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NEW SOURCES OF WHEAT YELLOW RUST (*PUCCINIA STRIIFORMIS* F. *TRITICI*) SEEDLING RESISTANCE

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Abstract

Wild relatives of wheat as new sources of genetic diversity are a potent resource for addressing biotic and abiotic stress constraints that limit wheat productivity. These are distributed in the three gene pools of the Triticeae and over the last two decades are being extensively utilized in breeding programmes globally. In this study, 200 accessions of *Triticum turgidum* (2n=4x=28; AABB), 40 accessions of *Aegilops tauschii* (2n=2x=14;DD), and 6 accessions of *Ae. triuncialis* (2n=4x=28;CCUU) were screened against stripe rust of wheat (*Puccinia striiformis tritici*) at seedling stage. Bulk inoculum collected from environmentally diverse wheat growing areas of Pakistan was used for the seedling screening. The innoculum had virulence for genes *Yr1, Yr3, Yr4, Yr5, Yr6, Yr7, Yr8, Yr9, Yr18, YrSp, YrSD, and YrCV.* Infection types (ITs) ranging from low to high were recorded within each germplasm category where 35 durum lines (*T. turgidum,*) *and* 13 of *Ae.tauschii* had good seedling resistance (0-2). Another 20 durum and 12 *Ae. tauschii* lines were found moderately resistant. Frequency distributions of the ITs was higher for *Ae. tauschii* lines (34%) as compared to the durum wheats (20%). Advanced germplasm testing involving synthetic hexaploid wheats have made available several lines that are resistant to stripe rust. The source of resistance in this germplasm is attributed to alleles on the A and B genomes of durum parents, or on the *Ae. tauschii's* D genome, or is a combination of genes that are pyramided as a consequence of A, B and D genome hybridizations. Ample diversity has been identified that warrants exploitation in wheat breeding.

Introduction

Stripe rust, caused by the obligate parasite *Puccinia striiformis* Westend. f. sp. *tritici* Eriks., is one of the most important diseases of wheat (*Triticum aestivum* L.). This forma speciales infects numerous wheat cultivars, as well as few barley cultivars and certain grass species (Stubbs, 1985). Historically, stripe rust has been more important in areas with cool, wet environmental conditions and, therefore, occurs regularly in northern Europe, the Mediterranean region, Middle East, Western United States, Australia, East African highlands, China, the Indian subcontinent, New Zealand, and the Andean regions of South America (Dennis & Brown, 1986). Stripe rust is also important in more tropical areas of higher altitude such as the North African countries, the Himalayan foothills of India and Pakistan, and Mexico (McIntosh, 1980). In comparison with the leaf rust and stem rust pathogens of wheat, the global distribution of *P. striiformis* f. sp. *tritici* is more restricted. Stripe rust did not occur in Australia until 1979 (O'Brian *et al*., 1980), and was not found in New Zealand until 1980 (Bayles *et al*., 1989).

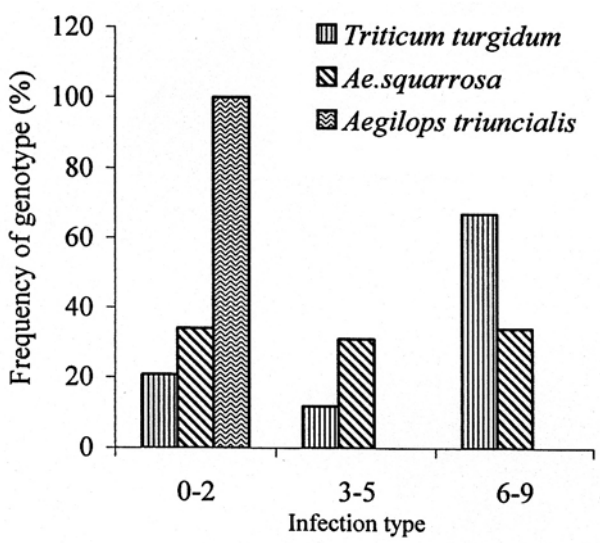

Fig. 1. Frequency distribution of the Its of *T. turgidum, Ae. Squarrosa* and *Ae. Triuncialis*.

