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CONFIRMATION OF RESISTANCE IN WHEAT GERMPLASMS AGAINST STRIPE/YELLOW RUST THROUGH SEEDLING RESISTANCE TEST (SRT) AND ADULT PLANT RESISTANCE (APR)

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ABSTRACT
In the present study, nine hundred and eighty wheat germplasm accessions of exotic collection of NBPGR were screened against stripe rust in 2012-13 seasons under natural condition. Out of these, 30 accessions showing resistance response were randomly selected with the aim of studying that to screen wheat germplasm accessions against yellow rust at seedling resistance test (SRT) and adult plant resistance (APR) during 2013-2014 seasons. During the study, results revealed that 8 accessions viz. EC-635612, EC-635627, EC-635881, EC-664208, EC-664244, EC-692246, EC-693271 and EC-693322 were resistant towards all the tested pathotypes of stripe rust in seedling as well as at adult plant stage. Remaining 19 germplasms showed adult plant resistance response except 3 germplasms, *i.e.* EC-636264, EC-635861 and EC-664299. Additionally, the relationship between the plot yield and A-value of all the thirty germplasm accessions were also observed and was found to be negatively correlated with highly significant level (r = -0.603**). Present study suggests that out of 30 accessions, 27 wheat germplasm accessions were having resistance response that could be used for further incorporation in breeding programmes for developing stripe rust resistant wheat varieties.

INTRODUCTION

Wheat is the second most important cereal crops in the world. Rust disease has challenged the wheat cultivation in all over the world. Among the three rust of wheat, stripe/yellow rust caused by *Puccinia striiformis* is an important constraint to wheat production in cool environment. It is reported in over 60 countries world-wide. It needs low temperature to survive for whole year in the Himalayas, which is of epidemic consequences in North Hill Zone, North West Plain Zone in India. Stripe rust is principally an important disease of wheat during winter or early spring or at higher elevations (Roelfs *et al.,* 1992). Yellow rust appeared in epidemic form in various places i.e. plains of Jammu and Kashmir, foot hills of Punjab and Himachal Pradesh, parts of Haryana, tarai regions of Uttarakhand during 2010- 11, (Sharma and Saharan, 2011). Stripe rust can cause 100% yield loss if infection occurs very early (Afzal *et al.,* 2009). Besides, for the management of rust chemical fungicides play an important role like Tilt (propiconazole), Quadris (azoxystrobin), Stratego (propiconazole + trifloxystrobin), Headline (strobilurin), and Quilt (azoxystrobin + propiconazole) restricts the spread of stripe rust (Chen, 2005). Foliar application of fungicide Quadris proved most effective in reducing the final rust severity in all the varieties viz., PBW-343, RSP-561, PBW-550 and Agra local (Ahanger *et al.,* 2014). The systemic fungicides, propiconazole found the best for inhibition of 86.03 per cent uredospores germination of leaf rust of wheat followed by hexaconazole and penconazole with 77.40 and 72.29 per cent, respectively (Chaudhary *et al.,* 2013). But since chemical fungicides are not economically feasible on large scale and create pollution to the environment along with the development of resistance against some chemicals due to emergence of new pathotypes. Thus, relaying on resistant cultivars is the most economic means of controlling rusts and also environmental friendly (Chen, 2005).

The fungus has an ability to mutate, multiply rapidly and spread over large areas has led to widespread epiphytotics in India (Nagarajan and Joshi, 1975). The breakdown of resistance gene led to investigation of adult plant resistance and slow rust resistance. Wheat cultivars with slow rusting genes are often susceptible at the seedling stage, but may be moderately to highly resistant to all pathotypes at the adult plant stage in the field. Slow rusting is not affected by the types of patho types (Knott, 1989). Both types of resistance sources i.e. seedling and adult plant durable resistance studied in wheat germplasm offer a diverse sources of resistance and promising genetic stock for accumulating seedling and adult plant resistance to acquire durable and long lasting resistance against stripe and leaf rust pathogens. Deployment of race-specific resistance gene has the capability of providing highly effective protection by delaying the infection process (Shah *et* *al.,* 2010). Villasenor (2009); Singh and Bansal (2013) also studied genetics analysis of the stripe rust resistance in adult plant of wheat cultivars. Hadi and Muhammad (2011) have characterized wheat germplasm for stripe rust resistance in seedling stage. Kumar *et al.,* 2014 also studied different wheat cultivars and postulated three different combinations of yellow rust resistance genes viz. *Yr*2, *Yr*9 and *Yr*18. Therefore, the experiment were conducted with the objectives to observe the infection response of 30 wheat germplasms at seedling and adult plant stage against stripe rust and their disease progression for screening the resistant germplasm.

