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Screening and Molecular Characterisation for Durable Rust Resistance in Wheat Germplasm Resources

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Abstract Identification of slow rusting resistance sources against rusts is important for gene pyramiding, gene deployment and developing wheat varieties with durable rust resistance. In the present investigation resistance was assessed with slow rusting parameter i.e. area under disease progress curve (AUDPC). Three hundred thirty accessions were screened for their infection response during 2012–2013 in the field conditions and out of these thirty accessions possessing good agronomic attributes were evaluated again under field conditions in 2013–2014. These accessions were also subjected to molecular characterisation for *Lr34/Yr18/Sr57* with linked STS marker $csLV34_{150}$. Nine exotic accessions were found possessing *Lr34/Yr18/Sr57*. Lower AUDPC and RIR of all the tested accessions indicate their slow rusting nature against both leaf and stripe rusts. The relationship between the plot yield and AUPDC (A-value) was observed negative which signifies that plot yield decreases when A-value increases in both the diseases.

Keywords Wheat, Rust, Durable resistance, Area under disease progress curve (AUDPC).

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Introduction

Wheat (*Triticum aestivum* L.) is grown on more land area than any other commercial crop and is the most important staple food for humans globally. The production of wheat is constrained by several biotic and abiotic stresses. The warmer parts of the world are mainly affected by many diseases. Among these diseases, rusts are the most important. Wheat rusts caused by *Puccinia* spp. pose constant threat to sustainable wheat production and food security. Due to climate change break down of resistance due to major genes is a matter of great concern as it leads to epidmeic like situation when happens with a mega cultivar. Leaf (brown) rust caused by *Puccinia triticina* Erisk is the most widespread and regularly occurring among the three rusts found on wheat in India [1]. The yield losses caused by leaf rust range from 30—50%. It causes severe loss in yield when

Table 1. AUDPC (A-value) score of germplasm accession in epiphytotic and control conditions against stripe and leaf rust during 2013–2014.

Accessions No.	AUDPC (A-value) Stripe rust Epiphytotic condition	Stripe rust Control condition	Leaf rust Epiphytotic condition	Leaf rust Control condition
1) EC-692221	19.60	8.40	18.90	10.50
2) EC-635538	175.00	75.60	27.3	18.90
3) EC-636264	928.20	651.00	14.00	7.00
4) EC-693271	7.00	7.00	7.00	7.00
5) EC-664200	32.76	18.20	140.00	106.40
6) EC-693252	44.80	32.20	42.00	14.00
7) EC-692231	23.80	7.00	525.70	231.00
8) EC-693322	28.00	18.90	39.90	10.50
9) EC-663926	100.80	60.90	35.00	12.60
10) EC-635590	18.20	12.60	168.00	140.00
11) EC-635861	838.60	588.00	12.60	18.20
12) EC-635683	81.20	47.60	2.80	2.80
13) EC-664208	12.60	12.60	252.00	252.00
14) EC-635721	7.00	7.00	28.00	21.00
15) EC-635614	12.60	7.00	1656.20	1267.00
16) EC-635627	0	0	0	0
17) EC-664244	0	0	0	0
18) EC-635881	42.00	12.66	190.40	190.40
19) EC-597991	134.40	52.50	56.00	25.20
20) EC-620046	28.00	12.60	7.00	7.00
21) EC-664315	44.80	29.40	28.00	12.60
22) EC-597999	667.75	528.00	126.00	105.00
23) EC-635741	0	0	0	0
24) EC-635711	72.10	48.30	49.00	42.00
25) EC-635609	63.00	32.60	56.00	53.20
26) EC-635612	42.00	12.60	7.00	2.80
27) EC-693289	98.00	18.20	72.80	53.20
28) EC-635705	0	0	0	0
29) EC-635602	28.00	8.40	78.40	70.00
30) EC-664299	1263.50	957.60	42.00	14.00

weather conditions are favorable in the country. Stripe (yellow) rust caused by *Puccinia striiformis* f.sp. *tritici* continue to pose a major threat to wheat production over large areas, particularly in Asia. In most wheat producing areas, yield losses caused by stripe rust range from 10—70% [2]. Stem (black) rust caused by *Puccinia graminis* f. sp. *tritici* is an important disease of wheat worldwide. It occurs in warm climate and the yield losses caused by stem rust in susceptible varieties has been reported up to 40% in endemic area and up to 100% in epidemic condition.