The sexual stage of *P. striiformis* has not been encountered and alternate hosts have not been identified (Stubbs, 1985). Numerous, highly specified pathotypes of *P. striiformis* occur and are probably evolved by mutation and somatic recombination (Stubbs, 1985). Because of destructive nature of the rusts more efforts have gone in fighting rusts than any other disease. The emphasis has been two-fold around identifying sources of resistance and on incorporating new genes from new sources into wheat for durable resistance.

Related wild progenitor and non-progenitor species of wheat represent a large reservoir of useful variability that can be exploited for wheat improvement **(**Mujeeb-Kazi, 2006). Over the past few decades several agronomically important traits, including resistance to diseases, pests and abiotic stresses have been transferred from wild progenitors into wheat (Knott & Dvorak, 1976; Sharma & Gill, 1983; Jiang *et al*., 1994; Sharma, 1995; Friebe *et al*., 1996) and exploited commercially.

In Pakistan, the leading cultivars grown by the farmers are susceptible to stripe rust. The options other than varietal deployment are to first identify and then incorporate new genetic diversity in the wheat . Hence the objective of the present study was to evaluate genetic diversity for stripe rust resistance in genomically diverse germplasms.

Material and Methods

Host germplasm: Two hundred accessions of *Triticum turgidum* (2n=4x=28; AABB), 40 accessions of *Aegilops tauschii* (2n=2x=14, DD), and 6 accessions of *Ae. triuncialis* (2n=4x=28; CCUU) were obtained from Plant Genetics Resource Institute (PGRI) at the National Agricultural Research Center (NARC), Islamabad where this study was undertaken. In addition from the CIMMYT wide cross program of interspecific hybridization several synthetic hexaploid (*T. turgidum X Ae. tauschii*: 2n=6x=42, AABBDD) entries with their durum parental contributors were also screened.

The pathogen: Bulk innoculum collected across all the environmentally diverse regions of Pakistan was used. Virulence was prevalent for genes *Yr1, Yr3, Yr4, Yr5, Yr6, Yr7, Yr8, Yr9, Yr18, YrSp, YrSD and YrCV.*

Greenhouse evaluation: *T. turgidum*, *Ae. tauschii*, A*e. triuncialis* and synthetic hexaploid wheats were planted in disposable pots under glasshouse conditions. Four to six week old plants were inoculated with urediospore suspension of the bulk innoculum suspended in a mixture of 30:70 mineral oil: petroleum ether. Innoculated plants were placed in open air for two hours for evaporating the oil. Plants were then transferred to a dew chamber set at temperature and light regimes of 10^{o}C, 16h light and 8h dark for 48 hrs. The plants were then transferred to the glasshouse under minimum / maximum temperature controls of 10 and 18^{o}C.

Disease scoring for seedlings: Three weeks after inoculation infection types were recorded on the plants on a 0-9 scale (McNeal *et al*., 1971**),** when the susceptible check was showing maximum infection. Plants having infection types 0-2 were considered as resistant, those having infection types 3-5 with intermediate resistance and those scored between 6-9 ranged from susceptible to highly susceptible**.**

