Indian Journal of Agricultural Sciences **85** (3): 331–4, March 2015/Article
https://doi.org/10.56093/ijas.v85i3.47089

Genetic analysis of the stem rust resistance genes in synthetic hexaploid wheat (*Triticum aestivum*) lines

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Received: 19 June 2013; Revised accepted: 5 January 2015

ABSTRACT

The objective of this investigation was to examine the inheritance of stem rust (*Puccinia graminis* f. sp. *tritici*) resistance genes in synthetic hexaploid wheat lines and to identify the allelic relationship among the resistant lines. Genetic basis of stem resistance was studied in Synthetic 4, Synthetic 55, and Synthetic 86 and $F_{2:3}$ population, derived from (susceptible cultivar, i.e. Agra local × resistant lines (Synthetic 4, Synthetic 55, and Synthetic 86). Isolate 40A of *Puccinia graminis tritici* (most prominent stem rust pathotype in India) was used to examine the segregation pattern. The results revealed that resistance in Synthetic 4 and Synthetic 55 for pathotype 40A was governed by a single dominant gene, as F_2 seedlings segregates in a ratio of 3:1. While, resistance in Synthetic 86 was governed by two genes, i.e. one dominant and one recessive gene, as F_2 seedling depicted 13:3 segregation ratio, which further conferred by F_3 family data. Allelism studies (using as F_2 population derived from a cross between Synthetic 4 × Synthetic 55), revealed the resistant gene present in these line was same. However, F_2 population derived from Synthetic 4 × Synthetic 86 and Synthetic 55 × Synthetic 86 showed that the resistant gene in Synthetic 86 was different from Synthetic 4 and Synthetic 55 as seedling of F_2 population segregated in ratio of 61:3.

Key words: Genetics resistance, Inheritance, *Pucinia graminis* f. sp. *tritici*, Synthetic hexaploid wheat

In India stem rust (*Puccinia graminus* f sp. *tritici*) occurs in most of the wheat growing areas, particularly severe in central, peninsular and southern part of the country. The introgression of resistance gene from wild related or cultivated species has provided greater genetic diversity to rust resistance in wheat. So far several stem rust (*Sr*) resistance genes, confirming specific resistance to this rust has been identified and assigned to specific chromosome (McIntosh *et al*. 2003). The alien sources of rust resistance were incorporated into desirable agronomic background to facilitate the easy transfer of rust resistance gene into newly adopted genotypes (Sawhney *et al*. 1996). Many successful efforts to transfer desirable gene from wild relatives of wheat involving *T. tauschii*, and other sources of resistance genes have been introduced into *T. aestivum* (Cox *et al*. 1994). For diversification of genetic resistance, some synthetic hexaploid wheat's (SHWs) are produced at CIMMYT from *T. turgidum* × *T. tauschii* crosses with the objective to exploit new genetic variability available for resistance or tolerance to abiotic and biotic stress including rust resistance (Mujeeb-Kazi *et al*. 1996). The D-genome of *T. tauschii*, and AB genome of *T. turgidum*, are known to be a rich reservoir of valuable genes for resistance to diseases and pests of bread wheat (Cox *et al*. 1994). Since, direct transfer of resistance genes from diploid and tetraploid species to hexaploid wheat requires cytological follows up (Ma *et al*. 1995). Therefore, synthetic hexaploid wheat provides an excellent opportunity in easy transfer of resistant genes from *T. turgidum* and *T. tauschii* to cultivated wheat without cytological analyses.

In this study, material of three SHW's brought from CIMMYT, carrying high degree of resistance against the virulent races of stem rust were selected and were studied for the genetic analysis. The investigation was undertaken to determine the nature of inheritance of resistance genes and to study the allelic relationship among these resistant sources.

MATERIALS AND METHODS

Three stem rust resistant synthetic hexaploid wheat lines introduced from CIMMYT, Mexico (Table 1) were used to generate study material. The F_1, F_2 and F_3 populations of three resistant synthetic hexaploid wheat (SHW) × susceptible parents (Synthetic 4 × Agra local, Synthetic 55 × Agra local and Synthetic 86 × Agra local) and F_1 and F_2 populations of three (SHW) resistant × resistant parents (Synthetic 4 × Synthetic 55, Synthetic 4 × Synthetic 86 and Synthetic 55 × Synthetic 86) were generated during years 2006 and 2007. The material was

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Table 1 Synthetic hexaploid wheats, their ID number and parentages