MATERIALS AND METHODS

Nine hundred and eighty wheat germplasm accessions of exotic collection of NBPGR were screened against stripe rust in 2012-13 seasons under natural condition at Norman E. Borlaug Crop Research Centre, Pantnagar, India by using the modified Cobb's scale (Peterson *et al.,* 1948). Then, 30 germ plasm accessions i.e. EC-597991, EC-597999, EC-635590, EC-635598, EC-635602, EC-635609, EC-635612, EC-635614, EC-635627, EC-635683, EC-635705, EC-635711, EC-635721, EC-635741, EC-635861, EC-635881, EC-636264, EC-663926, EC-664200, EC-664208, EC-664244, EC-664299, EC-664315, EC-692221, EC-692231, EC-692246, EC-693252, EC-693271, EC-693289 and EC-693322, showing resistance were selected for screening at seedling and adult plant stage in the same way as indicated by Bhardwaj *et al.*, (2010) in order to observe their infection response in an epiphytotic condition in 2013-2014 seasons.

Growing of seedling and inoculations

The experiments were conducted at Directorate of Wheat Research, Regional Station, Flowerdale, Shimla, for seedling resistance test. All the selected 30 germplasm accessions were sown for testing at seedling stage against some pathotypes of stripe rust. 5 lines of each accession were sown in the aluminium trays using loamy soil having manure (3:1) that contain 5 g of N.P.K mixture. Checks accessions with known gene were evaluated for comparing infection response. When plants became one week old with fully expanded primary leaf they were inoculated by using an atomiser having 15 mg spores of specific pathotypes 14A, 20A, 31, I(38S102), K(47S102), L(70S69), P(46S103), T, 46S119 and 78S84 of stripe rust that are being suspended with 2ml light grade mineral oil. After the evaporation of oil spraying of fine mist of water was done and they were placed for 48 hours in dew chamber at 15 ± 3^{o}C temperature with 100% relative humidity and 12 hours day light (Bhardwaj *et al.,* 2010). The plants were transferred to a greenhouse and grown at 16 ± 2^{o}C temperature under relative humidity of 40-60% and light of 15000 lux for 12 hours.

Infection types on the test 30 accessions were recorded at 15 days after inoculation to check the infection response of each specific pathotypes (Stakman *et al.*, 1962; Bhardwaj *et al.,* 2010 and Kumar *et al.*, 2014). Infection types were characterized as 0 = no visible infection; 0; = small hypersensitive flecks, 1 (highly resistant) = minute uredia, surrounded by necrotic areas, 2 (moderately resistant) = small to medium uredia surrounded by chlorotic area, 3 (moderately susceptible) = uredia small to medium in size and chlorotic areas may be present, 3+ (highly susceptible) = uredia large with or without chlorosis, profusely sporulating and rings formed. Infection type 33+ (susceptible) is classified when both 3 and 3+ pustules occur together (Nayar *et al.,* 1997). Infection types of 0 to 2 were considered as resistant and infection types of 3 to 3+ and more were considered as susceptible.

