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SYNTHETIC HEXAPLOID WHEATS AS A NOVEL SOURCE OF GENETIC DIVERSITY AND RESISTANCE AGAINST YELLOW RUST

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Abstract

Special subsets of synthetic hexaploid wheats (SHWs) have been formed and one such subset is "Elite II" comprising of 33 SH entries. The subset was screened against yellow rust having virulence for *Yr1, Yr4, Yr6, Yr7, Yr8, Yr9, Yr12, Yr17, Yr18, Yr26, Yr*27, *YrSp, YrSu, YrSk* and *YrA*; collected from diverse wheat growing areas of Pakistan. Fifteen genotypes were found resistant at seedling stage by exhibiting infection type 2-4, indicating the presence of major resistant genes. Adult plant screening under field conditions showed 8 genotypes resistant, three moderately resistant and 4 susceptible. Six RAPD markers of 10-mer arbitrary sequences were applied for genetic diversity analysis of these 15 genotypes. Fifty eight amplicons were generated by 4 primers. Total 83.33% polymorphism was computed among 15 lines. Dendrogram showed 15 genotypes divided into two distinct clusters. The coefficients are in the range of 38.89% to 100% on similarity matrix. Genotypes Elite II-16 and 31 were most polymorphic with 38.89% similarity. Genetic diversity and resistance identified can be exploited in wheat breeding for yield and durable resistance against yellow rust, using limited and genetically diverse SHWs for commercial bread wheat improvement.

Introduction

Stripe rust caused by *Puccinia striiformis tritici* Eriks, is one of the most destructive and devastating pathogens to wheat production all over the world (Stubb, 1985)**.** Yellow rust is more important in the areas having cool and wet climatic conditions. It frequently attacks wheat crop in Indo-Pak subcontinent, especially the foothills of Himalaya, the North Eastern and Northern Pakistan. Being a potential threat to wheat production in cooler parts of the country, yellow rust is continuously jeopardizing the wheat production, thus most efforts have to be diverted to control this disease. Chemical applications and cultural practices have been adopted effectively in controlling the outbreak of rust diseases, however, the use of resistant cultivars remained most economical and environment friendly. Sustainable resistance to stripe rust should be enhanced by making the availability of major resistant genes and genes conferring durable resistance from the available genetic sources. The last two to three decades wheat breeding focused on high yielding cultivars with durable resistance against rusts. Identification of additional sources of resistance and their incorporation in to elite wheat cultivars for durable resistance is still required. The leading cultivars grown by the farmers are having narrow genetic base (Mukhtar, 2002), and very limited polymorphism throughout the world (Cadalen *et al*., 1997), making the crop more vulnerable to rust diseases. International wheat breeding has given major emphasis on genetic control of disease by introducing the new genetic diversity and resistant genes within elite commercial cultivars.

Wheat wild progenitors are potent sources of resistance and genetic diversity, due to their worldwide distribution, natural selection and process of evolution for millions of years evolved genes for resistance, especially the *Aegilops tauschii* (DD), the D-genome progenitor of bread wheat. Synthetic hexaploid wheats (AABBDD) were produced by hybridizing various *Ae. tauschii* (DD) accessions with elite durum wheat (*Triticum turgidum*: AABB) cultivars (Mujeeb-Kazi, 2006). Resistance sources against yellow rust have been identified by various scientists in *T. turgidum*, *T. durum*, *Ae. tauschii* and synthetic hexaploid wheat (Ma *et al*., 1997; Sumaira *et al*., 2007; Tariq-Khan and Ul-Haque, 2011). Over the past few decades, various important agronomic traits have been successfully transferred from wild progenitors into bread wheat advance lines (Yang *et al*., 2003; Lage *et al*., 2003; Khan & Khan, 2010). DNA molecular markers were successfully used for the assessment of genetic diversity among *Triticum dicoccoides* accessions (Fahima et al., 1999), among and within the CIMMYT landraces (Dreisigacker *et al*., 2005), landraces from Balochistan (Khan *et al*., 2010) and in improved wheat cultivars of Pakistan (Bibi *et al*., 2009).

CIMMYT produced a large number of synthetic hexaploid wheats (SHW), special subsets have been formed, "Elite-II" is a subset comprising of 33 entries. This subset from CIMMYT has been in extensive evaluation for finding resistance to major biotic stresses of global importance. In this study, Elite-II genotypes were screened at seedling and post seedling stages against yellow rust. The main objective of the study was to evaluate the major and minor gene for resistance lying in these synthetics, and molecular based diversity analysis had pinpointed the highly polymorphic genotypes. Phenological data will further help us in selection of superior type of SHWs for future breeding programmes regarding resistance against yellow rust and genetic diversity in commercial bread wheat improvement.