Seedling resistance, which is due to major genes

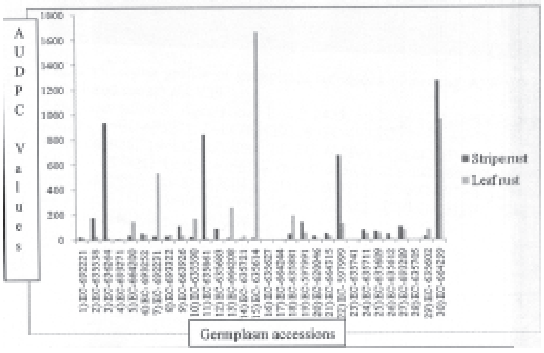

Fig. 1. AUDPC values for stripe and leaf rusts in epiphytotic.

and race specifc, is often transient once exposed in commercial cultivars due to genetic shifts in the pathogen population. Adult plant resistance can be race-specific or race non-specific. Both types of seedling and adult plant resistance identified in wheat germplasms offer promising genetic stocks for accumulating both resistances to acquire durable resistance and long lasting control against rust pathogens. Developing durable resistance in wheat cultivars against rapidly evolving new pathogens is one of most sustaining ways to control rust diseases. *Lr34/Yr18/Sr57* complex on chromosome 7DS [3] expresses resistance to all three i.e. leaf, stripe and stem rusts. The present study was conducted to screen the wheat germplasm against leaf and stripe rust (as Pantnagar location is hot spot for these two rusts and stem rust does not appear in normal conditions) for their slow rusting and adult plant resistance in the field and to confirm the presence of *Lr34/Yr18* with the use of linked STS marker,csLV34$_{150}$.

Materials and Methods

The experimental materials

Three hundred thirty germplasm comprising of indigenous and exotic accessions were screened for their infection response during 2012–2013 season in the field conditions under epiphtotic conditions. Out of these, thirty accessions having good agronomic attributes bearing EC numbers namely, 1) EC-692221, 2) EC-635538, 3) EC-636264, 4) EC-693271, 5) EC-664200,

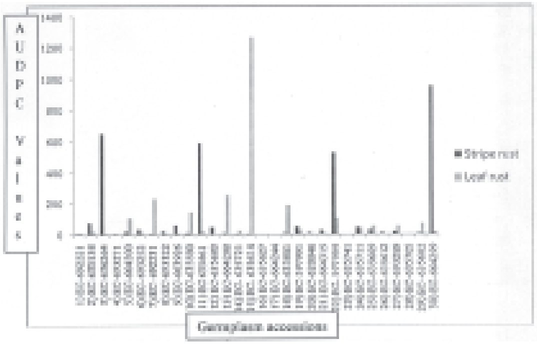

Fig. 2. AUDPC values for stripe and leaf rusts in control.

6) EC-693252, 7) EC-692231, 8) EC-693322, 9) EC-663926, 10) EC-635590, 11) EC-635861, 12) EC-635683, 13) EC-664208, 14) EC-635721, 15) EC-635614, 16) EC-635627, 17) EC-664244, 18) EC-635881, 19) EC-597991, 20) EC-620046, 21) EC-664315, 22) EC-597999, 23) EC-635741, 24) EC-635711, 25) EC-635609, 26) EC-635612, 27) EC-693289, 28) EC-635705, 29) EC-635602 and 30) EC-664299 were evaluated under epiphytotic conditions again in the crop season 2013–2014.