Field trial for adult plant resistance

In 2013-2014 seasons, 30 plot of 1m^2 area for each 30 germ plasm accessions in 3 replication and along with 30 plots of 1m^2 area for each germplasm accession as check plot were grown at Norman E. Borlaug Crop Research Centre, Pantnagar, India for adult plant resistance. Then when seedling became one month old they were inoculated for 3 times after 7 days interval with mixture of pathotypes *i.e.* K(47S102), L(70S69), P(46S103), 46S119, 78S84 of stripe rust spore which were brought from Directorate of Wheat Research, Regional Station, Flowerdale, Shimla, in the month of January to create an epiphytotic condition (Kumar *et al.,* 2014). But control plots were not inoculated with pathotypes and they were allowed to grow under natural conditions, only for comparing the infection response in the inoculated one and control for each germplasm accessions respectively. After 2 weeks of inoculation, disease observations were taken by using the modified Cobb's scale (Peterson *et al.,* 1948). Disease ratings for adult plant stage were characterized as 0 (Immune) = No visible infections, R (Resistance) = Necrotic areas with or without uredia, MR (Moderately resistance) = Necrotic areas with small uredia, MS (Moderately susceptible) = Medium uredia with no necrosis but some chlorosis, S (Susceptible) = Large uredia with no necrosis and no chlorosis, X (Intermediate) = Variable sized uredia and fully susceptible and data were recorded 8 times. Area under disease progress curve (AUDPC) was also calculated in order to check the disease progression during the study by using the formula as given by Wilcoxson *et al.*, (1975). The relationship between plot yield and A-values of each wheat germplasm accessions were calculated by using Pearson correlation of coefficient (Cochran and Cox 1967).

RESULTS AND DISCUSSION

Seedling resistance test

The infection responses of all the tested 30 germplasm accessions at seedling stage against several pathotypes of yellow rust were presented in Table 1. Among them, the germ plasm accessions EC-597991, EC-636264 and EC-664299 were highly susceptible to all the tested pathotypes of stripe rust. The accessions EC-635612, EC-635627, EC-635881, EC-664208, EC-664244, EC-692246, EC-693271 and EC-693322 were resistant towards all the pathotypes with infections type of less than 3. The accession EC-635741 was resistant to all the pathotypes except pathotype *i.e.* 46S119. According to Hadi and Muhammad (2011); Kumar *et al.* (2014), these 8 accessions which showed seedling resistant may be due to presence of some seedling resistant *Yr* genes which confer complete resistant phenotype and normally race-specific.

Adult plant resistance

Table 1: Seedling infection response of stripe rust in 30 wheat germplasm accessions inoculated with different pathotypes

Accessions No.	Stripe rust pathotypes								
	14A	20A	31 (67S64)	I (38S102)	K (47S102)	L(70S69)	P(46S103)	T	46S119	78S84
EC-597991	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +
EC-597999	0;	0	0	0;	0;	3 +	0;	0	3 +	3 +
EC-635590	0;	3 +	3 +	0;3 +	0;3 +	3	2 +	0;	3 +	3 +
EC-635598	0;	0;2 +	0;	;-	0;	3 + 0;	;	3 + ,0;	3 +	3 +
EC-635602	3 +	3 +	3 +	3 +	0;2 +	3	3 +	3 +	3 +	3 +
EC-635609	;-	1	2	0;	0;	0	0	3 +	0	3 +
EC-635612	0;	0	0	0	2	;-	1,2	0;	2	1
EC-635614	0;	3 +	0;	;-	0;	3 +	;	3 +	3 +	3 +
EC-635627	0;	0;	0;	;-	;-	1	;-	0;	2	1
EC-635683	0;	3 +	0;	3 +	0;	3 +	;	3 +	3 +	3 +
EC-635705	0;	;	0;	0;	0;	3 +	;	0; 2	3 +	3 +
EC-635711	0;	3 +	0;	;-	0;	1	3 +	0	1	;-
EC-635721	0;	0;	0;	0;	0;	3	;	0;	3 +	3C
EC-635741	0;	;-	0;	0;	0;	0;	0;	0;	3 +	;-
EC-635861	0;	0;	0;	;-	0;	3 +	0;	0;	3 +	3 +
EC-635881	0;	0;	0;	0;	0;	0	0;	1	2	1
EC-636264	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +
EC-663926	-	0;	0;	0;	0;	3 +	0;	0;	3 +	3 +
EC-664200	0;	3-	0;	;	0;3 +	3 +	;	3 +	3 +	3 +
EC-664208	0;	2	0;	0;	0;	1	0;	0;	2	1,2
EC-664244	0;	0;	0;	0	0;	2	;	0;	1	2
EC-664299	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +	3 +
EC-664315	0;	0;	0	;	0;	3 +	;	0;	0;	3 +
EC-692221	0;	0;2	0;	;-	;-	3 +	3 +	0;	;-	;-
EC-692231	0;	3 +	0;	0;	0;	3 +	;	3 +	3 +	3 +
EC-692246	;-	0;	0;	0;	0;	2	;	0;	1	2
EC-693252	0;	3 +	0;	0;	0;	;-	0;	0;	1,2	3 +
EC-693271	0;	0;	0;	0;	0;	;-	0;	2	1	2
EC-693289	0;	0;	0;	0;	0;	3 +	0;	0;	3 +	;-
EC-693322	0;	0;	0;	0;	0;	0;	0;	0;	1	2

