Characterization of Durable Resistance gene Yr18/Lr34 against Stripe Rust (*Puccinia striiformis* f. sp. *tritici*) in different Pakistani Wheat Cultivars by Using Molecular (STS) and Morphological (LTN) Markers

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Abstract: Stripe rust, caused by Puccinia striiformis f. sp. tritici, is an important disease of wheat in many countries. Its significance was seemed to be increased to many serious wheat growers at that time when more than 70% of the wheat crop got loss due to it. Hundreds of diverse methods was developed to control that very stupid and indirect enemy of human but failed because of mutation of pathogen in the universe is so quick. Durable resistance is considered the most impact giving way to slowly control the spread of disease in whole plant. In this study we tried to characterize durable rust resistance gene, Yr18, in Pakistani wheat cultivars using molecular marker, csLV34, categorize under STS and morphological marker for leaf tip necrosis (LTN). We used a hexaploid population from Pakistani selected wheat genotypes that were moderately resistant to moderately susceptible (MRMS) to stripe rust in field trials. The wheat genotypes were evaluated for stripe rust at, Pir Sabaq (Nowshera Khayber Pukhtoonkhwa, KPK), Pakistan which is considered as hotspot for stripe rust pathogen. Characterization included seedling testing, field evaluation, morphological marker studies and marker assisted selection (MAS). MAS of all cultivars revealed that varieties (C-518, Mexipak, Kohinoor-83, Faisalabad-83, Zardana-93, Shahkar-95, Moomal-2002, Wattan-94, Pasban-90, Kiran-95, Haider-2000) possessed the desirable marker. Same varieties showed susceptibility at seedling stage and moderate resistance to resistance (MRR) at adult stage under field conditions with either presence or absence of LTN as a morphological marker. The slow-rusting gene, Yr18, can be utilized in combination with other slow-rusting genes to develop high levels of durable adult plant resistance (APR) to stripe rust in wheat.

Keywords: Yellow rust, Adult Plant Resistance (APR), csLV34, Marker assisted selection (MAS)

1 Introduction

Wheat *(Triticum aestivum* L.em.Thell) is chief among the cereals (wheat, maize, rice), being cultivated worldwide due to having adaptability to
altered climatic conditions. Wheat covers an area, globally, of 215 million ha, 44% (62 million ha) of which is grown in Asian countries like China, India and Pakistan (1). The current wheat production in South Asia is around 95 million tones and demand for 2020 is estimated to be around 137 million tones. Among the most important diseases in wheat that significantly reduce wheat production are those caused by the rusts (leaf, stem, and stripe). Stripe rust caused by *Puccinia striiformis* West, is most likely the most widely distributed, and infect both spring and winter wheat cultivars across diverse cultivated regions in the world (2–5). Various methods have been used to control the stripe (yellow) rust of wheat worldwide which are; plant resistance, use of fungicides and cultural control.

The most efficient, cost-effective and environment-friendly approach to prevent the losses caused by rust epidemics is the development of genetic resistance to biotic stress. The use of cultivars with single-gene resistance (race-specific resistance) permits the selection of mutations at a single locus to render the resistance effective in a relatively short time. However, due to loss of variation and selection pressure, and evolution, new virulent races of the fungus appear which increase the need to develop durable resistance. Hence, the use of combinations of resistance genes has been suggested as the best method for genetic control of stripe and other rusts. This can be achieved by pyramiding effective resistance genes, but expression of individual resistance genes is difficult to monitor in the field. Conversely, a group of race-nonspecific resistance

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mechanisms has been described in wheat which is mainly polygenic. This has often been described as slow rusting (6), adult plant resistance and partial resistance (5,7) and is known to be long-lasting and more durable (8,9).

A more durable resistance to stripe rusts involves slow rusting (10,11) which is defined as a form of partial resistance in which host genotypes delay rust development (12) through different mechanisms (13). In many cereal-rust patho-systems, the quantitative aspects of cultivar resistance have been described and estimated by means of disease severity at a certain crop development stage, the area under disease progress curve (AUDPC) and Coefficient of Infection (CI) values for adult plant resistance (14–16). Two loci, Lr34/Yr18 complex on chromosome 7DS (17) and the Lr46/Yr29 complex on 1BL (18,19), express resistance to both leaf (brown) (P. triticina) and yellow rusts. The Lr34/Yr18 locus, in particular, is of immense importance since it has contributed to durable resistance against the two rust pathogens. Both types of resistance have always been inherited jointly and reported to be either pleiotropic or completely linked (2,18,19). This gene pair is reported to co-segregate with other traits such as leaf tip necrosis, powdery mildew (designated as Pm38), and tolerance to Barley yellow dwarf virus (Bdv1) (20,21).This multi-pathogen protection provided by Yr18/Lr34 locus has made it one of the most valuable genetic regions for disease resistance wheat breeding.

