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Study of stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici*) severity of Bread wheat and validation of resistance in identified lines with the help of molecular marker

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Abstract
Wheat (*Triticum aestivum* L.) is an annual monocot cereal belonging to the family *Poaceae* (earlier *gramineae*), originated from south west Asia. The field experiments were conducted at Norman E. Borlaug Crop Research Centre of Govind Ballabh Pant University of Agriculture and Technology, Pantnagar during 2015-2016 and 2016-2017 in Rabi season. The experimental material consists of ninety elite lines from Indo-Australian project on triple rust resistance. The lines were evaluated in field conditions for stripe rust. These lines were categorized into resistance and susceptible on the basis of modified Cobb's scale. Disease severity was noted at different intervals and Area under disease progress curve (AUDPC) was generated to calculate A-value. The laboratory work was done in order to confirm the presence of effective rust resistant gene(s) with linked molecular markers in elite lines. The SSR markers, Psp 3000 linked to effective yellow rust resistance gene *Yr10*.In the field experiment the lines, namely, P-2, P-3, P-46, P-62, P-73 and P-90 for stripe rust showed low coefficient of disease level (CDL), low A-value and low rate of infection with moderately resistance and moderately susceptible type of response. The use of molecular markers confirmed the presence of *Yr10* gene in 14 lines. The lines possessing resistant genes and showing phenotypic resistance in field conditions can be used in breeding programme for developing resistance wheat varieties.

Keywords: Stripe rust (*Puccinia striiformis* Westend. f. sp. *tritici*), Molecular Markers, Area under disease progress curve (AUDPC), A-value, Cobb's Scale

1. Introduction
Wheat (*Triticum* spp., most commonly *T. aestivum* L.) is an annual monocot cereal belonging to the family *Poaceae* (earlier *Gramineae*) which was originated from south west Asia. Botanically wheat grain is a type of fruit called caryopsis. Common wheat or bread wheat (*T. aestivum*) is a hexaploid species cultivated all over the world and contribute about 95% production among all other cultivated wheat species along with the highest monetary yield compared to other cereals.

Today wheat is cultivated worldwide throughout the subtropics and tropics where it plays an important role in agricultural economic systems of these regions. Wheat is the third most-produced cereal after maize and rice and staple food of millions of people. The estimated world production of wheat in 2015-16 was 734.1 million tons which was slightly higher than 2014-15 production (729.8 million tons). China (130.19 million MT) is the highest producer of wheat followed by India (88.94 million MT), Russia (61 million MT), United States of America (55.84 million MT), Canada (27.6 million MT), Ukraine (27.25 million MT), Australia (26 million MT), Pakistan (25.47 million MT), Turkey (19.5 million MT) and Iran (14 million MT). These countries contribute about 76% of the total world Wheat production. The normal world productivity is 30.39 q/ha.

In India genetic diversity at the farmer's field level is very limited although a large number of varieties have been released. Wheat is prone to various diseases and insect-pests both field and storage which cause losses in quantity and quality in different agro-ecological system and their comprehensive list can be found in many compendiums (Wilcoxson and Saari, 1996) [31].

The major limiting factors in successful production of wheat in India are growing threat of new virulence of diseases such as wheat rusts (yellow, brown and black rust) and leaf blight, heat stress and drought due to climate change.

Among the fungal diseases rusts, powdery mildew, leaf blight, Karnal bunt and loose smut are important. The rust fungi are the most damaging amongst all pathogens affecting wheat (Chaves *et al*., 2013) [5]. The stripe rust also known as yellow rust pathogen; *Puccinia striiformis* Westend. f. sp. *tritici* produces yellow-colored stripes consisting urediniospores parallel along the venations of each leaf blades. Favorable condition for infection by rust fungi includes suitable weather and age of the plants during late October till December. The uredospores and teliospores of the rust fungus are killed during summer months in the plains of North India due to prevailing high temperatures after harvest of wheat crop.

The three rusts vary in their optimum temperature requirements for different developmental stages. The highest temperatures (15–30°C) is tolerated by stem rust fungi followed by leaf rust fungi i.e., 15-20ºC while lowest (9–15°C) is by stripe rust fungi (Roelfs *et al*., 1992) [23]. Stripe rust is a destructive disease of wheat that can cause significant yield losses in severe epidemics due to reduction in kernel number and size (Ma and Singh, 1996) [19]. Stripe rust of wheat has caused serious wheat-yield losses around the globe over the years. There are *Yr* gene (1-48) have been reported till date (Hovmoller *et al.,* 2011 and De Vallavieille-Pope *et al*., 2012) [14, 7]. The rusts affect the photosynthetic ability of the plant and transportation of photosynthates from green part of the plant, which results shriveled grains. This adversely affects the yield, moreover, the grain quality is also badly affected resulting lower price in market. Since rusts are obligate parasites, any resistance genes in host cultivars that curtail or eliminate rust reproduction will place tremendous selection pressure on variants that are virulent to the resistance genes.

