



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

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### 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<sub>2,3</sub> 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<sub>2</sub> 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<sub>2</sub> seedling depicted 13:3 segregation ratio, which further conferred by F<sub>3</sub> family data. Allelism studies (using as F<sub>2</sub> population derived from a cross between Synthetic 4 × Synthetic 55), revealed the resistant gene present in these line was same. However, F<sub>2</sub> 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<sub>2</sub> population segregated in ratio of 61:3.

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

In India stem rust (*Puccinia graminis* 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<sub>1</sub>, F<sub>2</sub> and F<sub>3</sub> 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<sub>1</sub> and F<sub>2</sub> 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           |                              | <i>Ae. tauschii</i><br>accession |
|--------|--------------|--------------------|------------------------------|----------------------------------|
|        |              | <i>T. turgidum</i> | <i>Ae. tauschii</i>          |                                  |
| SHW 4  | CIGM 87-2775 | ALTAR<br>84        | <i>Ae. tauschii</i><br>(193) | W×188                            |
| SHW 55 | CIGM 90-799  | GAN                | <i>Ae. tauschii</i><br>(180) | W×180                            |
| SHW 86 | CIGM 93-229  | DOY1               | <i>Ae. tauschii</i><br>(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<sub>1</sub>, P<sub>2</sub> and F<sub>1</sub> and 60 seeds from F<sub>2</sub> 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<sub>2</sub> population having >30 seeds were progeny tested (as F<sub>3</sub> families with their F<sub>2</sub> 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 500°C for 24 hours. Disease reaction was recorded twice at 10<sup>th</sup> and 12<sup>th</sup> days after inoculation. The segregating (families having both resistant and susceptible plants) and non-segregating (families having only resistant plants from resistant F<sub>2</sub> plants and susceptible plants from susceptible

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. |

F<sub>2</sub> plants) F<sub>3</sub> families were identified.

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<sub>2</sub> plants and susceptible plants from susceptible F<sub>2</sub> plants) F<sub>3</sub> 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<sub>1</sub> 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 ratio | $\chi^2$ 23:1 | Df | P     |
|---|------------------|----|-----|----------------|---------------|----|-------|
|   | R                | S  | T   |                |               |    |       |
| F <sub>1</sub> (Syn4×AL)                | 9                | 0  | 9   |                |               |    |       |
| <sup>a</sup> F <sub>2</sub> (Syn4×AL)   | 122              | 49 | 171 | 3:1            | 1.22          | 1  | 0.269 |
| F <sub>1</sub> (Syn55×AL)               | 10               |    | 10  |                |               |    |       |
| F <sub>2</sub> (Syn55×AL)               | 110              | 29 | 139 | 3:1            | 1.269         | 1  | 0.260 |
| F <sub>1</sub> (Syn 86×AL)              | 8                |    | 8   |                |               |    |       |
| <sup>b</sup> F <sub>2</sub> (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 4 Mode of segregation of seedlings in F<sub>3</sub> families of crosses between Synthetics and Agra local against pathotypes 40A

| Population                                      | Pathotype | No. of Families |             |    | Total Expected ratio | $\chi^2$ |
|---|-----------|-----------------|-------------|----|----------------------|----------|
|   |           | HR              | *Seg(MR-MS) | HS |                      |          |
| <sup>a</sup> F <sub>3</sub> (Synthetic 4 × AL)  | 40A       | 21              | 42          | 12 | 75 (1:2:1)           | 3.24     |
| F <sub>3</sub> (Synthetic 55 × AL)              | 40A       | 14              | 47          | 16 | 77 (1:2:1)           | 3.86     |
| <sup>b</sup> F <sub>3</sub> (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<sub>2</sub> 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<sub>2</sub> 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<sub>3</sub> families derived from individual F<sub>2</sub> plants based on their disease reaction were harvested individually. According to reaction three groups of F<sub>3</sub> 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<sub>3</sub> generation were considered as HR, whereas, families which were susceptible in F<sub>3</sub> generation were categorized homozygous susceptible (HS). Similarly segregating families which showed moderate reaction (resistance as well as susceptible) were classified as SEG. In F<sub>3</sub> families the number of homozygous susceptible, segregating and

Table 5 Segregation pattern at seedling stage in F<sub>2</sub> generation of crosses between different synthetic hexaploid wheat with Agra local, inoculated with pathotype 40A

| Populations                        | (test of allelism) |    |       | $\chi^2$ 61:3 | P     |
|------------------------------------|--------------------|----|-------|---------------|-------|
|                                    | No. of plants      |    |       |               |       |
|                                    | R                  | S  | Total |               |       |
| F <sub>2</sub> (Synthetic 4 × 55)  | 175                | 0  | 175   |               |       |
| F <sub>2</sub> (Synthetic 4 × 86)  | 171                | 12 | 183   | 1.430         | 0.232 |
| F <sub>2</sub> (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).

The F<sub>2</sub> 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<sub>3</sub> 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<sub>1</sub> 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<sub>2</sub> population of this cross with 146 resistant and 24 susceptible individuals exhibited 13:3 (P>0.05) ratio of fitness (Table 3). In F<sub>3</sub> 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<sub>2</sub> and F<sub>3</sub> 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  $\times$  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  $\times$  resistant crosses Synthetic 4  $\times$  Synthetic 86 and Synthetic 55  $\times$  Synthetic 86 showed segregation for disease reaction. In cross Synthetic 4  $\times$  Synthetic 86 out of 183  $F_2$  individuals, 171 were resistant and 12 were susceptible, while in Synthetic 55  $\times$  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.

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