However, information on slow rusting resistance conferred by Yr18/Lr34 complex in Pakistani wheat was not available. Therefore, this study is conducted to characterize 30 Pakistani wheat cultivars for slow rusting resistance at both phenotypic and genotypic levels and to test the efficiency of different epidemiological parameters in selecting slow rusting genotypes.

2 Materials and Methods

2.1 Plant Growth

Cereal Crops Research Institute (CCRI) Pir Sabaq, (Noshehra KPK, Pakistan) was selected as an experimental site because yellow rust come to occurs more severe over there every year (22) and over-summering is frequent in the region. Therefore, this location is known as hotspot for the yellow rust (23). Being situated at the gateway of the new rust races entering from neighboring, this area has got geographic distinctiveness (Coordinates: 34°0'55N 71°58'29E) in Pakistan. A set of thirty selected Pakistani wheat varieties were grown at CCRI along with a susceptible control Morocco as shown in Table. 1. Each variety was planted in 2m long row with 30 cm distance between the rows. Mixture of Morocco was sown around the trial as a spreader row to make possible development of rust epidemic.

2.2 Disease Testing

Data for disease severity and infection types were assessed in the field at least thrice during adult plant stages: firstly, when the first spikelet was visible, then at the peak period of disease development when the susceptible control Morocco reached a disease severity of 100S and lastly at maturity. The severity was recorded as percent of the rust infection on the plants according to the modified Cobb scale (24) incorporating both percent leaf area affected and the host response (Table 2).

2.3 DNA extraction, quantification and amplification

DNA was extracted from 10 days-old seedlings of 19 cultivars (only MRMS cultivars) by the CTAB method (25). Fresh leaves were cut from the plants and placed in 1.5 ml eppendorf tubes. The tubes were subsequently dropped in liquid Nitrogen for rapidly freezing the leaf material. The plant material was then crushed with a micro pestle while inside the tube by adding 1 ml of preheated (65 ºC) 2X CTAB solution. The homogenized leaf tissues were transferred to two 1.5 ml eppendorf tubes and were incubated in a water bath at 65 ºC for 30 min. 0.5 ml of chloroform and isoamyl-alcohol (24:1) were added and were inverted vertically 5-10 times, followed by centrifugation at 10,000 rpm for 10 minutes. After centrifugation, supernatant was transferred to fresh tubes and 0.6 volume of 3M sodium acetate was added. Then 500µl cold isopropanol was added and mixed properly by inverting the tubes a few minutes. The DNA was pelleted and washed with 70% cold ethanol. The pellet was air dried and re-suspended in 40µl 0.1X TE buffer. (Purified DNA samples were stored at -20°C for further use).

Purity of the dissolved DNA in the samples was analyzed by the checking the absorbance ratios at 260/280 nm on spectrophotometer while concentration was calculated assuming that 1 O.D. (optical density) at 260 nm corresponds to 50ng/µl of DNA. Screening of twenty wheat varieties was carried out using STS marker (csLV34) linked to stripe rust resistance gene Yr18 (Gene link, NY USA (Table 3).The 10µl reaction mixture consisted of 60-70ng of template DNA, 1.0 µl Mg-free 10 X PCR Buffer (Fermentas), 0.6µl (5unit/µl) of Taq DNA polymerase (Fermentas), 25 mM of $MgCl_2$, 2.5 mM dNTPs (Sigma Chemical Co., St. Louis, MO) and 30ng of a single primer synthesized by Gene link (NY, USA). After 5 min of denaturation at 94°C,

amplifications were programmed for 40 consecutive cycles each consisting of 1 min at 94°C, 1 min at 55°C annealing, 2 min at 72°C followed by a 7 min extension step at 72°C. A total of nineteen varieties were studied for the identification of stripe rust resistance genes using STS markers reported to be linked with stripe rust resistance genes. Morocco susceptible was used as negative control while Chinese spring with known gene, *Yr18* for stripe rust resistance were used as a positive check. The sequence of STS marker Yr18 is shown in Table 3.