SHW	ID No.	Pedigree		*Ae. tauschii* accession
		T. turgidum	*Ae. tauschii*	
SHW 4	CIGM 87-2775	ALTAR 84	*Ae. tauschii* (193)	W×188
SHW 55	CIGM 90-799	GAN	*Ae. tauschii* (180)	W×180
SHW 86	CIGM 93-229	DOY1	*Ae. tauschii* (372)	W×372

SHW, Synthetic hexaploid wheat; I D No., Identification Number, Ae, *Aegilops*

evaluated against stem rust during 2008-09. The surface sterilized seeds (10 each from P_1, P_2 and F_1 and 60 seeds from F_2 generation) from each cross were sown in three replications in pots (6" × 6") containing sterilized medium made up of the decomposed agropeat, vermiculite and sand in the ratio of 2: 1: 1 in the glasshouse at National Phytotron Facility, Indian Agricultural Research Institute, New Delhi. Five seeds were sown in each pot with a uniform depth of about 1.5 cm. After sowing, pots were placed under controlled environmental conditions in the greenhouse. The pots, containing seedlings of Agra local (highly susceptible check) were placed after every two rows of pots having study material. Simultaneously, each resistant and susceptible individual of F_2 population having >30 seeds were progeny tested (as F_3 families with their F_2 identities).

The urediospore inoculum of most virulent race (40A) of *P. graminis* f. sp. *tritici*, received from DWR, Regional Station, Flowerdale, Shimla was multiplied on susceptible variety Agra local in glasshouse. The multiplication of inoculum and testing of material in the glass house was done following Joshi *et al.* (1988). About 7–9 days old seedlings of Agra local were sprayed with inoculums and incubated in the humid glass chamber for 48 hours. Urediospore dust was collected after 12–15 days of inoculation. The purity of pathotype was tested on differential sets before use.

The inoculum was sprayed when seedlings were eight to ten days old. Before inoculation, the plants were sprayed with water to provide a uniform layer of moisture on the leaf surface. Freshly collected urediospores were suspended in water, fortified with Tween- 20 and sprayed on seedlings. The inoculated material was incubated for 36 hr in humid glass chambers at a temperature of 23 ± 2 °C and more than 85% relative humidity. After incubation, seedlings were shifted to muslin cloth chambers at the same temperature to avoid contamination and spread of the rust pathogen. Before inoculation, the incubation and growth chambers were thoroughly sterilized with the spray of ethyl alcohol and baking at 500ºC for 24 hours. Disease reaction was recorded twice at 10th and 12th days after inoculation. The segregating (families having both resistant and susceptible plants) and non-segregating (families having only resistant plants from resistant F_2 plants and susceptible plants from susceptible F_2 plants) F_3 families were identified.

Table 2 Stem rust rating scale described by Peterson (1948)

Class	Infection type (IT)	Description of symptoms
Immune	0	No uredia nor other indications of infection
Nearly immune	;	No uredia, but hypersensitive flecks present
Very resistant	1	Uredia minute; surrounded by distinct necrotic areas
Moderately resistant	2	Uredia small to medium; usually in green island surrounded by a decidedly chlorotic or necrotic border
Mesothetic (Heterogeneous)	X	Uredia variable, sometimes including all infection types and intergradations between them on the same leaf.
Moderately susceptible	3	Uredia medium in size; coalescence susceptible infrequent; no necrosis, but chlorotic areas may be present
Very susceptible	4	Uredia large, and often coalescing; no necrosis, but chlorosis may be present under unfavorable growing conditions.

The severity of disease was scored on 0-4 scale as described by Peterson *et al.* (1948) after 12 and 15 days of inoculation. Infection types from 0-2 were considered as a low response, indicating a resistant or moderately resistant host. Infection types from 3 to 4 were considered as a high response, indicating a moderately susceptible or susceptible host.

The families showing disease severity between TR, 0 to 2 were considered as R and the progeny showing uniform level of it 3 and 4 were considered as S (Renu Khanna *et al.* 2005). The segregating (families having both resistant and susceptible plants) and non-segregating (families having only resistant plants from resistant F_2 plants and susceptible plants from susceptible F_2 plants) F_3 families were identified. The Chi-square ($\chi 2$) test for goodness of fit, was used to compare the actual ratios with those calculated for Mendelian segregation.

