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# Deciphering the genetic basis of stripe rust resistance of exotic winter wheat cultivars and their utilization in pre-breeding

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**Genetic basis of stripe rust resistance was investigated in two exotic winter wheat cultivars namely, Spaldings prolific and China84-40022. Inheritance was studied with the most virulent stripe rust race 46S1119 (avirulent on *Yr1, 5, 10, 15, 27, SP, Su, CV* and virulent on *Yr2, 3, 4, 6, 7, 8, 9, 22, 23, 25*). The stripe rust resistance of Spaldings prolific and China84-40022 were governed by single dominant gene. Test of allelism demonstrated that the genes imparting resistance against the pathotype 46S119 in Spaldings prolific, China84-40022, Mega and CappelleDesprez are different. The rust resistance genes of these cultivars were incorporated in to the spring wheat. Three genetic stocks namely, UP2338<sup>\*3</sup>/China84-40022 (FLW3), UP2338<sup>\*3</sup>/Mega (FLW12) and UP2338<sup>\*3</sup>/CappelleDesprez (FLW40) were developed for their subsequent utilization in resistance breeding.**

**Key words:** Stripe rust resistance in winter wheat, anticipatory pre-breeding.

## INTRODUCTION

Stripe (yellow) rust (*Puccinia striiformis* West end. f. sp. *tritici*) of wheat, is an important cereal disease in many wheat growing regions of the world, especially, in areas with cool and wet environmental conditions (Roelfs et al., 1992). Cultivation of resistant varieties is the most effective, eco-friendly, adopted worldwide and an economically viable method of combating the rust disease (Line and Chen, 1995). Understanding the genetic basis of resistance is of prime importance for their use in breeding programmes. It not only generates information about the nature and number of genes in the donor parents but also helps in formulating efficient strategy for the incorporation of rust resistance. Generally, resistance conferred by single effective seedling resistance genes is neutralized within few years of its commercial cultivation (Line and Chen, 1996; Chen et al., 2002).

There are only a few varieties with narrow resistance base that are occupying large area in India (Punjab, Haryana and West-Uttar Pradesh) making this region more vulnerable to rust epidemics as these are susceptible to the newly evolved races of stripe rust. It is a cause of concern that none of the release varieties or pre-released/advanced lines of wheat are resistant to these races of stripe rust (Nayaret al., 2001; Prashar, 2005). Therefore, it is imperative that the diversity of rust resistance is enhanced through exploitation of new sources of resistance from exotic materials. As these sources, winter wheat in particular, tend to generate large number of undesirable segregants, efforts were made to develop pre-breeding lines carrying diverse resistance genes with desirable agronomic characteristics. An elaborate pre-breeding programme was initiated for the development of superior and agronomically desirable genetic stocks with yet unexploited resistance genes from exotic winter wheat. This was the first step towards diversifying the genetic base of resistance and subsequently pyramiding them. The present investigation was initiated

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with the dual objectives of understanding the genetic basis of stripe rust resistance of some of the very important winter wheat cultivars including CappelleDesprez and the variety known for durable resistance to stripe rust (Johnson, 1984), and development of resistant lines that carry resistance genes from the donor winter wheat as well as the recipient parent.

## MATERIALS AND METHODS

The experimental material comprises of CappelleDesprez, Mega, Spaldings Prolific, China84-40022, UP2338 and Agra Local and. The F<sub>1</sub>, F<sub>2</sub> population and F<sub>3</sub> families of the crosses of Agra Local with Spaldings Prolific and UP2338 with China84-40022 and F<sub>2</sub> generation of the crosses between CappelleDesprez/Mega, CappelleDesprez/China84-40022, Mega/China84-40022, Spaldings Prolific/China84-40022, Spaldings Prolific/Mega and Spaldings Prolific/CappelleDesprez. UP2338 was back crossed thrice with CappelleDesprez, Mega and China84-40022 for developing agronomically superior stripe rust resistant lines.