Results and Discussion

Hexaploid bread wheat (*Triticum aestivum*) was first cultivated around 6,000 years ago. It contains an enormous amount of variation and is grown globally under many environmental conditions and is thus exposed to diverse biotic and abiotic stresses that influence its productivity. New variation for resistance traits could increase wheat yields without requiring an increase in inputs of pesticides, fertilizers, and irrigation water. Only a small proportion of the available genetic variation in the gene pool of wheat, has been exploited for crop improvement in modern breeding programs. Hence exploiting sources of diversity within the Triticeaae has developed as a potent means to enrich the genetic composition of cultivated wheats (Mujeeb-Kazi, 2003, 2006). Of the two options available for utilizing the unique germplasm emphasis has been attached to those forms that are closely related to wheat and thus the diploid progenitors are held in high priority currently (Mujeeb-Kazi *et al*., 2007). Of these, the D genome diploid progenitor *Ae. tauschii* occupies the top spot (Mujeeb-Kazi, 2006) and the route that incorporates its alleles via bridge crossing has been more exploited by breeders since this also allows the access of the other two genomes present in the germplasm used known as synthetic hexaploid wheats. Synthetics are a product of combinations that involve elite durum cultivars (*T. turgidum,* 2n=4x=28, AABB) combined with diverse D genome accessions (*Ae. tauschii*, 2n=2x=14, DD) yielding hybrids that are ABD genomically which upon induced chromosome doubling by colchicine (Mujeeb-Kazi *et al*., 2006) form the hexaploid synthetics; 2n=6x=42, AABBDD.

In this study two aspects have been investigated: a) Screening of various durum wheats and various *Aegilops* accessions from the gene bank, and b) Screening of synthetic hexaploid wheats of the elite I and II international sets of CIMMYT Mexico together with their durum cultivars.

The focus has been on *Ae. tauschii* accessions in each aspect since this D genome progenitor of bread wheat is recognized to be a rich reservoir of genes for many biotic and abiotic stress (Knott, 1979, Gill *et al*., 1986). Numerous accessions of this diploid grass have been screened globally for their resistance to many diseases and pests of wheat. *Lr41* and *Yr28* resistant genes from *Ae. tauschii* (Kerber & Dyck, 1969; Dyck & Kerber, 1970; Kerber, 1987; Cox & Gill, 1992; McIntosh, 1988) and many agronomically important traits have been transferred from Ae. *tauschii* to bread wheat.

In this study, high frequency of the seedling resistant accessions of *Ae. tauschii* confirm the above mentioned fact. These 13 accessions of *Ae.tauschii,* collected from Balochistan province of Pakistan, while providing a diverse source of resistance to this disease, can also be further tested and exploited for other stress factors that limit wheat production. The resistant selections made from the 200 accessions of durum wheats are an added resource for adding A and B genome diversity that has been under-exploited in wheat breeding. They have yielded 35 entries that have IT values of 0-2 (Table 1). Another 20 are moderately resistant and could also be used when their diversity data becomes available.

Although a high level of resistance (0-2) was found in the durum wheats tested the frequency of such accessions was low when compared to *Ae.tauschii*. This could be due to the low frequency of resistant genes in the test samples. Genotypes of durum wheats from Central, Southern or Western Europe and South America have a high frequency of resistant genotypes. But we cannot say unequivocally that this is the resistance status of durums for stripe rust resistance in Pakistan. There are hundreds of accessions stored in the gene bank that could be screened for stripe rust resistance in Pakistan to establish this fact, thus permitting the unlocking of unique alleles for practical utility.

The strategy for using these resistant durums is via crossing them onto high yielding but stripe rust susceptible bread wheats and then advancing the F1 pentaploid (2n=5x=35, AABBD) output to extract new durums and bread wheats based upon recombination events involving the variable A and B genomes present in the cross. The additional route that exploits both germplasms is to combine the durum cultivars with each *Ae. tauschii* accession, test the synthetic hexaploid products and utilize it in wheat breeding *via* bridge crossing (Mujeeb-Kazi *et al*., 2007). If targeted transfers are desired then the resistant accessions of *Ae. tauschii* (Tables 1 and 2) can be hybridized with ideal bread wheat cultivars and direct transfers into the D genome of such cultivars be achieved (Gill & Raupp, 1987). Both the above strategies have shown to be effective in wheat improvement.