RESULTS AND DISCUSSION

Screening and resistance reactions

The studies on host pathogen interaction between SHWs and 40A pathotype of stem rust produced a spectrum of infection type (IT) ranging between; 0, ; and 1. The Synthetic 4 showed reaction of 0 and ; whereas in synthetic 55 and synthetic 86, the reaction type was of ; with a maximum of 1. Agra local was highly susceptible with infection type rating of 4 at seedling stage in glasshouse.

Inheritance of resistance

The F_1 seedling generations of crosses Synthetic 4 ×

Table 3 Mode of segregation of seedlings in different generations of crosses between Synthetics and Agra local inoculated with stem rust pathotype 40A

Population	No. of seedlings			Expected	$\chi 2$	Df	P
	R	S	T	ratio	23:1		
F_1 (Syn4×AL)	9	0	9				
[a]F_2 (Syn4×AL)	122	49	171	3:1	1.22	1	0.269
F_1 (Syn55×AL)	10		10				
F_2 (Syn55×AL)	110	29	139	3:1	1.269	1	0.260
F_1 (Syn 86×AL)	8		8				
[b]F_2 (Syn 86×AL)	146	24	170	13:3	2.39	1	0.122

Syn, Synthetic lines; AL, Agra Local a susceptible cultivar; R, Resistance; S, Susceptible; T, total; DF, Degree of Freedom; P, probability; R denotes with very small uredinia surrounded by necrosis (Resistance); S denotes large uredinia lacking necrosis/ chlorosis (Susceptible)

Table 5 Segregation pattern at seedling stage in F_2 generation of crosses between different synthetic hexaploid wheat with Agra local, inoculated with pathotype 40A

(test of allelism)

Populations	No. of plants			$\chi 2$	P
	R	S	Total	61:3	
F_2 (Synthetic 4 × 55)	175	0	175		
F_2 (Synthetic 4 × 86)	171	12	183	1.430	0.232
F_2 (Synthetic 55 × 86)	181	13	194	1.765	0.184

R, Resistant; S, susceptible; $\chi 2$, Chi-square value; P, probability

resistance families observed were 21, 42, and 12 respectively, which showed 1:2:1 ratio (P>0.05) of goodness of fitness which again confirmed the presence of single dominant gene in Synthetic 4 against pathotype 40A (Table 4).

Table 4 Mode of segregation of seedlings in F_3 families of crosses between Synthetics and Agra local against pathotypes 40A

Population	Pathotype	No. of Families			Total Expected	$\chi 2$
		HR	*Seg(MR-MS)	HS	ratio	
[a]F_3 (Synthetic 4 × AL)	40A	21	42	12	75 (1:2:1)	3.24
F_3 (Synthetic 55 × AL)	40A	14	47	16	77 (1:2:1)	3.86
[b]F_3 (Synthetic 86 × AL)	40A	57	77	5	139 (7:8:1)	2.64

HR, Homozygous resistance plants; "*Seg" denote family segregating for resistant and susceptible plant; HS denotes highly susceptible homozygous plants

Agra local and Synthetic 55 × Agra local showed resistance reaction (IT, 1) to stem rust pathotype 40A, indicating that stem rust resistance in Synthetic 4 and Synthetic 55 is governed by dominant gene. Based on infection types, the F_2 seedlings were divided into two groups, i.e. resistant (infection type similar to Synthetic 4) and susceptible (infection type similar to Agra local). The F_2 seedlings of Synthetic 4 × Agra local with 122 resistant and 49 susceptible individuals, segregated in a ratio of 3:1 ($\chi 2$=1.22) indicates that resistance in Synthetic 4 against stem rust is governed by single dominant gene. Similarly several researchers, viz. Nzuve *et al.* (2013), Ghazvini *et al.* (2012), Babiker *et al.* (2009), Yin *et al.* (2008), Nanthakumar and Tomar (2003) on various background wheat cultivars also reported resistant reaction against stem rust in wheat is governed by single dominant gene, On-contrary Dyke and Skyes (1995) reported single recessive gene controls resistant reaction against some Ethiopian wheat collections.