### Seedling resistance test

The F<sub>2</sub> and F<sub>3</sub> seedlings were raised in the aluminum trays with the 7<sup>th</sup> row of each tray as a susceptible check (Agra Local). Fully expanded primary leaves were inoculated with uredospores suspended in non-phytotoxic isoparaffinic oil (Soltrol 170, Philips Chevron). The inoculated seedlings were kept in a humid glass chamber for 48 h and then transferred to the temperature glass house benches at 16°C. Infection types (IT's) were recorded 14 days after inoculation and were classified as follows: IT's 0; (zero fleck), ; (fleck) and ;2 (fleck two) were classified as the resistant reactions whereas, IT's 3 (three) and 3+ (three plus) were designated as susceptible reactions. Race 46S119 (avirulent on *Yr1*, 5, 10, 15, 27, *SP*, *Su*, *CV* and virulent on *Yr2*, 3, 4, 6, 7, 8, 9, 22, 23 and 25) was used for inheritance study. Chi squared tests were applied to check the compliance of postulated and observed genetic ratios.

### Adult plant resistance (APR) test

The parents and checks were analyzed with the highly virulent race 46S119. Each test entry was replicated four times and planted in a two meter row bed. Adult plants were inoculated after the appearance of flag leaf with uredospores suspended in non-phytotoxic isoparaffinic oil (Soltrol, 170). APR test for leaves, stems and stripe rusts were done in separate green houses which were kept humid for 48 h with the help of humidifiers. Temperature was maintained at the desired level with the help of sensor attached exhaust fans. Terminal disease severity scores were taken and rust severity was recorded as per the modified Cobb's scale (Peterson et al., 1948).

### Test of resistance of pre-breeding lines

Resistance of genetic stocks were based on seedling resistance test (SRT) under temperature controlled glass house conditions with appropriate checks, screening under natural epiphytotic at summer nursery Wellington, the hot spot for leaf rust and stem rust, and evaluation in the main crop season at Karnal by artificial inoculation with virulent races of stripe rust. Resistance genes were

postulated on the basis of differential host-pathogen interaction and substantiated with pedigree. Data on morpho-agronomic traits were based on the replicated trial at Karnal, planted in RBD with three replications.

### Selection scheme for the development of rust resistant lines

The F<sub>1</sub> generation was backcrossed thrice with the recurrent parent UP2338. Continuous backcrosses were made (BC<sub>2</sub> was derived from BC<sub>1</sub>/UP2338 and BC<sub>3</sub> from BC<sub>2</sub>/UP2338). Selection could not be done for the backcrosses of Mega and CappelleDesprez as they produced only susceptible backcross seedlings. Therefore, BC<sub>2</sub> were made on 20 random BC<sub>1</sub> plants and backcross seed obtained from each plant was kept in a separate packet. After obtaining the selfed seed (BC<sub>1</sub>F<sub>2</sub>) from 20 BC<sub>1</sub> plants that were used for backcross, the BC<sub>1</sub> plants carrying recessive resistance genes in heterozygous condition were identified through progeny testing. Only those BC<sub>2</sub> seeds were retained that was harvested from BC<sub>1</sub> plants carrying resistance gene. Similarly, BC<sub>3</sub> that were carrying resistance gene were identified. About one hundred BC<sub>3</sub>F<sub>2</sub> progenies of each of the crosses were tested with stripe rust race 46S119 to identify the resistant seedlings. About one hundred resistant BC<sub>3</sub>F<sub>2</sub> seedlings were transplanted in the field and harvested individually. The BC<sub>3</sub>F<sub>3</sub> progenies were planted at the summer nursery of IARI, Regional Station, Wellington, India for the evaluation of stem rust resistance and agronomic traits. One to five stem rust resistant and agronomically desirable plants were selected from each of the BC<sub>3</sub>F<sub>3</sub>. The BC<sub>3</sub>F<sub>4</sub> families were tested at the seedling stage with stripe rust race 46S119 and the homozygous families were identified and transplanted in the field. The BC<sub>3</sub>F<sub>5</sub> progenies were screened at Wellington for resistance and agronomically desirable plants were selected individually. The selected BC<sub>3</sub>F<sub>6</sub> progenies were tested at the seedling stage separately with stripe rust race 46S119, stem rust race 62G29-1 and leaf rust race 109R63 in the temperature controlled glass house. The families that were homozygous resistant to all the pathotypes were transplanted in the field and harvested in bulk. These bulk harvested BC<sub>3</sub>F<sub>7</sub> lines along with checks were planted in RBD with three replications at Karnal for agronomic evaluation and rust resistance test.