Materials and Methods

Screening of host germplasm against yellow rust: Thirty three accessions of Elite-II synthetic wheat (AABBDD; 2n=6x= 42) were obtained from CIMMYT Wide Crosses Program (Mujeeb-Kazi, 2006). Elite-II SHWs were planted in disposable pots under glass house conditions at CDRP Sunny Bank Murree. Plants were inoculated with urediniospore suspension of yellow rust having virulent genes against *Yr1, Yr4, Yr6, Yr7, Yr8, Yr9, Yr12, Yr17, Yr18, Yr26, Yr*27, *YrSp, YrSu, YrSk* and *YrA* in the form of mixture of 30:70 mineral oil and petroleum ether**.** Inoculation process was same adopted by Rizwan *et al*., (2007). Disease severity was recorded according to the McNeal Scale 0-9 (1971), when susceptible check was showing maximum infection. Experiment was repeated twice in 2006-07 and 2007-08 summer. Field screening was done at National Agricultural Research Centre Islamabad (NARC) fields during 2006-07 and 2007-08 with same inoculum. Out of 33 genotypes, fifteen (Table 2) found resistant at seedling stage were selected for molecular studies.

Phenological data: Data relating to phenology of each genotype was taken during the winter season 2006-07 and 2007-08. It contained general features of the genotype, awn colour, number of tillers per plant, plant height etc.

DNA extraction: The DNA was extracted by using Weining & Langridge (1991) protocol. To remove RNA from DNA, DNA was treated with 40µg RNAse-A (0.20µl of commercially supplied RNAase-A purchased from Gene Link, USA) at 37°C for 1 hour. After RNAase treatment DNA quantification was done with the help of spectrophotometer. DNA samples were run on 1.0% Agarose gel to check the quality of DNA and then stored at 4ºC.

Polymerase chain reaction (PCR): To use in PCR, a 1:5 dilution of DNA was made in double distilled, deionized and autoclaved water. Six RAPDs of OPA series (Table 1) were used for PCR studies. It was carried out in 25 µl reaction buffer containing 50-100 ng total genomic DNA template, 0.25 µM of each primer, 200 µM of each dATP, dGTP, dCTP, dTTP, 50 mM KCl, 10 mM Tris, 1.5 mM $MgCl_2$ and 2.5 units of Taq DNA polymerase. The amplification conditions were as an initial step of denaturization for 1 minute at 94°C followed by 45 cycles each consisting of a denaturization for 1 minute at 94°C. An annealing step of 1 minute at 34°C and an extension step of 2 minutes at 72°C. Seven minutes were given after the last cycle to the extension step at 72°C to ensure the completion of the primer extension reaction. Gene Amp PCR system 2700 was used for all amplification reactions. For electrophoresis of the amplification products, 1.5% agarose gel was used. Gels were visualized by Ethidium Bromide under the UV light chamber and observed using the computer program UVIPhotoMW. Amplification conditions were essentially the same as described for RAPDs. For electrophoresis of the amplicons produced by RAPDs, 3.0% agarose gel was used.

RAPDs analysis: For statistical analysis of RAPDs, all the scorable bands were considered as single locus/allele. The loci were scored as present (1) or absent (0). Bivariate (0-1) data matrix was generated. Genetic distances were calculated using Un-weighed Pair Group of Arithmetic Means (UPGMA) procedure as GDxy=1-dxy/dx+dy-dxy, where GDxy = Genetic distance between two genotypes, dxy = total number of common loci (bands) in two genotypes, dx = Total number of loci (bands) in genotype 1 and dy=Total number of loci (bands) in genotype 2 (Nei & Li, 1979). Dendrogram was constructed by using bivariate data matrix of primers with the help of computer programme, "Popgene32" version 1.31.

Results

Seedling stage resistance: Fifteen genotypes of wheat showed resistance against yellow rust. Infection types (0-4) recorded representing resistant (R) and moderately resistant (MR) (Table 2) reaction against yellow rust at seedling stage under ideal conditions and flux of pathogen. It indicated the presence of major genes for resistance against yellow rust.

Adult plant stage resistance: Results of selected 15 wheat genotypes having major genes for resistance against yellow rust showed variability of resistance reaction at adult plant stage in the field. Eight lines were found resistant, 3 lines moderately resistant and four genotypes susceptible to yellow rust (Table 2).