2.4 Analysis of data at adult plant stage

The disease severity data were used to calculate the area under the disease progress curve (AUDPC) using a computer program developed at CIMMYT. The relative percentage of area under the disease progress curve for each entry was calculated by setting AUDPC of Morocco as 100% (5).

3 RESULTS

3.1 Evaluation of wheat germplasm for stripe (yellow) rust resistance at adult stage under natural conditions in the field

Thirty selected Pakistani wheat genotypes were evaluated for stripe rust resistance at the adult plant stage after natural infection in Pir Sabaq (Nowshera KPK, Pakistan). Disease observations were recorded as reaction types of stripe rust and its severity on affected leaves of the diseased plants.

3.1.1 Reaction type & Severity

Based on reaction type (26) and severity measured in percentages (27); of the thirty wheat varieties, 3 (10%) of the genotypes (ZA-77, Soghat-90, Rawal-87) were resistant (with infection type (IT) R, severity 1-10%), 19 (63.34%) cultivars (C-518, mexipak, chenab-70, kohinoor-83, faisalabad-83, rohtas-90, bakhtawar-93, zardana-93, kaghan-93, shahkar-95, moomal-2002, punjab-96, bahawalpur-95, haider-2000, pasban-90, sarsabz, anmol, wattan-94 and kiran-95 and Pasban-90) were intermediates or moderately resistant to moderately susceptible (with IT MRMS, severity 11-20%), 3(10%) genotypes (Suleman-96, Kohsar-95 and Khybar-87) were moderately susceptible to susceptible (with IT MS-S, severity 21-30%) and 4 (13.34%) of genotypes (INQ-91, Kohistan-97, Punjnad-88 and Morocco) were susceptible (with IT S, severity 31-100%) (26) as shown in Figure 1 and Figure 2.

3.1.2 Coefficient of Infection values (CI)

Based on derived Coefficient of Infection values, Cultivars with CI values of 0-10, 11-20 and above 20 were regarded as possessing high, moderate and low levels of adult plant resistance, respectively. Results revealed that 3 (4%) cultivars (ZA-77, SOGHAT-90 and RAWAL-87) showed high level of adult plant resistance with coefficient of infection values between 0-10, 19 (70%) cultivars (C-518, Mexipak, Chenab-70, Kohinoor-83, Faisalabad-83, Inq-91, Rohtas-90, Soghat-90, Bakhtawar-93, Zardana-93, Kaghan-93, Shahkar-95, Suleman-96, Kohistan-97, Moomal-2002, Punjab-96, Bahawalpur-95, Kohsar-95, Haider-2000) displayed moderate level of adult plant resistance and coefficient of infection ranged between 11-20. Low level of adult plant resistance shown by remaining 8 (30%) cultivars (Suleman-96, Kohsar-95, Khybar-87, Pirsabak-2004, Kiran-95, Khybar-87, Sarsabz and Punjnad-88) with CI values above 20 as shown in Figure 3.

3.1.3 rAUDPC value

Based on rAUDPC values (26), Cultivars were categorized into three distinct groups. The first group included genotypes exhibiting rAUDPC values up to 10% of check were considered resistant. While genotypes showing rAUDPC values 11 to 20% of check were placed in second group of moderately resistant to moderately susceptible Cultivars. Third group comprised of genotypes with rAUDPC values above 20% of the check were rated as susceptible. Results revealed that, 3(%) Cultivars (ZA-77, Soghat-90 and Rawal-87) were resistant (Raudpc 1-10%). While 12(%) cultivars (C-518, Mexipak, Chenab-70, Faisalabad-83, INQ-91, Rohtas-90, Soghat-90, Bakhtawar-93, Zardana-93, Shahkar-95, Suliman-96, Kohistan-97, Punjab-96, Bahawalpur-95, Kohsar-95, Moomal-2002, Kiran-95, Sarsabz, Rawal-87 and wattan-94) were moderately resistant to moderately susceptible (rAUDPC 11-20%) . Remaining three Cultivars (Punjab-96, Bahawalpur-95, Kohsar-95) were susceptible (rAUDPC>20%) as shown in Figure.4.