Inoculation and disease assessment

One Month old seedlings were inoculated 3 times at 7 days interval with each individual pathotypes i.e. K, L, P, 46S119, 78S84 of stripe and 2, 77-2, 77-5, 104-2 of leaf rust in the month of January to create an epiphytotic conditions. After 2 weeks of inoculation infection type and disease severity were recorded 8 times by using the modified Cobb's scale where, 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 [4].

DNA extraction and molecular marker analysis

The DNA from the leaves of young seedlings of 30 germplasm accessions was extracted using CTAB method (Cetyl trimethyl ammonium Bromide). The SSR marker, with csLV34 primer sequence forward 5'GTTGGTTAAGACTGGTGATGG-3´, reverse 5´-TGCTTGCTATTGCTGAATAGT-3´ was used to know the presence of *Lr34/Yr18* in the selected germplasms through DNA amplification.PCR amplification includes the reaction mixture of DNA templates (50ng/ul) of 2.0 ul, dNTPs mix (25mM each) of 0.4 ul, *Taq* DNA polymerase (5Uul) of 0.22ul, buffer (10X) of 1.5ul, primer (40 ng/ul) of 2.0 ul, de-ionized water of 6.6 ul in one reactionat 94°C for 5 min. 50—60°C for 1 min. and 72°C for 2 min. for 35 cycles followed by final extension step of 10 min at 72°C. PCR products were resolved by means of electrophoresis in 2.5% agarose gel and visualized under UV light transilluminator.

Results and Discussion

Disease severity

Leaf rust

In the 1st week of disease observation the leaf rust infection were absent in all tested 30 germplasm accessions except accession 15 (EC-635614) with trace severity. There were no infections in all control germplasm accessions in 1st and 2nd week of disease observation except accession 15 (EC-635614) with trace and 10%, respectively. Four accessions, namely, 16 (EC-635627), 17 (EC-664244), 23 (EC-635741) and 28 (EC-635705) showed near to immune reaction to leaf rust as they were not infected by leaf rust till the last (8th) disease observation. Susceptibility response was recorded up to 60% in the accession 15 (EC-635614).

Strip rust

In the first week of disease observation the accession number 3 (EC-636264) was found to have disease severity of 2% followed by the accessions, 11 (EC-635861), 22 (EC-597999) and 30 (EC-636264) with trace disease severity whereas other 26 accessions were found to be free from stripe rust infestation. There was no infection in all control lines also. In 8th week of disease observation the highest disease severity was observed in accession 30 (EC-636264) with 70% disease severity followed by accessions, 3 (EC-636264)

Fig. 3. Molecular profile of germplasm accessions possessing APR gene *Lr34/Yr18* with linked marker csLV34. *Lane description:*S: Agra Local, R=UP 2425, 1=EC-692221, 2=EC-635538, 3=EC-636264, 4=EC-693271, 5=EC-664200, 6=EC-693252, 7=EC-692231, 8=EC-693322, 9=EC-663926, 10=EC-635590, 11=635861, 12=EC-635683, 13=EC-664208, 14=EC-635721, 15=EC-635614, 16=EC-635627, 17=EC-664244, 18=EC-635881, 19=EC-597991, 20=EC-620046, 21=EC-664315, 22=EC-597999, 23=EC-635741, 24=EC-635711, 25=EC-635609, 26=EC-635612, 27=EC-693289, 28=EC-635705, 29=EC-635602, 30=EC-664299.

and 11 (EC-635861) each having 60% of disease severity. Out of the 30 accessions tested, the four accessions, 16 (EC-635627), 17 (EC-664244), 23 (EC-635741) and 28 (EC-635705) were observed showing zero disease severity, that is near to immune response forstripe rust.