Table 1. Primers used for genetic analysis along with their oligo name and sequences.

Primer No.	Oligo name (OPA)	Primer sequence	Molecular weight	EC	Size
1	DecamerA-01	CAGGCCCTTC	2963.97	92.8	10
2	DecamerA-02	TGCCGAGCTG	3044.01	101	10
3	DecamerA-03	AGTCAGCCAC	2996.98	107.5	10
4	DecamerA-04	AATCGGGCTG	3068.02	109	10
5	DecamerA-08	GTGACGTAGG	3108.04	119.8	10
6	DecamerA-09	GGGTAACGCC	3053.01	107.7	10

Table 2. Stripe rust screening results of Elite-II synthetic hexaploid wheat (Partial data from Tariq-Khan and Ul-Haque 2011).

Elite-II Accession	Pedigree	Seedling stage reaction against yellow rust at CDRP Sunny Bank Screen House Murree	Field reaction against yellow rust at NARC nursery, Islamabad
05	TK SN1081/*Ae. tauschii* WX222; (TA1599)	3 4	MR
09	STY-US / CELTA // PALS /3/ SRN 5 /4/ *Ae. tauschii* (WX431)	1 2	R
10	LCK59.61 / *Ae. tauschii* (WX693)	3 4	MR
11	SKARV 2 / *Ae. tauschii;* (WX304; TA2449)	2 3	R
13	DOY 1 / *Ae. tauschii* (WX1027)	1 2	R
16	CETA / *Ae. tauschii* (WX533)	2 3	S
17	CPI / GEDIZ /3/ GOO // JO / CRA /4/ *Ae. tauschii* (WX1018)	1 2	R
18	CETA / *Ae. tauschii* (WX1031)	3 4	S
22	CROC 1 / *Ae. tauschii* (WX212)	2 3	S
24	ARLIN 1 / *Ae. tauschii* (WX430)	3 4	S
27	GAN / *Ae.tauschii* (WX206)	2 3	MR-MS
30	68111 / RGB-U // WARD RESEL /3/ STIL /4/ *Ae. tauschii* (WX385; TA2549)	1 2	R
31	CETA / *Ae. tauschii*WX417	0 1	R
32	68.111 / RGB-U // WARD RESEL /3/ STIL /4/ *Ae. tauschii* (WX431)	0 1	R
33	DOY 1 / *Ae. tauschii* (WX534)	0 1	R

R= Resistant, MR= Moderately Resistant, MS= Moderately Resistant, S= Susceptible

Genetic diversity evaluation: Out of six applied primers, four amplified the DNA scorable bands. The primers OPA-8 amplified 9 samples, OPA-3 amplified 8, OPA-4 amplified 7 and OPA-2 amplified only 2 out of 15 genotypes. Allele size ranged from 750bp-1500bp for OPA-2, 750bp-3000bp for OPA-3, 500bp-1500bp for OPA-4, 500bp-2500bp for OPA-8 for all the fifteen genotypes. Total 58 scorable bands with an average of 14.5 bands per primer and 3.86 bands were generated for each genotype.

Every single band was considered as a single locus/allele. Un-weighed Pair Group of Arithmetic Means (UPGMA) function (Nei & Li, 1979) was used to estimate genetic distances between the genotypes. Dendrogram (Fig. 1) was constructed with the help of bivariate data showing genetic relationship of the Elite-II genotypes, showing two clusters, A and B. Two genotypes "05 and 16", found outlier, Coefficient of Variation (COV) for genotype05 is 50-66.67% and for genotype16 as 38.89-55.56%. Cluster A divided into two sub-clusters, where genotype31 found most diverse with COV range of 77.78-83.33%; B comprised of 5genotypes with COV range of 83.33-94.4% (Table 3).

Phenological characterization: Phenological data of SHWs during 2006-07 and 2007-08 covers over all phenotypic expressions of genotypes (Table 4). Breeders and agronomist are most concerned to these parameters for their future selection. Elite-II 09, 10, 24 and 30 has excellent tillering quality; 1000 grain weight is prime factor for future yield and Elite-II 9, 10, 17, 24, 27, 30, 31, 32 and 33 has high grain weight (Table 4).