3.1.4 Leaf Tip Necrosis (LTN)

Leaf tip necrosis, a morphological marker (28) was recorded in wheat varieties after heading stage under field conditions. In present study, of 30 selected Pakistani wheat varieties, 18 Cultivars (C-518, Mexipak, Chenab-70, ZA-77, Kohinoor-83, Soghat-90, Kohsar-95, Haider-2000, Pirsabak-2004, Kiran-95, Khybar-87, Sarsabz, Punjnad-88, Rawal-87, Anmol, Wattan-94 Pasban-90 and Morocco) lacked LTN phenotype. While remaining 12 genotypes (Bahawalpur-95,

Bakhtawar-93, Zardana-93, Suleman-96, Punjab-96, Faisalabad-83, Inq-91 and Rohtas-90) expressed LTN as shown in Figure 5.

3.2 Molecular Characterization of germplasm using STS marker linked to Yr18/Lr34

Out of 30 wheat varieties evaluated against stripe rust under field conditions, a sub set of 20 Cultivars with reaction types MRMS, were selected for molecular Characterization. STS marker (csLV34F/csLV34R) was used for a specific gene Yr18 in Pakistani selected wheat germplasms. The STS marker showed polymorphism for Yr18 in wheat genotypes. Results revealed that STS marker csLV34 amplified two alleles, a band of 150 bp size that has been reported to be tightly linked with resistant gene Yr-18 and another fragment of 230 bp size, not associated with resistance shown in Table 5. Cultivars; C-518, Mexipak, Kohinoor-83, Zardana-93, Shahkar-95, Moomal-2002, Haider-2000, Kiran-95, Wattan-94 and Pasban-90 showed amplified band of 150bp and the remaining 10 cultivars such as Chenab-70, Rohtas-90, Bakhtawar-93, Kaghan-93, Punjab-96, Bahawalpur-95, Sarsabz and Anmol amplified a fragment of 230 bp shown in Figure 6.

4 Conclusion

Seedling and field testing revealed that majority of (total 22) cultivars (C-518, Mexipak, Chenab-70, Faisalabad-83, INQ-91, Rohtas-90, Soghat-90, Bakhtawar-93, Zardana-93, Shahkar-95, Suliman-96, Kohistan-97, Punjab-96, Bahawalpur-95, Kohsar-95, Moomal-2002, Kiran-95, Sarsabz, Rawal-87 and Wattan-94) had higher reaction (susceptibility) at seedling stage and were classified as moderately resistant to moderately susceptible at adult plant stage with low rAUDPC (15.2-27.8%), CI reaction type and severity (11–20%). Cultivars carrying slow rusting display high infection type in the seedling and lower rAUDPC at adult stage may have race-nonspecific resistance. Similarly, cultivars with CI values of 0-20, 21-40 and 41-60 at adult stage are regarded as possessing high, moderate and low levels of adult plant resistance, respectively. Based on the seedling and field data, 22 cultivars may have race nonspecific resistance for stripe rust. This type of resistance remains effective for longer time, even if pathogen changes its genotype, because durable resistance, such as slow rusting and High-Temperature Adult Plant resistance (HTAP), is controlled by more than one genes (at least 2-3).

Leaf Tip Necrosis (LTN), a morphological trait, shows complete linkage or pleiotropism in some cultivars with Yr18/Lr34 genes and could be used as a morphological marker to identify wheat lines carrying these genes (Singh 1992). In the present study its expression was observed positive for eight varieties (Faisalabad-83, INQ-91, Rohtas-90, Bahawalpur-95, Zardana-93, Suleman-96, Punjab-96, and Bakhtawar-93). Varieties with LTN also have been rated as moderately resistant based on phenotypic parameters of rAUDPC, CI and severity of stripe rust at adult stage. However, this parameter carries a few drawbacks. Its expression can be masked by genetic background and variable influences of environments. Shah et al. (2010) evaluated a set of Pakistani wheat cultivars at seedling and adult plant stage for resistance to stripe rust with selected Pak isolates of Puccinia striiformis f. sp. triticiand reported similar results. In our study some varieties have shown leaf tip necrosis but others not which shown durability or slow rusting under molecular marker csLV34, So LTN is not more perfect and trustworthy morphological marker as it can be easily encountered under different environmental stresses. For further validataion, molecular markerfor Yr18 has been used to determine the presence or absence of the gene in these cultivars. STS marker showed ponlymorphism for Yr18 in wheat genotypes. Of thirty, 11 genotypes (C-518, Mexipak, Kohinoor-83, Faisalabad-83, Zardana-93, Shahkar-95, Moomal-2002, Haider-2000, Pasban-90, Wattan-94 and Kiran-95) showed amplification of band of 150 bp reported to be linked with Yr18 (Lagudah et al., 2006). This indicates that these genotypes have gene Yr18. Based on STS marker data, Genetic relationship among 19 wheat genotypes was determined. UPGMA-based dendrogram grouped 19 genotypes into two clusters. 11 genotypes (C-518, Mexipak, Kohinoor-83, Faisalabad-83, Zardana-93, Shahkar-95, Moomal-2002, Haider-2000, Pasban-90, Wattan-94 and Kiran-95) formed a distinct and largest group with closely related genotypes showing amplification of 150 bp (linked to gene Yr18) in first cluster (A). Second cluster (B), with 8 genotypes with 230bp fragment formed the small group. Both these clusters were grouped together at similarity level of 80%.Tabassum et al.,(2010) carried out molecular characterization and reported similar clustering pattern of wheat genotypes based on STS marker data. Our results found similar to both Tabassum et al., (2010) and shah et al., (2010) when compared on the bases of Molecular marker, csLV34, and field data(29).