The second option is where internationally available synthetics were studied alongwith their durum parents. The data of Table 3 indicates the seedling resistance values of this germplasm. The durum cultivars have 17 resistant entries with IT values of 2 to 3 on a 0 to 9 scale. Synthetic hexaploid wheats in Elite 1 range in IT values from 0 to 3 and this range is between 2 to 3 for the Elite 11 sub-set of synthetics. The interpretations suggest that a synthetic wheat can be resistant due to three reasons; the first due to the A and B genome of its durum parent, or due to its *Ae. tauschii* accessional genome, or due to both the A and B genomes and the *Ae. tauschii's* D genome. Data of Table 3 indicates those entries that define these classes of resistance contribution. The notations At mean it is *Ae. tauschii* source, Td is from the durum parent and At, Td from both the *Ae. tauschii* and durum contributors.

Table 1. Seedling yellow rust resistance evaluation of the progenitor germplasm under controlled glasshouse conditions.

Sr. No	Value	Symbol	*Triticum turgidum* (200)	*Ae. tauschii* (40)	*Ae. triuncialis*
1.	0-2	Resistant	35	13	1
2.	3-5	Moderately Resistant	20	12	0
3.	6-9	Susceptible	114	13	0
Total entries			**169**	**38**	**1**

Table 2. Seedling resistance (IT<3 on a 0-9 scale) to stripe rust in tested lines.

Triticum turgidum	Accession Numbers
	16611, 16615, 16673, 16675, 16538, 16556, 16553, 16696, 16700, 16532, 16624, 16692, 16552, 16697, 16697, 16643, 16641, 16629, 16626, 16531, 16623, 16534, 16621, 16649, 16554, 16695, 16699, 16698, 16525, 16412, 16588, 16662, 16664, 166686, 16687, 16711
Aegilops tauschii	18278, 16386, 16405, 16410, 16411, 16409, 16406, 16392, 16396, 17322, 17328, 17330
Aegilops triuncialis	16398

Conclusions

Virulence presence for stripe rust is a lingering threat to national wheat production that also extends across all geographical boundaries. The dynamics of this pathogen's character imposes a major stress that can jeopardize wheat crops productivity. Thus breeders continue their search for new genes or assemble gene combinations that can give a genetically durable varietal performance system. Search for new gene sources is spread across the three wheat gene pools and of these a focus on the primary gene pool holds priority. The choice is narrowed down to the D genome diploid progenitor species *Ae. tauschii* (2n=2x=14) essentially because of its maximum proximity to the D genome of bread wheat and due to its extensive global distribution that exposes the accessions to a multitude of biotic and abiotic stress environments. Screening and identification of resistant accessions is paramount to breeding programs and in this study several sources have been identified from this *Ae. tauschii* resource. These alleles will significantly enrich the D genome of bread wheat. Additional sources of resistance are present in the A and B genomes and thus genetic pyramiding would enlarge the resistance security across all three wheat genomes. Not included here is the involvement of molecular diversity analysis that would significantly add merit to picking the best of the best materials for wheat improvement, and for enhancing breeding efficiency. RAPD's would be the initial source of establishing DNA diversity followed next at least by exploiting the genome and chromosome specific microsatellite markers (Roder *et al*., 1998).

Table 3. Sources of seedling resistance (0-9 scale) to stripe rust in *Triticum turgidum* and synthetic hexaploid wheats tested under glasshouse conditions.