0 = Immune ;- = Nearly immune; 1, 2 = Resistant 2 + = Moderately resistant; 3 = Moderately susceptible; 33 + = Susceptible 3 + = Highly susceptible

The infection responses of 30 germplasm accessions at adult plant stage in the form of weekly severity were presented (Table 2). Among all germplasms, the highest disease severity was observed in the accessions EC-636264, EC-635861 and EC-664299 having 60-70 per cent of disease severity with MRMS response. The remaining 27 germplasms showed severity of less than 40 per cent. In accordance to Khan *et al.,* 2002, the disease severity of 40 per cent range which are characteristic of moderately resistant to moderately susceptible response, were taken as phenotypically resistant to stripe rust and they were still acceptable for selecting as resistant cultivars. Similar results were obtained in the experiment at adult plant stage of wheat studied by Singh and Bansal (2013) in durum wheat cultivars; Kumar *et al.*, (2014) in some old wheat varieties that support the result of present finding. Therefore, all the tested 27 accessions were phenotypically confirmed as resistant to stripe rust in adult plant stage as they were showing low disease severity of less than 40 per cent.

Relationship between Area under disease progress curve (AUDPC) and plot yield

AUDPC value over an interval of 8 weeks was calculated (Table 3) for both epiphytotic and control plot in order to check the disease progression. AUDPC is inversely proportional to the degree of resistance therefore, germplasm accessions having very low AUDPC are resistant while those with high AUDPC are susceptible. The highest A-values were observed in three accessions viz. EC-636264, EC-635861 and EC-664299 with 928.2, 838.6 and 1263.5 respectively. The results of Seyed *et al.* (2013) showed that AUDPC of 800% per day indicated a susceptible cultivar and AUDPC > 800 indicated a too susceptible one. These 3 accessions were categorised as susceptible based on their high amount of severity per cent and A-value. The remaining 27 accessions were categorised as resistant as their low level of disease severity and A-value. The plot yield were also calculated for both epiphytotic and control plot to observe the effect of disease on yield (Table 3). Even though the values were not consistent as the different germplasm accessions have different characters and attributes towards stripe rust that affects in yield. It was statistically observed that the plot yield and A-value for the disease in all 30 germplasm accessions were negatively correlated at highly significant level (r = -0.63**) which signify that plot yield decreases when A- value increases (Table 4). Hence, from the results the 8 accessions *i.e.* EC-635612, EC-635627, EC-635881, EC-664208, EC-664244, EC-692246, EC-693271 and EC-693322 were resistant at both seedling as well as adult stage which may be due to the presence of some resistant *Yr* genes which confer complete resistant phenotype and are normally race- specific. And the remaining 19 germplasm accessions viz. EC-597991, EC-597999, EC-635590, EC-635598, EC-635602, EC-635609, EC-635614, EC-635683, EC-635705, EC-635711, EC-635721, EC-635741, EC-663926, EC-664200, EC-664315, EC-692221, EC-692231, EC-693252

Table 2: Weekly disease severity of stripe rust under epiphytotic condition during 2013-14