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List of abbreviations

Yr: Yellow rust
Lr: Leaf rust
STS: Sequence Tag Specific
LTN: Leaf Tip Necrosis
MAS: Marker Assisted Selection
APR: Adult Plant Resistance
AUDPC: Area under Disease Progress Curve
CCRI: Cereal Crops Research Institute

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S.No.	Varieties	Pedigrees
1	C-518	T9 x 8A

2	MEXIPAK	PJ62 'S'-GB-55
3	CHENAB-70	C271-WT(E) x SON64
4	ZA-77	NORTENO 67 X SIETE CROSS 11 30367-IM-IY-3M-0Y
5	KOHINOOR-83	(OREF1.158-FDL x MEXIFEN 'S'-TIBA632) COC
6	FAISALABAD-83	FURY x KAL-BB
7	INQ-91	WL711/CROW 'S'
8	ROHTAS-90	1N1A66/A/DISTT//1N166/3/GEN81
9	SOGHAT-90	PAVON/SODIUM 223DE
10	BAKHTAWAR-93	KAUZ'S'
11	ZARDANA-93	CNO S/8156 TOB 66 CNO6-PVN
12	KAGHAN-93	TEETER (SIB)/(SIB) JUNCO
13	SHAHKAR-95	WL711//F3.71/TRM
14	SULEMAN-96	F6.74/BUN//SIS/3/VEE#7
15	KOHISTAN-97	V-1562//CHRC 'S'/HORK / 3 / KUFRA-1 /4/CARP 'S'/BJY
16	MOOMAL-2002	VEE/TRAP#1//SOGHAT-90
17	PUNJAB-96	INIA/SON64-P.4160(E)XSON64
18	BAHAWALPUR-95	CNO 'S'-LR4 X SON 64/SON (AMBER)
19	KOHSAR-95	PSN 'S' /BOW 'S'
20	HAIDER-2000	CHIL/WUH3
21	PIRSABAK-2004	KAUZ/STAR
22	KIRAN-95	WL711 NaN3 (MUTANT 7/81) X CROW 'S'
23	KHYBAR-87	LIRA'S'
24	SARSABZ	PI-FROND/PI-MAZOE
25	PUNJNAD-88	K.4500*2/BJY
26	RAWAL-87	MAYA-MONCHO 'S'/KVZ-TRM
27	ANMOL	LIRA 'S'
28	WATTAN-94	*
29	PASBAN-90	INIA66/A.DISTT//INIA66/3/GEN81
30	MOROCCO	*

* Pedigree not known.

Table.1. Names and Pedigrees of Pakistani wheat varieties selected in our experiment.

Reaction/response	Observation
No disease	O
Resistant	R
Moderately Resistant- Resistant	MRR
Moderately resistant	MR
Moderately resistant-Moderately Susceptible	MRMS
Moderately Susceptible	MS
Moderately Susceptible-Susceptible	MSS
Susceptible	S

Table.2. Infection scale used for severity of stripe rust development at adult plant stage.

Name of STS marker	Sequance (5'-3')	Gene	Size (bp)	Reference
csLV34F	GTTGGTTAAGACTGGTGATGG	Yr18	150	Lagudah et al., 2006
csLV34R	TGCTTGCTATTGCTGAATAGT			

Table.3. Sequences (F/R) of STS marker used for yellow rust resistance gene Yr18.