## RESULTS

The seedling infection of the parents and other lines with known resistance genes (McIntosh et al., 1995, Nayar et al., 2001, McIntosh et al., 2005) are presented in Table 1. The infection type of Spaldings Prolific, China84-40022 and Mega was zero fleck and the IT's of CappelleDesprez varied from fleck one to fleck two whereas Agra Local and UP2338 were susceptible (IT's 3+). The observations on the F<sub>2</sub> and F<sub>3</sub> generations are presented in Table 2.

### Inheritance of stripe rust resistance to race 46S119 in China84-40022

The F<sub>2</sub> generation of the cross between UP2338 and China84-40022 was segregated for 0; and 3+ one hundred and sixty nine seedlings showed resistance and 68 were susceptible (Table 2). The data were good fit to 3 resistant: 1 susceptible (P = 0.18). Among the one

**Table 1.** Rust response of some *Yr* genes against yellow rust races in the seedling resistance test.

Cultivar/line	Documented genes	SRT 67S8	SRT 47S102	SRT 70S69	SRT 46S119	SRT 78S84	APR <sup>a</sup> 46S119
Agra Local	-	3+	3+	3+	3+	3+	100S
UP2338	<i>Yr9</i>	0;	0;	3+	3+	3+	100S
<i>TcLr26</i>	<i>Yr9</i>	;	0;	0;	3+	3C	100S
Vilmorin23	<i>Yr3</i>	;2+	3+	3+	3+	3+	100S
Hybrid46	<i>Yr3, Yr4</i>	;2	3C	23	3C	;-	80S
Maris Huntsman	<i>Yr2, Yr3, Yr4, Yr13</i>	0;	0;	0;	0;	3+	80S
CappelleDesprez	<i>Yr3, Yr4, Yr16</i>	;-	0;	;1 to ;2	;1 to ;2	;-	R
CD-Mara-2D	<i>Yr3, Yr4</i>	;-	0;	;1 to ;2	;1 to ;2	0;	R
China84-40022	<i>UnkownYr</i>	0;	0;	0;	0;	0;	R
Mega	<i>Yr3, Yr4, Yr12</i>	0;	0;	0;	0;	0;	R
Spalding Prolific	<i>YrSP</i>	0;	0;	0;	0;	0;	R
TSA	<i>Yr5</i>	0;	0;	0;	0;	0;	R
Moro	<i>Yr10+YrMor</i>	0;	0;	0;	0;	0;	R
CN25087	<i>Yr15</i>	0;	0;	0;	0;	0;	R

SRT, Seedling resistance test; APR, adult plant resistance; TSA, *Triticumspelta album*; <sup>a</sup> terminal disease severity of flag leaf under polythene house.