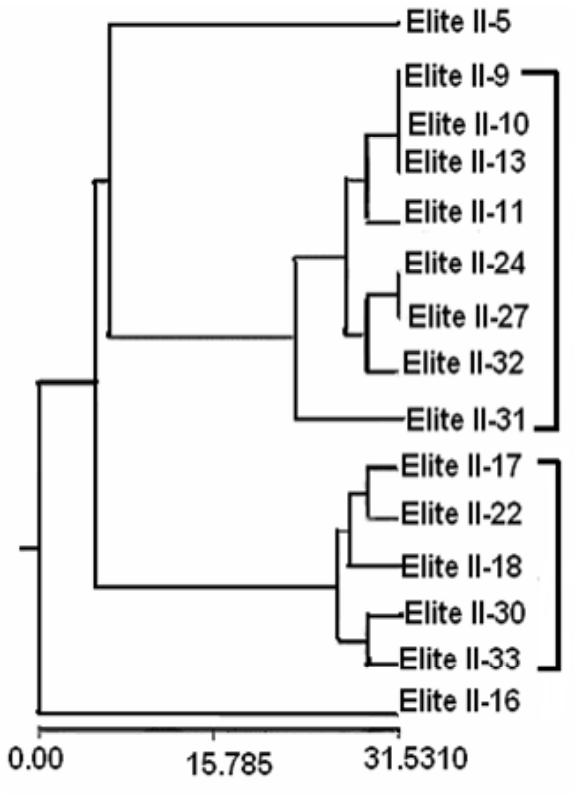

Fig. 1. Dendrogram based Nei's (1972) genetic distance: Method = UPGMA modified from Neighbor procedure of PHYLIP version 3.5.

Table 3. RAPDs Similarity Matrix for Synthetic hexaploid (SHs) wheats resistant to stripe rust at seedling stage.

Pop ID	Elite II -05	Elite II -09	Elite II-10	Elite II-11	Elite II-13	Elite II-16	Elite II-17	Elite II-18	Elite II-22	Elite II-24	Elite II -27	Elite II -30	Elite II -31	Elite II -32	Elite II -33
Elite II -05	****	0.6667	0.6667	0.6111	0.6667	0.5556	0.5556	0.6667	0.6111	0.5556	0.5556	0.5000	0.5000	0.6111	0.5556
Elite II -09		****	1.0000	0.9444	1.0000	0.5556	0.5556	0.5556	0.6111	0.8889	0.8889	0.5000	0.8333	0.9444	0.5556
Elite II -10			****	0.9444	1.0000	0.5556	0.5556	0.5556	0.6111	0.8889	0.8889	0.5000	0.8333	0.9444	0.5556
Elite II -11				****	0.9444	0.6111	0.5000	0.5000	0.5556	0.9444	0.9444	0.5556	0.7778	0.8889	0.5000
Elite II -13					****	0.5556	0.5556	0.5556	0.6111	0.8889	0.8889	0.5000	0.8333	0.9444	0.5556
Elite II -16						****	0.5556	0.4444	0.5000	0.5556	0.5556	0.6111	0.3889	0.5000	0.5556
Elite II -17							****	0.8889	0.9444	0.6667	0.6667	0.9444	0.7222	0.6111	0.8889
Elite II -18								****	0.9444	0.5556	0.5556	0.8333	0.7222	0.6111	0.8889
Elite II -22									****	0.6111	0.6111	0.8889	0.7778	0.6667	0.9444
Elite II -24										****	1.0000	0.6111	0.8333	0.9444	0.5556
Elite II -27											****	0.6111	0.8333	0.9444	0.5556
Elite II -30												****	0.6667	0.5556	0.9444
Elite II -31													****	0.8889	0.7222
Elite II -32														****	0.6111
Elite II - 33															****

Nei's genetic identity (above diagonal)

Table 4. Phenolgical data results of Elite-II syntehtic hexaploid wheats.