AccessionsNo.	Disease severity 3-2-14Week 1	10-2-14Week 2	17-2-14Week 3	24-2-14Week 4	3-3-14Week 5	10-3-14Week 6	17-3-14Week 7	24-3-14Week 8
EC-597991	0	TMRMS (0.0)	2MRMS (1.2)	5MRMS (3.0)	5MRMS (3.0)	5MRMS (3.0)	10MRMS (6.0)	10MRMS (6.0)
EC-597999	TMR (0.0)	5MS (4.0)	23.3MS (18.6)	26.6MS (21.2)	30MS (24.0)	33.3MRMS (26.6)	36.6MS (29.2)	36.6MS (29.3)
EC-635590	0	0	0	TMR (0.0)	TMR (0.0)	2MR (0.8)	2MR (0.8)	5MR (2.0)
EC-635598	0	5MR (2.0)	5MR (2.0)	5MR (2.0)	10MR (4.0)	15MR (6.0)	15MR (6.0)	15MR (6.0)
EC-635602	0	TMR (0.0)	TMR (0.0)	TMR (0.0)	TMR (0.0)	TMR (0.0)	5MR (2.0)	10MR (4.0)
EC-635609	0	0	TMR (0.0)	TMR (0.0)	TMR (0.0)	5MRMS (3.0)	5MRMS (3.0)	10MRMS (6.0)
EC-635612	0	0	0	0	0	0	5MRMS (3.0)	10MRMS (6.0)
EC-635614	0	0	0	TMR (0.0)	TMR (0.0)	TMR (0.0)	2MR (0.8)	5MR (2.0)
EC-635627	0	0	0	0	0	0	0	0
EC-635683	0	0	TMR (0.0)	2MR (0.8)	2MR (0.8)	5MR (2.0)	10MR (4.0)	20MR (8.0)
EC-635705	0	0	0	0	0	0	0	0
EC-635711	0	0	2MR (0.8)	2MR (0.8)	2MRMS (1.2)	5MRMS (3.0)	5MRMS (3.0)	5MRMS (3.0)
EC-635721	0	0	0	0	0	0	0	5MR (2.0)
EC-635741	0	0	0	0	0	0	0	0
EC-635861	TMR (0.0)	5MR (2.0)	20MRMS (12.0)	20MRMS (12.0)	33.3MRMS (19.9)	33.3MRMS (19.9)	60MRMS (36.0)	60MRMS (36.0)
EC-635881	0	0	TMR (0.0)	TMR (0.0)	TMR (0.0)	TMR (0.0)	5MRMS (3.0)	10MRMS (6.0)
EC-636264	2MRMS (1.2)	5MRMS (3.0)	15MRMS (9.0)	30MRMS (18.0)	30MRMS (18.0)	50MRMS (30.0)	60MRMS (36.0)	60MRMS (36.0)
EC-663926	TS (0.0)	TS (0.0)	TS (0.0)	2MRMS (1.2)	2MRMS (1.2)	5MRMS (3.0)	10MRMS (6.0)	10MRMS (6.0)
EC-664200	0	0	TMR (0.0)	2MR (0.8)	2MR (0.8)	2MR (0.8)	5MR (2.0)	5MR (2.0)
EC-664208	0	0	0	0	0	0	2MR (0.8)	5MR (2.0)
EC-664244	0	0	0	0	0	TMR (0.0)	TMR (0.0)	TMR (0.0)
EC-664299	TMR (0.0)	6.6MR (2.6)	20MR (8.0)	45MRMS (27.0)	63.3MRMS(37.9)	70MRMS (42.0)	70MRMS (42.0)	70MRMS (42.0)
EC-664315	0	0	0	TMR (0.0)	3MR (1.2)	3MR (1.2)	2MR (2.0)	10MR (4.0)
EC-692221	0	0	0	TMS (0.0)	2MR (0.8)	2MR (0.8)	2MR (0.8)	2MR (0.8)
EC-692231	0	TMR (0.0)	TMR (0.0)	TMR (0.0)	TMR (0.0)	3MR (1.2)	3MR (1.2)	5MR (2.0)
EC-692246	0	0	0	0	0	0	5MR (2.0)	10MR (4.0)
EC-693252	0	0	TMR (0.0)	TMR (0.0)	3MR (1.2)	3MR (1.2)	5MR (2.0)	10MR (4.0)
EC-693271	0	0	0	0	0	0	0	5MR (2.0)
EC-693289	0	0	TMR (0.0)	5MR (2.0)	5MR (2.0)	10MR (4.0)	10MR (4.0)	10MR (4.0)
EC-693322	0	0	0	0	0	0	5MR (2.0)	10MR (4.0)