The germplasm accessions which were recorded with disease severity in the range of 5 to 40% were taken as resistance to leaf and stripe rust. They were still acceptable for selecting resistant accessions for further breeding program for the development of resistance variety of wheat against leaf rust [4, 5].

Area under disease progress curve (AUDPC)-A value

Leaf rust

The highest A value of 1656.2 was observed in the accession 15 (EC-635614) where the infection was more as compared to other germplasm accessions followed by the accessions 7 (EC-692231) and 13 (EC-664208) with A value of 525.5 and 252.0, respectively. The accession 16 (EC-635627), 17 (EC-664244), 23 (EC-635741) and 28 (EC-635705) were having A values of zero as there were no infection. The remaining 23 accessions were having the A values in the range of 2.8 to 190.4 (Table 1 and Fig. 1).

Stripe rust

The highest A value of 1263.5 was observed in the accession 30 (EC-664299) followed by the accessions 3 (EC-636264) and 11 (EC-635861) with A value of 928.2 and 838.6, respectively. The lower A-value was recorded in remaining 23 accessions which were in the range of 7 to 134 whereas the accessions 16 (EC-635627), 17 (EC-664244), 23 (EC-635741) and 28 (EC-635705) shows zero A-value because of no stripe rust infection (Table 1 and Fig. 2).

Molecular analysis

The STS merker csLV34$_{150}$% on 7D chromosome linked with adult plant resistance (APR) gene *Lr34/Yr18* [6, 7], was used to screen the germplasm accessions for the presence of this APR gene. A specific 150 bp size band was observed in the genotype check, UP 2425, which clearly differentiated nine accession 1 (EC-692221), 8 (EC-693322), 14 (EC-635721), 16 (EC-635627), 17 (EC-634244), 23 (EC-635741), 24 (EC-635711), 25 (EC-635609) and 28 (EC-635705) from others (Fig. 3). So, the possibility of its linkage with the resistance genes was present in these accessions. In accordance to Kadkhodaei et al. [8] the leaf rust resistance gene, *Lr34/Yr18*, is known as "slow rusting gene" which provides durable and non specific APR and is located on the short arm of chromosome 7D. Thus, the result confirms that the STS marker csLV34 is reliable in the identification of effective adult plant resistance *Lr34/Yr18* gene. The result was also correlated with the utility of csLV34 marker in wheat breeding in postulating the likely occurrence of *Lr34/Yr18* across a wide range of wheat germplasm [9]. In general adult plant resistance genes confer a partial and slow rusting resistance [5].

The AUDPC score for both leaf and stripe rust indicated that most of the germplasm accessions showed low disease severity except four accessions, 3 (EC-636264), 11 (EC-635861), 22 (EC-597999) and 30 (EC-664299) for stripe rust and only one accession i.e. accession number 15 (EC-635614) for leaf rust. The results of Seyed [10] showed that AUDPC of 500% – 800% indicates a susceptible cultivar and AUDPC more than 800 indicates aboutthehighly susceptible one. Thus, 27 out of 30 accessions except 3 (EC-636264), 11 (EC-635861) and 30 (EC-664299), and 29 accessions except one i.e. 15 (EC-635614) were phenotypically resistance to stripe and leaf rust, respectively. While comparing the AUDPC score for both leaf and stripe rust, the germplasm accessions positive to *Lr34/Yr18* gene show lower AUDPC value and some germplasm accessions non-positive to *Lr34/Yr18* gene also show lower AUDPC value which may be due to the presence of some other effective *Lr/Yr* genes. Nine accessions which were phenotypically resistant were also confirmed as genotypically resistant with the presence of resistant gene *Lr34/Yr18* as the csLV34 locus is tightly linked to *Lr34/Yr18* and the results were also correlated with the reports of [6]. Thus, observation on AUDPC and the use of a reliable STS marker, csLV34, may help in resistance breeding of wheat and resistance gene pyramiding, providing durable resistance against leaf and stripe rusts.

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