Molecular markers, such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and SSR (Simple sequence repeats) have been widely used to tag fungal resistance genes in wheat.

2. Materials and Methods

The proposed investigation was conducted during rabi seasons of 2016-17.

2.1 Screening of Indo-Australian elite lines for stripe rust

During crop season 2016-17, a field experiment was conducted and ninety elite lines of Indo-Australian Programme on Triple Rust Resistance were screened against stripe rust.

2.2 Disease rating

The observation was recorded after the first appearance of symptoms of the disease. The stripe rust severity was recorded as percent of infection from the individual line according to the modified Cobb's scale (Peterson *et al.,* 1948) [21]. The severity was determined by visual observations, below 5 per cent severity the intervals used were Trace to 2 per cent. Usually 5 per cent interval was used from 5 to 20 per cent severity and 10 per cent intervals between 20-100 per cent.

Table 1: Rusts infection types at adult plant stage

Reaction type	Response value	Category	Visible symptoms
0	0.0	Immune	No visible infection
R	0.2	Resistance	Necrotic areas with or without uredia
MR	0.4	Moderately Resistance	Necrotic areas with small uredia
MS	0.8	Moderately Susceptible	Medium uredia with no necrosis but some chlorosis
S	1.0	Susceptible	Large uredia with no necrosis and no chlorosis
X	0.6	Intermediate	Variable sized uredia with necrosis or chlorosis and fully susceptible

The severity and response types were recorded at the same time.

Table 2: Combining severity and response reading

Scale	Description
TR	Trace severity of a resistant type of infection
5MS	5 per cent severity of a moderately susceptible type of infection
10MR	10 per cent severity of a moderately resistant type of infection
30S or 100S	30 per cent or 100 per cent severity of a susceptible type of infection

2.3 Harvesting and threshing

After maturity, the individual row of 90 elite lines was harvested manually and threshed separately by using electric operated thresher.

2.4 Analysis

In order to find out the statistical differences in effect of inoculation among the elite lines under epiphytotic condition and natural condition, paired t test was used for the analysis and area under disease progress curve (A values), coefficient of disease level (CDL) and infection rate per day (r-vaues). The relationship between plot yield, test weight and A values was also calculated separately for both the disease by using Pearson coefficient of correlation.

2.5 Determination of coefficient of disease level (CDL)

CDL was calculated to quantify the rust observations including disease rating and disease incidence. Average CDL is expected to give a good estimate of the relative resistance of varieties and it takes into account both the response and the percentage of rust infection, calculated from the field reading data. The response for different infection types were assigned from 0-1 (Loegering, 1969) [17]. The CDL was calculated by the formula given by Gupta, 1979 [9].

CDL = UIV×MCL
CDL= Coefficient of disease level
UIV= Unit incidence value
MCI= Modified coefficient of infection

Where, UIV= % incidence ÷ 100
MCI= Loegering's coefficient of infection ÷ 100
Loegering's coefficient of infection = Severity × Response value
CDL values are used for the calculation of rate of infection and its maximum value will be 1.

2.6 Determination of rate of infection (r) of disease
It is calculated by using formula given by Vander Plank (1963) [19].

$$r = \frac{2.3}{t}\left(log_{10}\frac{X_2}{1-X_2} - log_{10}\frac{X_1}{1-X_1}\right)$$

Where, r = Average rate of infection of disease per days
t = total days between the first and last date of observation of disease
X_1 = CDL at the first date of disease observation
X_2 = CDL at the last date of disease observation
Where, 1- X_1 and 1- X_2 are the correction factors in which one is considered as the maximum disease.

2.7 Area under disease progress curve (A values)
It is calculated by the formula given by Wilcoxson *et al.*, 1975 [30].

$$A = \sum_{i=1}^{K}\frac{1}{2}(S_i + S_{i-1})$$

Where, S_i = Rust severity at the end of the week
K = Number of successive evaluation of rust

2.8 Determination of coefficient of correlation (r)
Coefficient of correlation was calculated to find out the relationship between the plot yield and A value of each line and test weight and A value of each line separately. The correlation coefficient gives two kinds of information (i) indication of the magnitude of the relationship and (ii) information about the direction of the relationship (whether positive or negative). It is calculated by the formula given by Cochran and Cox (1967) [3].

$$r = \frac{\sum XY - \frac{\sum X \sum Y}{n}}{[\sum X2 - \frac{(\sum X)2}{n}][\sum Y2 - \frac{(\sum Y)2}{n}]}$$

Where
r = correlation coefficient
X = variables (A values)
Y = variable (plot yield and test weight)
n = Total numbers of observations