The F_3 families derived from individual F_2 plants based on their disease reaction were harvested individually. According to reaction three groups of F_3 families were obtained, which were classified as homozygous resistant (HR), homozygous susceptible (HS) and segregating (SEG). Families which were resistant and did not segregate in F_3 generation were considered as HR, whereas, families which were susceptible in F_3 generation were categorized homozygous susceptible (HS). Similarly segregating families which showed moderate reaction (resistance as well as susceptible) were classified as SEG. In F_3 families the number of homozygous susceptible, segregating and

The F_2 population of Synthetic 55 × Agra local produced 110 resistance and 29 susceptible individuals respectively against 40A pathotype of stem rust (Table 3), which perfectly fits in expected segregation ratio of 3:1 for single dominant gene ($\chi 2$=1.269). In F_3 families, the number of homozygous resistance, segregating and homozygous susceptible individuals were 14, 47 and 16 respectively. The ratio observed was showing goodness of fit (P>0.05) with the expected ratio of 1:2:1. It also confirmed the presence of single dominant gene in Synthetic 55 (Table 4).

The F_1 population of Synthetic 86 × Agra local exhibits resistance reaction (IT; 1) against stem rust pathotype 40A, indicates that resistant in Synthetic 86 is dominant at seedling stage. The F_2 population of this cross with 146 resistant and 24 susceptible individuals exhibited 13:3 (P>0.05) ratio of fitness (Table 3). In F_3 families the number of homozygous resistance, segregating and susceptible families observed were 57, 77 and 5 respectively, which showed goodness fits with the expected ratio of 7:8:1, indicating the presence of one dominant and one recessive gene for resistance in Synthetic 86.

The inheritance studies of various F_2 and F_3 populations of three crosses, indicates that the resistance in Synthetic 4 and Synthetic 55 was monogenic and dominant, whereas in Synthetic 86, it was di-genic and governed by one dominant and one recessive gene.

Test of allelism

Test of allelism was performed to discover whether resistant gene (s) present among three resistance sources

were same or different. The F_1 plants from all the crosses showed only a resistance reaction to stem rust pathotype 40A, indicates dominant allele is responsible for resistant reaction. All the F_2 individuals of Synthetic 4 × Synthetic 55 cross were resistant to stem rust. The absence of susceptible segregants in this cross suggested that these synthetic lines carry the same gene for stem rust resistance to pathotype 40A. Therefore, dominant allele for stem rust resistance present in Synthetic 4 and Synthetic 55 is same. Similarly Bahadur *et al.* (2003) in their study found that the gene for stem rust resistant in different resistant parents was same.

The F_2 population of resistant × resistant crosses Synthetic 4 × Synthetic 86 and Synthetic 55 × Synthetic 86 showed segregation for disease reaction. In cross Synthetic 4 × Synthetic 86 out of 183 F_2 individuals, 171 were resistant and 12 were susceptible, while in Synthetic 55 × Synthetic 86, out of 194 F_2 seedlings, 181 were resistant and 13 were susceptible. These results indicated that the dominant genes present in Synthetic 4 and Synthetic 55 was different from that of Synthetic 86. Hence, the test for goodness of fit was calculated to work out expected ratio, which was 61:3. This ratio indicates that the resistant reaction in Synthetic 86 is supposed to be controlled by two dominant and one recessive genes (Table 5). From the above results it was confirmed that the resistance gene present in Synthetic 4 and Synthetic 55 was same. However, resistance genes present in Synthetic 86 were different from that of Synthetic 4 and Synthetic 55 tested against same stem rust pathotype 40A at seedling stage.

In conclusion, the resistant reaction against stem rust in Synthetic 4 and Synthetic 55 was governed by one dominant gene while it was controlled by one dominant and one recessive gene (di-genic) in Synthetic 86. Most of the disease resistance genes are dominant in nature and are expected to produce active gene product in respect of recessive genes the role of dosage effect is emphasized. These stem rust resistant genes could be used further, in order to pyramid into the desired line for future plant breeding programme.

ACKNOWLEDGMENT

The financial support in the form of contingency provided by Council of Scientific and Industrial Research, New Delhi and research facilities provided by IARI, New Delhi is sincerely acknowledged.

REFERENCES

Assefa S and Fehrmann H. 2000. Resistance to wheat leaf rust in *Aegilops tauschii* Coss. and inheritance of resistance genes in hexaploid wheat. *Genetic Resources and Crop Evolution* **47:** 395–8.

Babiker E, Ibrahim A M H, Yen Y and Stein J. 2009. Identification of a microsatellite marker associated with stem rust resistance gene Sr35 in wheat. *Australian Journal of Crop Sciences* **3** (4):195–200.

Bhadur P, Charan R, Gandikumar and Selvakumar. 2003. Inheritance of resistance in five wheat cultivars to stem rust resistance. *Indian Phytopathology* **56** (2): 142–6.