**Table 2.** F<sub>2</sub> and F<sub>3</sub> segregation for rust response and tests of allelism.

Cross	Generation	Pathotype	Number of seedlings/ families		Expected ratio	$\chi^2$ value	P	
			resistant	segregating susceptible				
UP2338/CH84	F <sub>2</sub>	pt. 46S119	169		68	3R:1S	1.72	0.18
UP2338/CH84	F <sub>3</sub>	pt. 46S119	22	59	28	1R:2Seg:1S	1.4	0.49
AL/SP	F <sub>2</sub>	pt. 46S119	221		66	3R:1S	0.61	0.43
AL/SP	F <sub>3</sub>	pt. 46S119	21	56	27	1R:2Seg:1S	1.3	0.52
<b>Test of allelism</b>								
CD/CH84	F <sub>2</sub>	pt. 46S119	133		49	1R:0S	$\infty$	<0.01
CD/Mega	F <sub>2</sub>	pt. 46S119	158		61	1R:0S	$\infty$	<0.01
CD/SP	F <sub>2</sub>	pt. 46S119	234		88	1R:0S	$\infty$	<0.01
Mega/SP	F <sub>2</sub>	pt. 46S119	275		98	1R:0S	$\infty$	<0.01
Mega/CH84	F <sub>2</sub>	pt. 46S119	156		43	1R:0S	$\infty$	<0.01
SP/CH84	F <sub>2</sub>	pt. 46S119	265		18	1R:0S	$\infty$	<0.01
CH84/TSA ( <i>Yr5</i> )	F <sub>2</sub>	pt. 46S119	273		23	1R:0S	$\infty$	<0.01
CH84/Moro ( <i>Yr10</i> )	F <sub>2</sub>	pt. 46S119	256		19	1R:0S	$\infty$	<0.01
CH84/CN2580 <i>Yr15</i>	F <sub>2</sub>	pt. 46S119	301		22	1R:0S	$\infty$	<0.01
CH84/ <i>TcLr26/Yr9</i>	F <sub>2</sub>	pt. 46S103	321		0	1R:0S	0.0	>0.9

AL, Agra Local; CD, CappelleDesprez; SP, Spaldings prolific; CH84, China84-40022; TSA, *T. album*.

hundred and nine F<sub>3</sub> families, 59 were segregated, 28 were homozygous susceptible, and 22 were homozygous resistant (Table 2). The F<sub>3</sub> family segregation was in compliance with 1 resistant: 2 segregating: 1 susceptible (P = 0.49).

#### Inheritance of stripe rust resistance to pathotype 46S119 in Spaldings Prolific

The F<sub>1</sub> generation of Agra Local and Spaldings Prolific

were resistant. The F<sub>2</sub> was segregated for two infection types (0; and 3<sup>+</sup>). Two hundred and twenty one seedlings were resistant and 66 showed susceptibility which was in compliance with 3 resistant: 1 susceptible (P = 0.43, Table 2). The F<sub>3</sub> family segregation was a satisfactory fit to single gene segregation (P = 0.52, Table 2).

#### Test of allelism

The F<sub>2</sub> population of the crosses of CappelleDesprez/

**Table 3.** Means of morpho-agronomic traits at Karnal.

Pedigree	Plant height (cm)	Maturity (days)	TGW (g)	Yield/meter row (g)
AGRA LOCAL	130	115	34.0	72.5
UP2338	92.3	123	44.6	102.2
Mega	81.0	161	36.4	38.0
CappelleDesprez	80.5	160	34.2	40.0
China84-40022	84.6	149	32.1	46.0
UP2338* <sup>3</sup> /China84-40022 (FLW3)	87.3	121	39.8	96.5
UP2338* <sup>3</sup> /Mega (FLW12)	125	125	43.0	85.3
UP2338* <sup>3</sup> /CappelleDesprez(FLW40)	85	127	40.5	72.0
CV %	3.6	1.1	2.8	8.3
CD at 5%	10.6	2.8	2.9	12.8

**Table 4.** Seedling resistance test (SRT) and field resistance (FR) of genetic stocks against virulent races of rusts.

Variable	Leaf rust			Stem rust			Stripe rust			
	SRT 109R63	FRW	FRW	SRT 62G29-1	FRW	FRKarnal, India	SRT 46S119	SRT 78S84	FRW	FRKarnal, India
AGRA local	3+	100S	80S	3+	80S	0	3+	3+	20S	70S
UP2338	0;	80S	60S	2-	20MR	0	3+	3+	R	40S
Mega	3+	60S	40S	3+	50S	0	;	;	R	R
CappelleDesprez	3+	60S	60S	3+	60S	0	; to ;2	;	R	R
China84-40022	0;	80S	60S	2+	40MR	0	0;	0;	R	R
FLW3	0;	80S	60S	2-	15MR	0	0;	0;	R	R
FLW12	0;	80S	80S	2-	15MR	0	;	0;	R	R
FLW40	3+	60S	80S	3+	60S	0	;	;	R	R