3. Determination of paired t test
It was calculated to find out the effect of inoculation in each 90 elite lines of both the diseases and were compared to their respective control lines. It can be calculated by the formula.

$$t = \frac{\sum d}{\sqrt{n(\sum d2) - (\sum d)2 \div n - 1}}$$

Where
d = Difference between the observations, and
n = Number of paired observations

3.1 Validation of resistance in identified lines with the help of molecular markers
3.2 DNA extractions from leaves
The DNA from the 20 selected resistant lines was extracted from the young seedling stage wheat for the evaluation of adult plant resistance through moleculer techniques. The procedure for DNA isolation is given below:

3.3 CTAB (Cetyl Trimethyl ammonium Bromide) method
The genomic DNA was extracted for molecular characterization studies by using CTAB method of Doyle and Doyle (1990) [6] with some modifications.

3.3.1 Principle
CTAB has the useful property of precipitating carbohydrates at high temperature and high salt concentration and it also precipitates nucleic acids at low salt concentration and low temperature as CTAB is a cationic detergent.
Reagents used and preparations

A) Stock prepared
1. 1M Tris Cl buffer = 100 ml
Amount of Tris base = 12.114g
Tris base was dissolved in 80 ml of de-ionized water and pH was adjusted at 8 with 6N HCL then the final volume was made up to 100 ml with de-ionized water. Then the total solution was autoclaved and stored at room temperature.

2. 0.5M EDTA = 100 ml
18.612g EDTA was dissolved in 80 ml of de-ionized water having pellets of NaOH by adjusting pH at 8 and the final volume was made to 100 ml. It was autoclaved and kept at room temperature.

B) Working solutions
1. DNA extraction buffer = 100 ml
2 % (w/v) CTAB = 2g
1.4 M NaCl = 8.19g
20mM EDTA = 4 ml (0.5M stock)
0.2% beta mercaptoethanol = 200 µl

2. 70 % Ethanol = 100 ml
Ethyl alcohol = 70 ml
De-ionized water = 30 ml
Stored at 4^0C

2. Chloroform: Isoamyl alcohol (24:1) = 100 ml
Chloroform = 96 ml
Isoamyl alcohol = 4 ml

3. Isopropanol
Stored at -20^0C

4. TE Buffer = 100 ml
Tris Cl buffer (10mM) = 1 ml (1M stock)
EDTA (1mM) = 0.2 ml (0.5M stock)

3.4 Genomic DNA isolation procedure
a) 1g sterilized leaf sample was weighed for DNA isolation.
b) Leaf sample was ground in liquid nitrogen to fine powder using pestle and mortar.
c) The powder was transferred to polypropylene tubes having 1 ml of TE buffer by using a spatula.

d) Samples were incubated at water bath for 1 hour at 60ºC.
e) Centrifuged the samples at 12000 rpm for 10 minute at room temperature (25ºC).
f) 1 ml of Cl (24:1) was added and mixed for 15 minute by inversion to emulsify.
g) Centrifuged again at 13000 rpm for 10 minute at room temperature (25ºC).
h) By using a micropipette aqueous phase was removed and transferred to a clean tube, added with 2/3 volume of Isopropanol and mixed by gentle inversion. Then the tubes were kept for overnight at -20ºC.
i) Centrifuged the samples for 12 min. at 10,000 rpm at 4ºC.
j) Decanted the solution without disturbing the DNA pellet.
k) With 70 per cent ethanol solution, the pellet was washed by centrifuging at 6000 rpm for 10 min.
l) Evaporate the ethanol by drying the pellet and finally dissolved in 2000µl TE buffer and stored at - 4ºC.

3.5 Quantification of DNA by UV Spectrophotometer

Principle

DNA absorbs UV light due to conjugated aromatic nature of bases. Maximum absorption of UV light by the bases occurs at the wavelength of 260nm which is distinct from the property of proteins which absorb maximum at 280nm. Therefore, simplest method for quantification is reading the absorbance at 260 nm at which an OD_{260} of 1 in a 1 cm path length is equal to 50 µg/ml for double stranded DNA and RNA. An absorbance ratio of 260nm and 280nm gives an estimate of purity of the solution. Pure DNA and RNA solutions have OD260/OD280 values of 1.8 and 2 respectively. So, a higher than 1.8 indicates RNA contamination and ratio lower than 1.8 indicates protein contamination. The concentration is related to the OD by the following equation:

$$\text{DNA conc. (µg/µl)} = \frac{OD_{260} X 50 X Dilution - factor}{1000}$$

Where, Dilution factor was 60 (1µl sample DNA and 59µl distilled water).