S.No	Varieties	APR	Mean coefficient of infection	Mean of AUDPC	Mean of rAUDPC
1	C-518	MRMS	12.5fgh	36.25efg	25.84efg
2	MEXIPAK	MRMS	16.67fgh	41.09ef	29.23def
3	CHENAB-70	MRMS	17.5fg	41.73ef	29.78def
4	ZA-77	R	1.83gh	13.78g	9.81fg
5	KOHINOOR-83	MRMS	20f	44.63ef	31.84de
6	FAISALABAD-83	MRMS	10.83fgh	33.81fg	24.11efg
7	INQ-91	S	93.33c	89.05c	63.50c
8	ROHTAS-90	MRMS	15.83fgh	39.31ef	28.05def
9	SOGHAT-90	R	1.33gh	15.39g	10.93fg
10	BAKHTAWAR-93	MRMS	18.33f	42.86ef	30.53de
11	ZARDANA-93	MRMS	12.5fgh	36.25fg	25.84efg
12	KAGHAN-93	MRMS	16.67fgh	41.32ef	29.48def
13	SHAHKAR-95	MRMS	17.5fg	41.73ef	29.79def
14	SULEMAN-96	MS	41.67e	61.29de	43.71d
15	KOHISTAN-97	S	153.33b	110.45b	78.78b
16	MOOMAL-2002	MRMS	15fgh	39.68ef	28.25def
17	PUNJAB-96	MRMS	15.5fgh	39.91ef	28.48def
18	BAHAWALPUR-95	MRMS	12.5fgh	36.25fg	25.84efg
19	KOHSAR-95	MS	58.33d	70.63d	50.38d
20	Haider-2000	MRMS	20f	44.63ef	31.83de
21	PIRSABAK-2004	0	0h	9.89	7.05g
22	KIRAN-95	MRMS	18.33f	42.22ef	30.10de
23	KHYBAR-87	MS	46.67e	63.37de	45.18d
24	SARSABZ	MRMS	10.83fgh	33.83fg	24.10efg
25	PUNJNAD-88	S	93.33c	89.45c	63.50c
26	RAWAL-87	R	1.833gh	16.82g	11.66fg
27	ANMOL	MRMS	18.33f	42.22ef	30.10de
28	WATTAN-94	MRMS	58.33d	70.63d	50.38d
29	PASBAN-90	MRMS	13.33fgh	35.98fg	25.65efg
30	MOROCCO	S	233.33a	140.36a	100.03a

Table.4. Adult Plant Infection Type and mean comparisons for coefficient of infection, AUDPC and rAUDPC in selected Pakistani wheat genotypes to yellow rust. (Mean comparison based on Duncan multiple testing*) *Means followed by the same letters in each column are not statistically significant at $p<0.05$.

S.No	Varieties	Yr-18 (150 bp)	LTN
1	C-518	Present	Absent

2	MEXIPAK	Present	Absent
3	CHENAB-70	Absent	Absent
4	ZA-77	NC	Absent
5	KOHINOOR-83	Present	Absent
6	FAISALABAD-83	Present	Present
7	INQ-91	NC	Present
8	ROHTAS-90	Absent	Present
9	SOGHAT-90	NC	Absent
10	BAKHTAWAR-93	Absent	Present
11	ZARDANA-93	Present	Present
12	KAGHAN-93	Absent	Absent
13	SHAHKAR-95	Present	Absent
14	SULEMAN-96	NC	Present
15	KOHISTAN-97	NC	Absent
16	MOOMAL-2002	Present	Absent
17	PUNJAB-96	Absent	Present
18	BAHAWALPUR-95	Absent	Present
19	KOHSAR-95	NC	Absent
20	Haider-2000	Present	Absent
21	PIRSABAK-2004	NC	Absent
22	KIRAN-95	Present	Absent
23	KHYBAR-87	NC	Absent
24	SASABZ	Absent	Absent
25	PUNJNAD-88	NC	Absent
26	RAWAL-87	NC	Absent
27	ANMOL	Absent	Absent
28	WATTAN-94	Present	Absent
29	PASBAN-90	Present	Absent

30	MOROCCO	NC	Absent

NC= Not Characterized

Table 5. Evaluation of selected Pakistani wheat germplasm for stripe rust resistance by molecular marker and morphological marker.