Figure in the parentheses are Loegering's coefficient of infection; T = Trace; MR = Moderately resistance; MS = Moderately susceptible; MRMS = Moderately resistance moderately susceptible;S = Susceptible

Table 3: AUDPC (A-Value) score of wheat germplasm accessions in epiphytotic and control conditions against stripe rust during 2013-14

Accessions No.	AUDPC (A value)		Plot yield (kg/1m^2)	
	Epiphytotic condition	Control condition	Epiphytotic condition	Control condition
EC-597991	134.4	52.5	1.0	1.1
EC-597999	667.7	528	0.7	0.7
EC-635590	18.2	12.6	1.2	1.3
EC-635598	175.0	75.6	1.3	1.3
EC-635602	28.0	8.4	1.1	1.3
EC-635609	63.0	32.6	1.6	1.7
EC-635612	42.0	12.6	1.5	1.7
EC-635614	12.6	7.0	0.7	0.9
EC-635627	0.0	0.0	1.4	1.5
EC-635683	81.2	47.6	1.3	1.4
EC-635705	0.0	0.0	1.5	1.5
EC-635711	72.1	48.3	1.4	1.8
EC-635721	7.0	7.0	1.4	1.5
EC-635741	0.0	0.0	1.5	1.5
EC-635861	838.6	588	0.8	0.9
EC-635881	42.0	12.6	1.5	1.7
EC-636264	928.2	651	1.0	1.0
EC-663926	100.8	60.9	1.1	1.2
EC-664200	32.7	18.2	1.2	1.2
EC-664208	12.6	12.6	1.4	1.4
EC-664244	0.0	0.0	1.5	1.5
EC-664299	1263.5	957.6	1.0	1.1
EC-664315	44.8	29.4	1.2	1.2
EC-692221	19.6	8.4	1.5	1.6
EC-692231	23.8	7.0	1.0	1.3
EC-692246	28.0	12.6	1.6	1.8
EC-693252	44.8	32.2	1.2	1.3
EC-693271	7.0	7.0	1.3	1.4
EC-693289	98.0	18.2	1.3	1.3
EC-693322	28.0	18.9	1.4	1.5

Table 4: Correlation coefficient (r value) of plot yield and A-values of 30 wheat germplasm accessions

S. No.	Description of characters	"r" value
1.	Stripe rust	-0.603**

** = Critical value at 0.01 level = 0.463

and EC-693289 showed susceptible at seedling stage but resistant at adult plant stage which may be due to presence of some resistant *Yr* genes which confer resistant only at adult plant stage as being race specific (Kumar *et al.,* 2014). The combination of seedling and adult plant resistance would certainly delay the onset of rust epidemics and use of different kinds of resistance would help in accumulating combinations of resistance genes in wheat varieties, which in turn might impart durable resistance (Singh and Rajaram, 1992; Bhardwaj *et al*., 2010). Singh *et al.* (2004) evaluated 44 cultivars of wheat for resistance to leaf rust and among them 14 wheat lines possessing seedling resistance against 77-5 also showed adult plant resistance against pathotypes 77-5, 77-2 and 104-2. Kumar *et al.,* (2014) have also screened some old wheat varieties against different pathotypes of *Puccinia striiformis* for yellow rust resistance both at seedling as well as adult plant stage, which support the present findings in the study. Hence, from the study it can de concluded that the 27 resistant germplasm accessions, with combination of resistant responses at both stage of crop, could be used for further study for the presence of resistance *Yr* genes so that they could be incorporated in breeding programmes for developing stripe rust resistant wheat varieties.

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