3.6 PCR Amplification

PCR is based on the enzymatic amplification of DNA fragments that is flanked by oligo-nucleotide primers hybridizing to opposite strands of the target sequence. It involves three steps which are given below:

- Denaturing of the target DNA at 92-94ºC.
- Annealing of the primers to the template DNA.
- Primer extension by addition nucleotides to the 3'end of the primers by enzyme DNA polymerase.

3.7 PCR ingredients

i. Design and synthesis of the primers

Primers have the sequence complimentarily to either ends of the target DNA segment called template DNA to be synthesized. SSR molecular marker were used to evaluate seedling and adult plant resistance.

Table 3: List of markers and their primers used in the experiment

Markers	Primers	Chromosome
Psp3000 (*Yr10*)	F 5'- GCAGACCTGTGTCATTGGTC -3' R 5'- GATATAGTGGCAGCAGGATACG -3'	1B

ii. Tag DNA polymerase

It is a thermos-stable enzyme that replicates DNA at 72-74ºC and has 5'-3' and 3'- 5' exonuclease activity.

iii. dNTPs

iv. Buffer (10X)

10 mMTris- HCL, pH 9, 17.5mM MgCl2, 50mM KCL, 0.01 % gelatin.

3.7.1 Determination of primer annealing temperature

Primer annealing temperature is depend on its Tm value which is given by.

$$Tm(^oC) = 4(G+C) + 2(A+T)$$

Where, A, T, G, C stand for number of corresponding nucleotides in the primer.

Table 4: PCR amplification reaction mixture

Componets conc.	Final conc.	Single tube (µl)
DNA templates (50ng/µl)	50ng	2.0
dNTPs mix (25mlM each)	10mM	0.4
Taq DNA polymerase (5U/µl)	1.5U	0.22
Buffer (10X)	1X	1.5
Primer (40ng/µl)	5pmole	2.0
De-ionized water	15µl	6.6
Total volume		12.72

Cycles	Denaturation		Annealing		Polymerization	
	Temp.	Time	Temp.	Time	Temp.	Time
First cycle	94	5 min	-	-	-	-
35 cycles	94	1 min	50-60	1 min	72	2 min
Last cycle	-	-	-	-	72	2 min

3.8 Gel electrophoresis using agrose

Reagents used

1. **DNA loading dyes = 10 ml**
   Bromophenol blue (0.25 % w/v) = 0.025g
   Xylenecynal FF (0.25 % w/v) = 0.025g
   Sucrose (40 % w/v)
   The components were dissolved in 8 ml of de-ionized water and pH was adjusted at 8 then the final volume was made up to 10 ml and stored at -20oC.

2. **Electrophoresis buffer (50X TAE) = 100 ml**
   Tris base (0.045M) = 24.2g
   Glacial acetic acid = 5.91 ml
   EDTA (0.001M) = 10 ml (1M stock)
   The components were dissolved in 80 ml of de-ionized ware. The pH was adjusted at 8 with 6N HCL. The final volume was made up to 100 ml, autoclaved and stored at 4oC. 0.5X was the working solution of TBE buffer.

3. **DNA staining solution and Ethidium Bromide (10ml/ml)**
   Ethidium bromide = 10 mg
   Sterilized de-ionized water = 1 ml
   Working solution for staining gel was made by dissolving 60 µl ethidium bromide stock (10 mg/ml) in 3000 ml of de-ionized water.

3.9 Procedure for gel electrophoresis

Gel electrophoresis unit was used for fractioning SSR markers

on agrose gel. Agrose gel (2.5%) was prepared by dissolving aapropriate amount of agarose in 0.5X TBE buffer. DNA loading dye and DNA samples were mixed in 1:6 ratios for each well and loaded with a micropipette. Electrophoresis was done at 50V for 4 h in 0.5X TBE buffer. The gel was stained in ethidium bromide solution. After de staining in de-ionized water, the gel image was viewed in UV transilluminator and stored in gel documentation system.

3. Result and Discussion

The results obtained from experiment conducted to identify promising wheat elite lines exhibiting yellow rust at adult plant stage and moleculer analysis of lines showing resistance are described below.

4. Evaluation of the occurrence of stripe rust

4.1 Stripe rust

During crop season 2016-17, one month old seedlings were inoculated four times at three days' interval with four individual pathotypes of stripe rust for creating the epiphytotic condition. After 1 weeks of inoculations eight times from 16th January 2017 to 6th March 2017 at one-week interval observation recorded on occurrence.

