : 22	43 : 21	0.324
N24 / *Lr9*	127	0	—	—	—
Lr24 / N24	80	6	13.3 : 1	15 : 1	0.078
N24 / *Lr24+Sr24*	157	0	—	—	—
Lr27+Lr31 / N24	122	7	17.4 : 1	15 : 1	0.149
Lr28 / N24	138	9	15.3 : 1	15 : 1	0.004
Lr29 / N24	61	4	15.3 : 1	15 : 1	0.001
N24 / *Lr38*	171	**0**	—	—	—
N24 / *LrTr*	181	**0**	—	—	—

We found the action of one main and two complementary inhibiting genes. F_2 hybrids between stocks with *Lr24, Lr27 + Lr31, Lr28,* and *Lr29* with Nemchinovskaya 24 segregated according to a dihybrid pattern (15 resistant: 1 susceptible). The F_2 progenies from lines with *Lr9*, *Lr24 + Sr24*, *Lr38*, and *LrTr* are interesting because no plants were susceptible to leaf rust. All the plants are