Bahadur P, Nagarajan S and Nayar S K. 1985. A proposed system of virulence designation in India. II. *Puccinia graminis* f.sp. *tritici. Proceeding of Indian Academy of Sciences Plant Science* **95:** 29–33.

Cox T S, Raupp W J and Gill B S. 1994. Stem rust resistance genes *LR*41, *LR*42, and *LR*43 transferred from *Triticum tauschii* to common wheat. *Crop Science* **34:** 339–43.

Dyck P L and Sykes E E.1995. The inheritance of stem rust & leaf rust resistance in some ethopian wheat collections. *Euphytica* **81**: 291–7.

Ghazvini H, Hiebert C W, Zegeye T, Liu S, Dilawari M, Tsilo T, Anderson JA, Rouse MN, Jin Y, Fetch T. 2012. Inheritance of resistance to Ug99 stem rust in wheat cultivar Norin 40 and genetic mapping of Sr42. *Theoretical Applied Genetics* **125** (4):817–24.

Joshi L M, Singh D V and Srivastava K D. 1988. Manual of wheat diseases. Malhotra Publishing House, New Delhi, pp 75.

Ma H, Singh R P and Mujeebkazi A. 1995a. Resistance to stripe rust in *Triticum turgidum, T. tauschii* and their Synthetic hexaploids. *Euphytica* **82:** 117–24.

Ma H, Singh R P and Mujeebkazi A. 1995b. Supression /expression of resistance to stripe rust in synthetic hexaploid wheat. *Euphytica* **83:** 87–93.

Mcintosh R A, Wellings C R and Park R F. 1995. Wheat rust: An atlas of resistance genes. CSIRO, Australia, 200.

Mcintosh R A, Yamazaki Y, Devos K M, Dubcovsky J, Rogers W J, Appels, R. 2003. Catalogue of gene symbols for wheat. (*In*) *Proceedings of the 10th international wheat genet symposium*, Paestum, Italy.

MujeebKazi A, Rosas V and Roldan S. 1996. Conservation of the genetic variation of *Triticum tauschii* (Coss.) Schmalh. (*Aegilops squarrosa* auct. Non L.) in synthetic hexaploid wheats (*T. turgidum* L. × *T. tauschii*; 2n=6x=42, AABBDD) and its potential utilization for wheat improvement. *Genetic Resources and Crop Evolution* **43:** 129–34.

Nzuve F M, Tusiime G, Bhanvani S, Njan P N and Wanyera R. 2013. Studies of the genetics of inheritance of stem rust resistance in bread wheat. *African Journal of Biotechnology* 12 (21): 3153–9.

Nanthakumar G and Tomar S M S. 2003. Inheritance of *Triticum militinae* Zhuk. derived leaf rust and stem rust resistance in common wheat. *Indian Journal Genetics* 63:18–23.

Petersion R F, Cambell A B and Hannah A E. 1948. A digramatic scale for estimating rust intensity on leaves and stems of cereals. *Canadian Journal of Forest Research* **26:** 496–500.

Renu K, Bansal U K and Saini R G.2005. Genetics of adult plant stripe rust resistance in CSP 44, a selection from Austrialian wheat. *Journal of Genetics* **84**(3): 337.

Sawhney R N and Sharma J B, 1996. Introgerssion of diverse genes for resistance into an improved variety, Kalyansona. *Genetica* **97:** 255–61.

Sharma J B and Singh D. 2000. Inheritance of resistance to leaf rust in a wheat-rye recombinant 'selection 212'. *Indian Journal of Genetics* **60:** 271–80.

Singh R P, Huerta-Espino J and Roelfs A P. 2002. The wheat rusts. FAO Corporate Document Repository. *http://www.fao.org*

Singh S S, Sharma D N and Mehta H. 1998. Resistance to *Puccinia recondita tritici* in synthetic hexaploid wheat. *Indian Journal of Genetics* ***58*:** 263–9.

Stakman E C, Stewart D M and Loegering W Q.1962.Idetification of physiologic races of *Puccinia graminis* var. *tritici*. U S Department Agriculture, Agric.Res. Serv. E-617, p 53.

Yin J, Wang G, Ma F, Zhang H, Xiao J, Sun, Y, Diao, Y, Huang J, Guo Q 2008. Genetic analysis and SSR mapping of stem rust gene from mutant D51. *Frontiers of Agriculture* **2** (2):131–6.