In the first week on 16th January of observation, the line no. 1, 2, 16, 18, 20, 29, 37,42, 43, 54, 55 and 90 were observed with rust severity of in traces. There was no infection in all the lines in natural condition. On 23rd January 2017, second observation was taken and rust severity was recorded 5 per cent in the lines 2, 6, 7, 10, 11 and 12 and in line no. 32,35, 43 and 87 with 5 per cent showing reaction type MRMS. The remaining lines were observed to have disease severity of 0.0 to trace. In 3rd week the maximum 10 per cent severity recorded in many lines followed by the severity 5 per cent with reaction type MR and 5 per cent with reaction type MRMS, respectively. The highest severity of 20 per cent recorded in the lines 6, 10, 11, 18, 29 and 90 followed by the lines 7 and 77 with 15 per cent disease severity respectively, in 4th week of disease observation i.e., 6th February 2017.The line 90 showed maximum severity *i.e.,* 20 per cent with reaction type MS as compared to the other lines. The lines 18, 29 and 39 were observed to have 15, 30 and 30 per cent severity, respectively on 5th week of disease observation i.e., 13th February 2017. Other lines in which disease were recorded in the previous weeks were found with increased severity as the week progress.

In 6th week (20th February 2017), the highest severity observed in line 90.*i.e.,* 30 per cent with MS infection type of response followed by the lines, 2, 6, 15, 19, 22, 29, 35 and 39 with severity 20 per cent with infection type MS and 40 per cent with infection type MR, respectively. On 27th February 2017 *i.e.,* seventh week the initiation of disease was recorded in lines 56 and 82 with low level of severity *i.e.,* 5 per cent. The highest rust severity was observed in line 90 of 40 per cent with MS type infection. On last day of observation *i.e.,* 6th march 2017 (8th week) the highest disease severity was recorded in lines 90 of 70 per cent. Out of the 90 lines tested, the lines 64, 65, 66, 68, 69, 70, 71, 75, 76, 80, 81, 83, 88 and 89 were observed free of disease which means these lines possess high level of resistance to stripe rust. In natural condition the highest disease severity of 30 per cent with MS response were observed in lines 1 and 90.

According to Ali *et al.* (2009) [1], cultivars with CI values of 0-20, 21-40 and 41-60 were regarded as possessing high, moderate and low level of adult plant resistance, respectively.

4.2 Rate of infection (r)

4.2.1 Stripe rust

The highest rate of infection (r) per day was observed in line 56 with 0.1380 followed by the line 67, 78 and 84 with 0.1241, 0.1196 and 0.1147, respectively. The line 57, 64, 65, 66, 68, 69, 70, 71, 72, 74, 75, 76, 80, 81, 83, 86, 88 and 89 were free from yellow rust therefore, rate of infection was zero. The line 67 rate of the disease level was same from the first appearance to last rate of observation.

4.3 Analysis of paired t test

4.3.1 Stripe rust

The lines P-23 and P-25 were statistically observed as highly significant with t value of 5.6124 and 5.0823 respectively, as compared to their respective control lines confirmed these lines highly effective with the inoculation of stripe rust pathotypes during the experiment. Few lines i.e., P-2, P-9, P-13, P-16, P-52, P-58, P-59, P-60, P-61, P-67, P-78, P-79, P-82 and P-85 were observed non-significant as compared to their respective control lines, which showed that there was no inoculation effect on these lines (Table 5).

Table 5: Paired t test analysis on the effect of inoculation among the inoculated 90 elite lines lines and control lines against stripe rust during 2016-17.

S. No.	Pair (Inoculated – Control)	Mean difference	Standard error of mean	t values
1.	P-1	3.1000	2.6251	3.340043 s
2.	P-2	8.0750	10.3625	2.204050 ns
3.	P-3	3.0000	2.5116	3.378319 s
4.	P-4	3.0000	3.2496	2.611165 s
5.	P-5	3.1514	6.7949	2.414273 s
6.	P-6	6.8000	5.3109	3.621474 s
7.	P-7	6.4000	6.1708	2.933435 s
8.	P-8	1.8250	1.3338	3.848201 s
9.	P-9	6.0000	3.5649	4.760448 ns
10.	P-10	3.2500	2.6742	3.437415 s
11.	P-11	5.3000	3.0293	4.948428 s
12.	P-12	5.1500	5.3726	2.711197 s
13.	P-13	3.0500	4.2971	2.007529 ns
14.	P-14	5.0000	3.5456	3.988620 s
15.	P-15	4.8000	4.1514	3.270316 s
16.	P-16	4.5000	6.7297	1.891253 ns
17.	P-17	4.2500	3.3936	3.542106 s
18.	P-18	7.9000	7.9505	2.810440 s
19.	P-19	6.8500	7.7310	2.506104 s