Elite-II No.	Pub + /-	Pig + /-	Awn color	Awn length in cm	Days to flowering	Days to maturity	Plant height in cm	Leaf shape N/B	Avg. no of tillers/plant	1000 Kernel weight in gms
05	-	-	Brw	7.5 cm	131 ± 3	179 ± 3	24 ± 1.3	N	5.0 ± 0.4	31.00 ± 2
09	-	+	Brw	4.5 cm	133 ± 5	179 ± 3	35 ± 2	N	11 ± 0.4	50.00 ± 3
10	+	+	Y	04 cm	152 ± 3	182 ± 4	34 ± 3	B	13 ± 0.4	46.00 ± 1.9
11	-	-	LY	4.5cm	154 ± 5	173 ± 4	35 ± 1	N	4.0 ± 0.3	31.00 ± 2
13	-	+	Brw	03 cm	134 ± 4	174 ± 3	27 ± 3	N	5.0 ± 0.6	34.00 ± 1.8
16	-	-	Brw	03 cm	130 ± 3	183 ± 2	37 ± 1	B	5.0 ± 0.5	41.00 ± 2
17	-	+	Brw	03 cm	133 ± 3	180 ± 5	34 ± 4	N	5.0 ± 0.2	53.00 ± 4.6
18	+	+	Y	1.5 cm	134 ± 2	180 ± 3	36 ± 2	N	4.5 ± 0.8	44.00 ± 3
22	-	+	Brw	03 cm	131 ± 3	180 ± 5	28 ± 4	B	5.0 ± 0.9	40.00 ± 2
24	-	+	Brw	03 cm	131 ± 2	183 ± 3	33 ± 4	B	7.0 ± 0.15	47.00 ± 3
27	-	-	LY	02 cm	134 ± 4	161 ± 2	34 ± 2	N	4.0 ± 0.14	49.00 ± 2.2
30	-	+	Y	2.5 cm	154 ± 3	181 ± 5	20 ± 1	B	7.0 ± 0.1	54.00 ± 2.5
31	-	+	Y	04 cm	131 ± 1	175 ± 4	27 ± 3	N	4.0 ± 0.2	52.00 ± 3
32	-	+	Y	3.5 cm	134 ± 4	176 ± 3	30 ± 2	N	5.5 ± 0.3	59.00 ± 2
33	-	+	Brw	03 cm	124 ± 2	176 ± 5	25 ± 3	B	4.0 ± 0.2	49.00 ± 3

Pub. : Pubescence, Pig: Pigment, Awn color: Brw : Brown, W: Whitish, Y: Yellow, LY: Light Yellow. **Leaf shape:** N: Narrow, B: Broad

Discussion

Yellow rust is continuous threat to wheat production all over the world. Newly emerged virulent races of *Puccinia striiformis tritici* continuously targeted the resistant genes. Newly emerged virulence successfully overcame *Yr2* (McIntosh, 2009), *Yr9* in Syria, Turkey, Iran, Pakistan and India (Singh *et al*., 2004) in past few years, portraying a potential threat to wheat production. In Pakistan, wheat losses for leading cultivars were estimated as 5.77%, 6.63% and 14.90% for Inqilab-91, Wafaq-2001 and Bhakkar respectively (Afzal *et al*., 2007). Cultivars with *YrA*, *Yr6* and *Yr22* genes subjected heavy yield losses due to newly emerged virulent races. *Yr27* which exist in high yielding cultivars like Inqilab-91 was already broken. In Pakistan, *Yr3*, *Yr5*, *Yr10*, *Yr15*, *Yr26*, *YrSP* and *YrCV* are still resistant to yellow rust. Breeders were advised to use *Yr18*, *Yr29*, *Yr36* and *Yr39* with quantitative trait loci for adult plant resistance (Bux *et al*., 2011). Most of the high yielding world bread wheat cultivars are being cultivated successfully with *Yr18* along with adult plant minor genes for resistance. Our study supported the results of Tariq-Khan & Ul-Haque (2011) regarding yellow rust resistance performance of Elite-II synthetic Germplasm with little variations in reaction at seedling stage and likely to adult plant stage.

SHWs has recognized as source of resistance to biotic and a-biotic stresses. Phenological data is a base for future selection for agronomic traits (Khan, 2007), very little has been done regarding exploitation of SHWs for bread wheat improvement (Rizwan *et al*., 2007). Novel genetic diversity residing in SHWs is a source of durable and sustainable outputs (Mujeeb-Kazi & Rajaram, 2002). Calderini & Ortiz-Monasterio (2003) pointed out increased amount of macro and micro nutrients in synthetic germplasm.

Incorporation of identified resistance for yellow rusts and genetic diversity residing in Elite-II selected genotypes into locally adopted high yielding wheat cultivars like Inqilab-91, Wafaq-2001 and Punjab-96 is necessary to produce high quality wheat. It can ensure our future food security by enhancing micro and macro nutrients, grain weight. Elite-II 09, 10, 17, 27, 30, 31, 32 and 33 proved resistance against yellow rust and possessed excellent agronomic traits (Table 4). Integration of the phonological data and COV (Table 3) can give sustainable potent genotypes. Cultivars with sustainable production and durable resistance against yellow rust can be achieved by pyramiding effect of multiple genes introgression by applying multiline breeding approach. It will ensure our future food security.

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