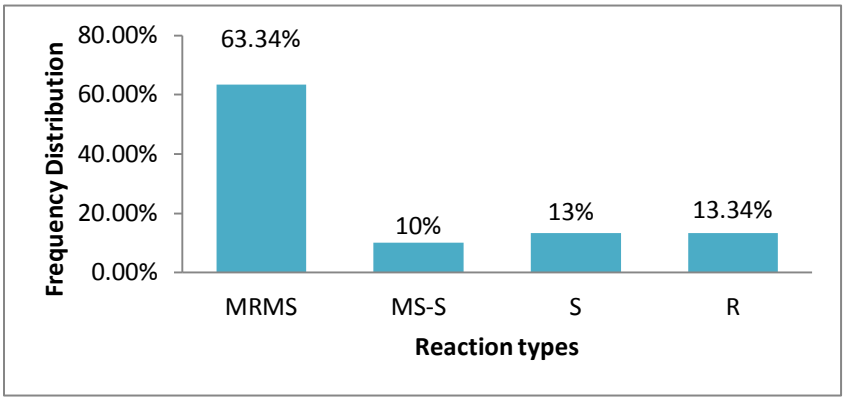

Figure 1. Frequency distributions of reaction types (Scored at the adult plant stage) in Pir Sabaq.

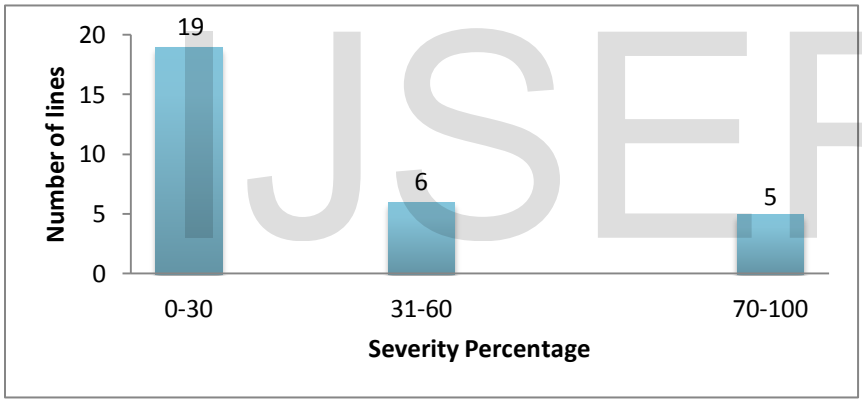

Figure 2. Frequency distributions of Severity %age (Scored at the adult plant stage) at Pir Sabaq.

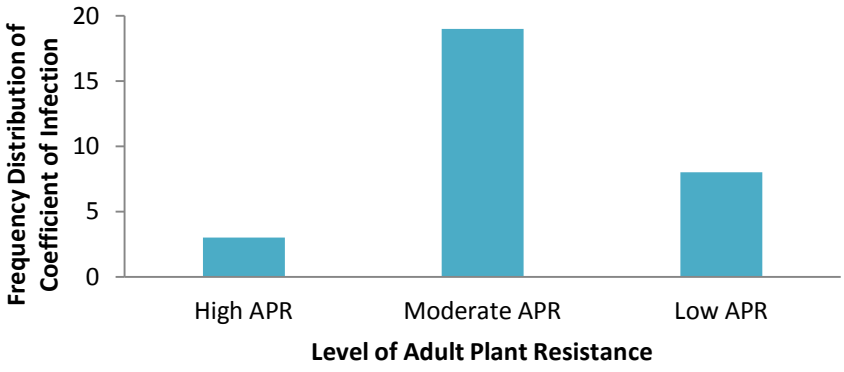

Figure.3. Frequency distribution of Coefficient of Infection (CI) against stripe rust data scored at adult plant stage, at Pir sabaq.

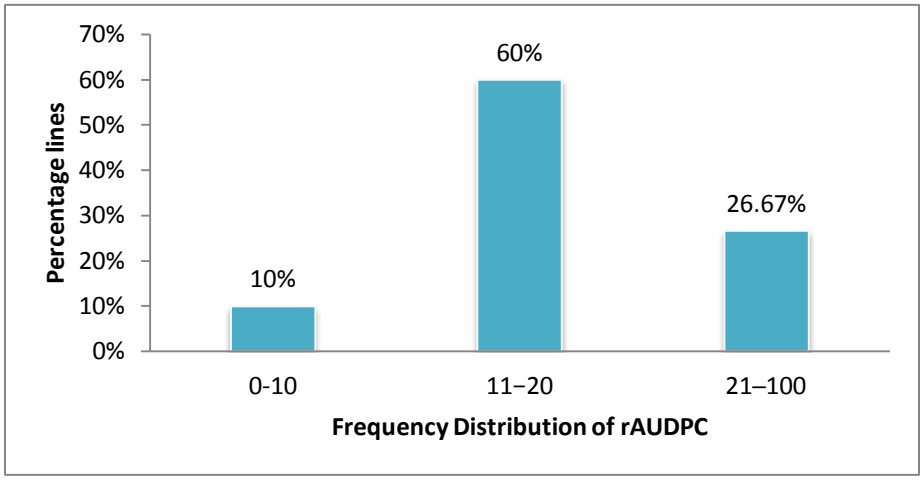

Figure.4. Frequency distributions of rAUDPC against stripe rust scored at the adult plant stage, at Pir sabaq.