20.	P-20	2.9000	1.9914	4.118910 s
21.	P-21	2.9000	3.1332	2.617884 s
22.	P-22	8.7000	9.6208	2.557718 s
23.	P-23	3.0000	1.5118	5.612486 s
24.	P-24	5.0000	5.8390	2.421696 s
25.	P-25	3.7000	2.0590	5.082338 s
26.	P-26	3.5000	2.2090	4.481291 s
27.	P-27	3.4500	3.1052	3.142398 s
28.	P-28	3.4500	2.1374	4.565347 s
29.	P-29	5.3000	3.0230	4.948426 s
30.	P-30	4.5000	2.7748	4.582576 s
31.	P-31	4.2000	2.5652	4.630064 s
32.	P-32	3.6000	3.0528	3.334313 s
33.	P-33	3.7500	2.2516	4.709894 s
34.	P-34	4.4000	2.8106	4.425361 s
35.	P-35	7.4000	6.5795	3.181089 s
36.	P-36	5.5500	6.5810	2.385266 s
37.	P-37	4.2000	2.7110	4.431791 s
38.	P-38	3.1000	2.8425	3.084615 s
39.	P-39	6.9500	6.2800	3.129782 s
40.	P-40	4.2500	3.2341	3.715775 s
41.	P-41	4.0000	2.8284	4.000000 s
42.	P-42	5.2000	5.3207	2.790484 s
43.	P-43	3.6000	3.0528	3.334313 s
44.	P-44	4.1500	4.0090	2.926404 s
45.	P-45	1.7500	1.4933	3.309300 s
46.	P-46	4.2500	3.3926	3.542106 s
47.	P-47	3.2000	2.8600	3.164043 s
48.	P-48	3.1500	3.0118	2.958133 s
49.	P-49	3.9000	2.9681	3.716091 s
50.	P-50	2.6500	2.5377	2.952920 s
51.	P-51	3.0500	3.0980	2.771501 s
52.	P-52	3.3000	4.3278	2.156619 ns
53.	P-53	2.9000	2.8896	2.837843 s
54.	P-54	1.2000	1.1313	3.000000 s
55.	P-55	3.4500	2.8513	3.421404 s
56.	P-56	0.0000	0.0000	0.0000
57.	P-57	0.0000	0.0000	0.0000
58.	P-58	3.1000	4.5541	1.925185 ns
59.	P-59	3.1000	4.4519	1.969069 ns
60.	P-60	2.3000	3.3511	1.940886 ns
61.	P-61	1.0000	1.6970	1.666667 ns
62.	P-62	5.7000	6.4768	2.489039 s
63.	P-63	3.7500	4.0730	2.603960 s
64.	P-64	0.0000	0.0000	0.0000
65.	P-65	0.0000	0.0000	0.0000
66.	P-66	0.0000	0.0000	0.0000
67.	P-67	1.0000	1.8493	1.527525 ns
68.	P-68	0.0000	0.0000	0.0000
69.	P-69	0.0000	0.0000	0.0000
70.	P-70	0.0000	0.0000	0.0000
71.	P-71	0.0000	0.0000	0.0000
72.	P-72	0.0000	0.0000	0.0000
73.	P-73	4.3000	4.5540	2.546212 s
74.	P-74	0.0000	0.0000	0.0000
75.	P-75	0.0000	0.0000	0.0000
76.	P-76	0.0000	0.0000	0.0000
77.	P-77	4.6000	3.3600	3.871937 s
78.	P-78	0.4250	1.2000	1.000000 ns
79.	P-79	3.2000	5.4083	1.673320 ns
80.	P-80	0.0000	0.0000	0.0000
81.	P-81	0.0000	0.0000	0.0000
82.	P-82	0.7500	1.4866	1.425573 ns
83.	P-83	0.0000	0.0000	0.0000
84.	P-84	1.4000	1.0246	3.861742 s
85.	P-85	3.0000	5.3702	1.579885 ns
86.	P-86	0.0000	0.0000	0.0000
87.	P-87	3.0000	3.0149	2.813285 s
88.	P-88	0.0000	0.0000	0.0000

89.	P-89	0.0000	0.0000	0.0000
90.	P-90	11.7500	11.5399	2.880981 s

d.f. = 7,* = t tab at 5% level of significance = 2.364624

** = $t_{\text{tab at 1\% level of significance}}$ = 3.49948

4.4 Area under disease progress curve (AUDPC)

Area under disease progress curve (A values) as given by Wilcoxson *et al.* (1975) [30] as better criteria to measure slow rusting or partial resistance.