Sr. No	Accession Number	Genotype	Seedling IT Range	Source of seedling resistance
		Triticum turgidum		
1.		68.111/RGB-U//WARDRESEL/3/STIL	2	
2.		68.111/RGB-U//WARD	2	
3.		SNIPE/YAV79/DACK/TEAL	2	
4.		DECOY1	2	
5.		GAN	2	
6.		SCOOP_1	2	
7.		LCK59.61	2	
8.		LARU	2	
9.		PI/GEDIZ/3/GOO/JO/CRA	3	
10.		STERNA-DW	3	
11.		SCAUP	3	
12.		TKSN1081	2	
13.		YARMUK	3	
14.		STY-US/CELTA//PALS/3/SRN_5	3	
15.		ACONCHI89	3	
16.		YAV_2/TEZ	3	
17.		6973/WARD.7463//74110	3	
		Synthetic hexaploid wheats : ELITE I		
18.	6	CROC-1/AE. SQUARROSA (205) *	0	At
19.	37	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE. SQUARROSA (629)	0	At
20.	45	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE. SQUARROSA (878)	0	At
21.	47	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE. SQUARROSA (882)	0	At
22.	54	CETA/AE. SQUARROSA(895)	0	At
23.	83	DOY1/AE. SQUARROSA (458)	0	Td At
24.	66	BOTNO/AE. SQUARROSA(625)	1	At
25.	74	YAV_2/TEZ//AE. SQUARROSA(895)	1	At
26.	77	RASCON/AE. SQUARROSA(312)	1	At
27.	91	CROC-1/AE. SQUARROSA(517)	1	At
28.	49	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE. SQUARROSA(890)	2	At
29.	50	CROC-1/AE. SQUARROSA (518)	2	At

Table 3. (Cont'd.)

Sr. No	Accession Number	Genotype	Seedling IT Range	Source of seedling resistance
30.	52	ALTAR 84/AE. SQUARROSA (JABANGOR)	2	At
31.	89	STY-US/CETA//PALS/3/SRN_5/4/AE. SQUARROSA(502)	2	Td At
32.	90	ALTAR 84/AE. SQUARROSA (502)	2	At
33.	3	ALTAR 84/AE. SQUARROSA (192)	3	At
34.	5	ALTAR 84/AE. SQUARROSA (198)	3	At
35.	16	ALTAR 84/AE. SQUARROSA (219)	3	At
36.	28	68.111/RGB-U//WARD/3/ AE. SQUARROSA (316)	23	At
37.	31	68112/WARD//AE. SQUARROSA (369)	3	At
38.	34	DOY1/AE. SQUARROSA (511)		Td At
39.	41	68.111/RGB-U// WARD RESEL/3/STIL/4/ AE. SQUARROSA (783)	3	Td At
40.	75	ARLIN/AE. SQUARROSA (283)	3	At
41.	76	FALCIN/AE. SQUARROSA (312)	3	At
42.	84	GREN/AE. SQUARROSA (458)	3	At
43.	93	CETA/AE. SQUARROSA (1024)	3	At
44.	12	DVERD-2/AE. SQUARROSA (1027)	3	At
45.	35	DOY1/AE. SQUARROSA (515)	4	Td At
46.	3	ROK/KML//AE. SQUARROSA (214)	4	At
47.	36	68.111/RGB-U// WARD/ 3/ AE. SQUARROSA (511)	4	At
48.	38	FGO/USA2111//AE. SQUARROSA (658)	4	At
49.	42	YAR/AE. SQUARROSA (783)	4	At
50.	44	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE. SQUARROSA (878)	4	At
51.	94	CETA/AE. SQUARROSA (1027)	4	At
		Synthetic hexaploid wheats: ELITE II		
52.	13	DOY1/AE. SQUARROSA (1027)	3	Td At
53.	16	CETA/AE. SQUARROSA (533)	3	At
54.	18	CETA/AE. SQUARROSA (1031)	3	At
55.	22	CROC_1/AE. SQUARROSA (212)	3	At
56.	24	ARLIN_1/AE. SQUARROSA (430)	2	At
57.	31	CETA/AE. SQUARROSA (417)	2	At

Td = *Triticum turgidum,* At = *Ae.tauschii syn. Ae. squarrosa*

● = *Ae. squarrosa* accession entry in the Wheat Wide Crosses Programs working collection at CIMMYT, Mexico.

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