R, Resistance; ; - ;1 2' 2, resistance; MR, moderate resistance; 2+, moderate resistance; s, susceptible; 3+, susceptible; 0, free from rust / no rust response; W, Wellington, India

China84-40022, Mega/China84-40022, CappelleDesprez/Spaldings Prolific, Spaldings Prolific/China84-40022, Spaldings Prolific/Mega and CappelleDesprez/Mega segregated confirmed that different genes were involved in the expression of stripe rust resistance in these cultivars. The infection types of China84-40022 against leaf and stem rust pathotypes indicated the presence of *Lr26/Sr31/Yr9* (data not presented). The F<sub>2</sub> seedlings of China84-40022/TcLr26 did not segregate when tested with race 46S103 (avirulent on *Yr9*) which confirmed presence of *Yr9* in China84-40022. Allelism test for *Yr5*, *Yr10* and *Yr15* was also done for China84-40022 because its pedigree information was not available and the infection types of lines carrying *Yr5*, *Yr10* and *Yr15* were similar to China84-40022. Absence of susceptible seedlings in the test crosses confirmed absence of *Yr5*, *Yr10* and *Yr15* in China84-40022.

### Development of rust resistant lines

The means of morpho-agronomic traits at Karnal are given in Table 3. The seedling reactions and field resistance of

the pre-breeding lines are presented in Table 4.

### FLW3, the line derived from China84-40022

In the BC<sub>3</sub>F<sub>2</sub> population of UP2338/China84-40022, 115 resistant seedlings were selected and transplanted. Sixty four agronomically desirable plants were selected at maturity. One advance line was named FLW3. In addition to 1BL.1RS (*Lr26*, *Sr31* and *Yr9*) this line also carries at least one more *Yr* gene from China-84-40022. It is immune to stripe rust and resistant to black rust. FLW3 has test weight of 39.8 g, short stature and matures in about 121 days.

### FLW12, the line derived from Mega

In the BC<sub>3</sub>F<sub>2</sub> population of UP2338/Mega, one hundred and twenty resistant seedlings were transplanted. Thirty agronomically desirable plants were selected at maturity and all of them produced homozygous stripe rust resistance to Indian stripe rust pathotypes might be an

undesigned gene since all the known genes of Mega namely; *Yr3*, *Yr4*, *Yr14* (McIntosh et al., 1995) are susceptible to race 46S119 of yellow rust (Table 1). FLW12 has long spikes and test weight of 43 g, average plant height was 125 cm and matures in about 125 days.

#### FLW40, the line derived from CappelleDesprez

In the BC<sub>3</sub>F<sub>2</sub> population of UP2338/CappelleDesprez, ninety six resistant seedlings were transplanted and twenty were selected at maturity. One agronomically desirable advance line (FLW40) was derived from BC<sub>3</sub>F<sub>4</sub> family that exhibited; CN IT's. FLW40 showed immune reaction at seedling as well as, adult plant stage. Based on SRT data and pedigree, FLW40 is expected to carry novel stripe rust seedling resistance genes from CappelleDesprez. FLW40 has small spikes and test weight 40.5 g, average plant height was 85 cm and matures in about 127 days.