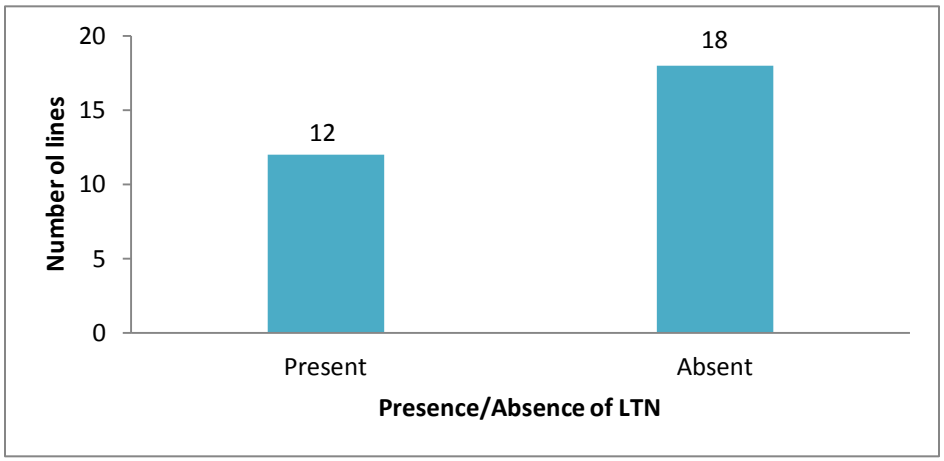

Figure 5. Frequency distributions LTN phenotype in wheat Cultivars scored at the adult plant stage, at Pir sabaq.

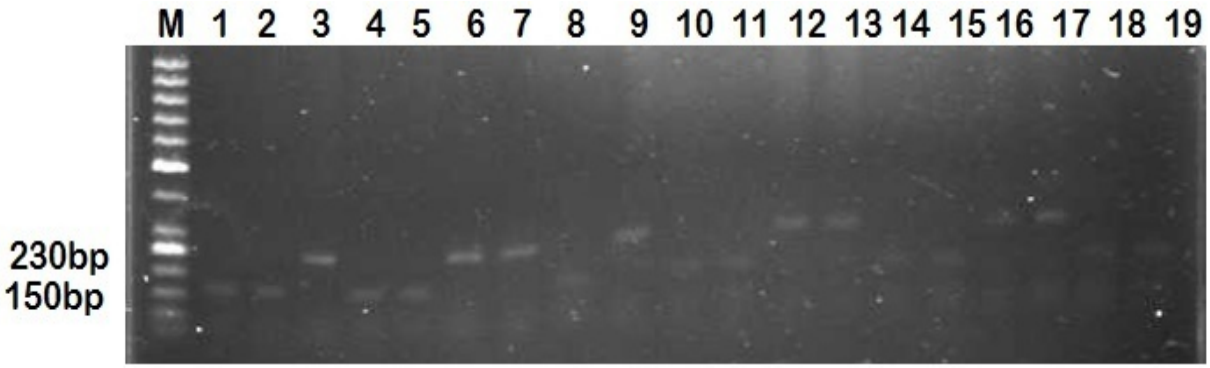

Figure.6 STS profiles obtained by Yr-18. A polymorphic band, present in all Pakistani selected varieties is indicated by the arrow. Products were separated on a 2% agarose gel. 1: Yr-18 source stock (Chinese spring), 2: Mexipak 3: Chenab-70, 4: Kohinoor-83, 5: Faisalabad-83, 6: Rohtas-90, 7: Bakhtawar-93, 8: Zardana-93, 9: Kaghan-93, 10: Shahkar-95, 11: Moomal-2002, 12: Punjab-96, 13: Bahawalpur-95, 14: Haider-2000, 15: Kiran-95, 16:Sarsabz, 17: Anmol, 18: Wattan-94, 19: Pasban-90.