4.4.1 Stripe rust

The highest A value of 869.75 was statistically observed in the line 90 followed by the line 20 with A value 588.0 and line 39 with A value of 565.95, respectively. The lower A-value was recorded in remaining 88 lines which were in the range of 36.85 to 497.35 whereas the lines which are highly resistant to stripe rust shows zero A-value because of no infestation. The lower AUDPC belonged to the lines that were resistant due to their effective race-specific resistance genes. The results of Seyed (2013) [27] showed that AUDPC of 500 – 800per cent per day indicated a too susceptible one. Thus, nearly all 89 lines except 90 were phenotypically resistant to stripe rust

Based on the AUDPC values, wheat cultivars were categorized into two distinct groups according to Ali *et al.* (2009) [1]. One group included the genotypes exhibiting AUDPC values up to 30%, and the other included the cultivars showing AUDPC values up to 60%. In these cultivars, rust initiated and sporulated but with final chlorotic and necrotic strips (MR and/or MS infection types). Subsequently, the progress of rust development remained slower and restricted. The cultivars in group 1 were marked as having better slow rusting and that of group 2 were marked as having moderately slow rusting. The reasons for the markings were because they also developed epiphytotic of very low potential as indicated by their AUDPC values, despite the ultimate expression of high infection type. Cultivars with such traits are expected to possess genes that confer partial resistance (Parlevliet, 1988) [20]. Apart from six cultivars having resistance 'TR' infection type at adult plant stage, the remaining genotypes that exhibited AUDPC value less than 30% were considered as having better level of partial resistance. This group was composed of cultivars with varying degrees of adult plant resistance, which was advocated to be more durable (Safavi, 2013). Moreover, cultivars with acceptable levels of slow rusting restricted the evolution of new virulent races of the pathogen because multiple point mutations were extremely rare in nature (Ali *et al.*, 2009) [1].

4.5 Plot yield

The line 70 gave the maximum plot yield i.e., 765 g/plot followed by line 52 having 705 g/plot. The lowest plot yield was recorded in the line 8 with 220 g/plot and line 1 with 290g/plot because the infection was high in these lines as compared to other therefore plot yield was less than 300 g the remaining lines produced average yield between 305 to 705 g/plot. Plot under epiphytotic conditions produces significantly less yield as compared to plot under natural conditions for all the lines.

4.6 Test weight

The highest test weight (1000 grain weight) was recorded in the lines 75 and 88 with 49.8 each followed by the line 89 (48.7g), which was significantly different from the line under natural condition. The minimum test weight was observed in the lines 53 with 17.7 followed by the line 6 producing 20.3g, the test weight recorded in the remaining line was found in between the range of 21.2-48.3g and less as compared to the line under natural conditions.

4.7 Correlation coefficient of plot yield, test weight and A values

4.7.1 Plot yield and A values

The relationship between plot yield and A value of different characters *viz*., stripe and leaf rusts were studied and the correlation coefficient for respective character are given in following (Table. 6)

Table 6: Correlation coefficient (r-value) of plot yield and A-values

S. No.	Description of characters	"r" value
1	Stripe rust	-0.61377

It was statistically observed that there was negative correlation coefficient in between the plot yield and AUDPC values, which refers that with the increase of A values there was decrease in plot yield. There was highly significant negative correlation coefficient between plot yield and A values in stripe rust

4.8 Test weight and a values

The relationship between test weight and A value of stripe rust was studied and the correlation coefficient for respective characters are given in following (Table 7).

Table 7: Correlation coefficient (r-value) of Test weight and A-values.

S. No.	Description of characters	"r" value
1	Stripe rust	-0.56009

It was observed that there was non-significant correlation coefficient with test weight and A value of stripe rust because the disease severity was not very high in all the line therefore test weight was not effected significantly.

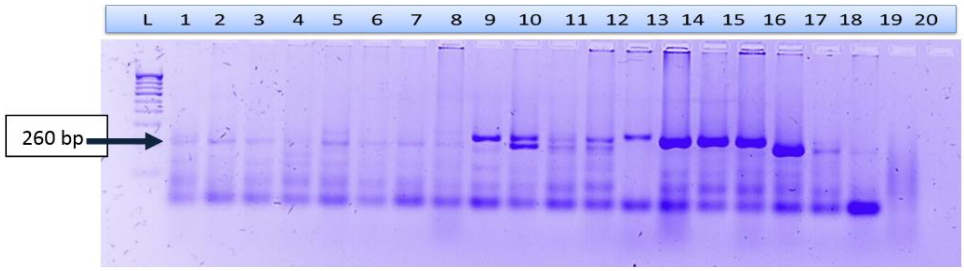
4.9 Moleculer analysis of presence of resistant genes in wheat elite lines/lines

Microsatellites markers are highly polymorphic even between closely related lines, stable, locus specific, co-dominant and also require a small amount of DNA (Roder *et al.*, 1998) [24]. The SSR marker (Psp3000) was used to confirm the presence of *Yr10* gene. Analysis of rust resistance through molecular techniques in 20 wheat elite lines P-56, P-57, P-64, P-65, P-66, P-68, P-69, P-70, P-71, P-72, P-74, P-75, P-76, P-80, P-81, P-83, P-86, P-88, P-89 and P-90 (although it has infection but it has taken as control) was carried out by using one SSR marker.