#### DISCUSSION

The stripe rust resistance of Spaldings prolific and China84-40022 were governed by the single dominant gene. Test of allelism clearly demonstrated that the resistance gene of Spaldings prolific, China84-40022, Mega and CappelleDesprez are different. As of now, there are no reports of seedling resistance genes other than *Yr3* and *Yr4* in CappelleDesprez and Mega (Chen and Line, 1993; McIntosh et al., 1995). Both of them have shown seedling resistance against highly virulent pathotypes 46S119 (avirulent on *Yr1*, 5, 10, 15, 27, SP, Su, CV and virulent on *Yr2*, 3, 4, 6, 7, 8, 9, 22, 23, 25) and 78S84 (avirulent on *Yr1*, 5, 10, 15, 25, SP, CV and virulent on *Yr2*, 3, 4, 6, 7, 8, 9, 22, 23, 27 and A) of stripe rust. Though, the seedling resistance genes of CappelleDesprez (*Yr3a*, *Yr4a* and unknown *Yr* gene) are not effective against many races of *P. striiformis* in Europe and other places, but in India, it showed that seedling resistance against all the races of stripe rust were detected (Datta et al., 2008). While CappelleDesprez is planted every year in the experimental field at Flowerdale, Shimla, India for the last 17 years, the races 46S119 (46E151+*Yr9*) and 78S84 (78E16) are quite virulent and existing since 1996 and 2001, respectively (Prashar et al., 2007). There is no incidence of stripe rust on CappelleDesprez both at seedling stage and adult plant stage under laboratory and field condition. This indicated that the resistance genes of CappelleDesprez may be effective against stripe rust in India. The stripe rust resistance of CappelleDesprez was effective for a long time in Europe (Johnson, 1984) probably because of the presence of adult plant Resistance gene (Powell et al., 2008) and it may also be resistant families. One advance line was named FLW12. It was completely resistant to stripe rust. In addition to

yellow rust resistance genes from Mega, it also carries *Lr26*, *Sr31* and *Yr9* from UP2338. The gene imparting durable in India. CappelleDesprez showed moderate susceptibility to stripe rust at Lincoln, Newzealand which suggested that either *Yr16* has become less effective or it conferred only a modest degree of resistance (Chng et al., 2011). The real durability of *Yr16* against stripe rust races in India can be judged when line possessing *Yr16* is cultivated in substantial area year after year for a long time. Therefore, in India, the role adult plant resistance gene *Yr16* on stripe rust can be studied if either the unknown seedling resistance gene (Datta et al., 2008) of CappelleDesprez is knocked out or is rendered susceptible by a new *P. striiformis* race.

Inheritance studies not only unveiled the genetic make-up of the lines under investigation but also generated valuable information for their use in breeding. The lines derived from the crosses of CappelleDesprez, China84-40022 and Mega were agronomically superior to their winter wheat parents for yield and maturity traits. The line derived from the cross between UP2338 and CappelleDesprez was inferior among the derived lines. The reason for its agronomic inferiority may be due to the fact that only one BC<sub>3</sub>F<sub>3</sub> line was selected for generation advancement which drastically minimized the probability to generate good progenies in the later generations. Though this line is not high yielding but its growth habit is spring type with good tillering capacity and hence, can be used in breeding programmes. The number of plants to be selected in the BC<sub>3</sub>F<sub>2</sub> generation is dependent on the number of genes governing resistance and the agronomic behaviour of the parents used in the crosses. Although, we recovered some agronomically desirable lines from the crosses of UP2338<sup>\*3</sup>/China84-40022 and UP2338<sup>\*3</sup>/Mega in advanced generations but it is recommended to advance at least 500 resistant BC<sub>3</sub>F<sub>2</sub> plants in order to derive agronomically superior plants from spring/winter wheat crosses in the cases where resistance is governed by a single gene. The number of resistant seedlings must be increased drastically for complementary or multigenic additive resistance as in the case of CappelleDesprez. The derived progenies of the pre-breeding programme are not expected to yield as much as the best checks of the variety trials but they will be good candidates as parents in breeding programmes. FLW3 possess good agronomic traits and high yield potential in addition to excellent rust resistance, and sensing the present threat of yellow rust, it should offer one of the best sources of stripe rust resistance to wheat breeding programme. Techniques should be devised to incorporate adult plant resistance genes like *Yr16* component and *Yr18* along with seedling resistance genes for better management of rust diseases.

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