The SSR marker i.e., Psp3000 with 260 bp showed its presence in the14 lines *viz*., P-56, P-57, P-64, P-65, P-66, P-68, P-69, P-70, P-71, P-72, P-74, P-75, P-88 and P-89. Therefore, 13 lines which were phenotypically resistant were also confirmed as genotypically resistance with the presence of resistant gene *Yr10*. According to Wang *et al*. (2002) [32] microsatellite markers Xpsp3000 is linked to *Yr10*.

Table 8: Amplification result of SSR marker in the genomic DNA of resistant and susceptible lines

Primers	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Psp3000	+	+	+	-	+	+	+	+	+	+	+	+	-	-	-	-	+	+	+	-



L = Ladder: 100 bp, 1=P-56, 2=P-57, 3=P-64, 4=P-65, 5=P-66, 6=P-68, 7=P-69, 8=P-70, 9=P-71, 10=P-72, 11=P-74, 12=P-75, 13=P-76, 14=P-80, 15=P-81, 16=P-83, 17=P-86, 18=P-88, 19=P-89 and 20=P-90

Fig 1: Psp3000 banding pattern

The lines P-1, P-2, P-3, P-5, P-6, P-7, P-8, P-9, P-10, P-11, P-12, P-17, P-18 and P-19 were observed as resistant to stripe rust at adult plant stage as there is zero CDL value, A values and rate of infection. These 14 lines were found to possess *Yr10* gene with the use of Psp3000 marker. The gene is responsible for resistance in adult plant stage. Lower severity of stripe rust and their resistance infection types, lower A values, lower coefficient of disease level, and lower rate of infection was observed which are promising adult plant resistance which was compatible susceptible with predominant and virulent pathotypes but expressed high resistance at adult plant stage (Bhardwaj *et al.,* 2010) [2]. According to the result of Yang *et al.,* (1987) [33] the slow rise in the infection rate and low infection frequency is an important characteristics of slow rusting phenomenon. Vander plank (1984) stated that varieties with slow rusting resistance have remained stable over the years apparently free from boom and bust cycle that have plunged varieties with vertical resistance.

5. Summary and Conclusion

Wheat (*Triticum aestivum* L.) is an annual monocot cereal belonged to the family *Poaceae* (earlier *Gramineae*), originated from south west Asia. Botanically wheat grain is a type of fruit called caryopsis. *T. aestivum* (Common wheat or bread wheat**)** is a hexaploid species cultivated all over the world and contribute about 95% production compare to all other cultivated wheat species along with the highest monetary yield compare to other cereals.

The field experiments were conducted during 2016-2017 rabi season. Ninety Indo-Australian wheat elite lines were evaluated for stripe *(Puccinia striiformis)* infestation under epiphytotic conditions. These accessions were categorized according to resistance and susceptibility shown by them following the modified Cobb's scale. These ninety elite lines evaluated for stripe rust resistance response in adult plant stage in the field experiment. In the crop season 2016-17, these 90 lines were planted and evaluated for resistance response to stripe rust under epiphytotic conditions. Severity of the infestation was recorded on regular interval and area under disease progress curve (AUDPC) was calculated. Simultaneously coefficient of disease level (CDL) and rate of infection (r) were also calculated. The laboratory work was done in order to confirm the presence of effective rust resistant gene(s) with linked molecular markers. One SSR markers i.e., Psp3000 linked to effective major gene for stripe rust was used for this purpose.

The salient findings of the study are summarized below

- In the field experiment under epiphytotic conditions out of ninety lines except the lines, namely, (P-2, P-3, P-46, P-62, P-73 and P-90 for stripe rust showed low disease level, low CDL, low A-value and low rate of infection with moderately resistance and moderately susceptible type of response. Statistically it was observed that there was negative correlation in between the plot yield and AUDPC values of all ninety elite lines, which indicate that with the increase of A-values there was decrease in plot yield.
- It was observed that there was significant negative correlation with test weight and A-value for both the diseases in all ninety elite lines.
- The fourteen lines, P-56, P-57, P-64, P-65, P-66, P-68, P-69, P-70, P-71, P-72, P-74, P-75, P-88 and P-89 were observed as showing resistant at adult plant stage of the crop, which were also confirmed with the presence of *Yr10* gene in all the 14 lines with linked marker Psp3000.
- Other remaining lines also showed adult plant resistance to stripe with low levels of CDL, A-values and rate of infection however, they were lacking either *Yr10* gene. Thus, it is assumed that these lines may possess some other effective *Yr*gene which confirm resistance in these lines and these could be traced deploying other linked molecular markers.
- The findings of study reveal that information generated based on AUDPC, CDL, r and use of reliable molecular markers together may help in resistance breeding of wheat and pyramiding of resistance gene for providing durable resistance against stripe rust.

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