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PHENOTYPIC CHARACTERIZATION OF WHEAT SELECTED LINES FOR RESISTANCE AGAINST LEAF STRIPE AND STEM RUST THROUGH PHENOTYPIC MARKERS APPLICATION

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ABSTRACT

Leaf, stripe and stem rust are considered one of the most important diseases of wheat in Pakistan. The use of rust resistant varieties is the most economical and environment friendly way to reduce losses due to rust diseases. For this purpose a study was planned to combine genes for enhancing rust resistance and high yield potential in wheat.The experiments for identification of durable rust resistance genes i.e. *Sr2/Yr30*, *Lr34/Yr18*, *Lr46/Yr29* on the basis of phenotypic markers like pseudo black chaff (Pbc) and leaf tip necrosis (Ltn1 & Ltn2) were conducted in augmented design during the years 2010-11 and 2011-12 at Wheat Research Institute, Faisalabad and Summar Agricultural Research Station, Kaghan. Total 750 head rows were selected from F6 generation and planted at SARS, Kaghan. The outstanding 345 lines were selected and planted for further evaluation and on the basis of rust reactions, phenotypic uniformity and high yield under the field conditions, 220 lines were selected for further testing. The results indicate that among 220 entries, 67 entries showed *Lr34/Yr18*, 23 entries showed *Lr46/Yr29* and 62 entries showed *Sr2/Yr30* genes combination. While in yield testing, among test entries, 99 entries were found to be high yielding ranging from 3973-4786 kg/ha. From the study, it is concluded that fourteen test entries i.e. V-11211,V-11212, V-11218, V-11227, V-11262, V-11288, V-11296, V-11304, V-11308, V-11319, V-11338, V-11353, V-11365 and V-11396 are the most prominent lines because these lines showed the combination of all three slow rusting genes as well as high yield potential. These selected genotypes will be very useful for the wheat breeders and pathologist of the country in planning of future hybridization program.

Key words: Breeding; resistance; slow rusting genes; phenotypic markers; *Triticum aestivum*; leaf and stripe rust.

INTRODUCTION

Wheat crop is hit by many biotic and a-biotic maladies which engendered to reduce its produce (Jellis, 2009). Leaf rusts also called as brown rust, stripe rust also called as yellow rust and stem rust also called as black rust, smuts, bunts and aphids as biotic stresses (Hussain *et al.*,2006) and terminal heat, drought, salinity, winds, hailstorms, fogs and excessive cloudy weather during crop season are the salient a-biotic stresses (Hussain *et al.*, 2011). Rusts are important worldwide due to the ability of rust pathogens to mutate and multiply rapidly, and to use their air-borne dispersal mechanism from one field to another and even over long distances.(Hussain *et al.*,2006) Rustsare currently the most important diseases of wheat worldwide, which threaten global food security (Hovmøller *et al.*, 2010). Major wheat growing areas of the world are facing repeated severe yellow rust epidemics since 2000, when two highly aggressive and high temperature tolerant *Pst* strains appeared (Hovmøller *et al.*, 2008). In Pakistan, rusts have been a constant threat to sustainable wheat production. The reason for the early collapse of varieties is linked to the evolution of new rust races, rendering resistance in the varieties ineffective based on major genes. The latest and current trend of genetic resistance in wheat is "the resistance based on the additive effects of minor genes accumulation" (Singh *et al.*, 1998). The durable resistance to leaf and stripe rusts of several cultivars is based on the slow rusting genes having additive effects (Singh *et al.*, 2005). The most efficient and economical management of wheat rusts is the evolution of rust resistant varieties and their on-farm cultivation (Chaudhary *et al.*, 1998; Kalappanavar *et al.*, 2008). In the present era of scientific advancement, the wheat research is focused to achieve durable rust resistance through incorporation of multiple minor genes or adult plant resistance genes (Singh *et al.*, 2005). The worst yellow rust epidemic in 2005 has wiped out almost all the commercial wheat varieties of Pakistan (Khan *et al.*, 2005). Yellow rust can reduce wheat yields by as much as 84% (Murray *et al.*, 1995). Rusted plots yielded 4% less crops, compared to fungicide-protected plots for cultivar with hypersensitive resistance (Singh and Rajaram, 1991). Yield and kernel weight average of rusted plots are 8% less for cultivars with partial resistance, but depending on cultivar, varied from 2 to 20% less. At present country is facing critical shortage of appropriate wheat varieties having both features of high yield and rust resistance to (Hussain *et al.,* 2006) leaf, yellow and stem rust. Now a days, pathologist and breeders have sought resistance mechanism based on minor genes which is called durable rust resistance (Singh *et al*., 2000). This type of rust resistance mechanism is more effective for many races rather than a single one and is long lasting (Hussain *et al*., 1999). A high level of resistance to yellow rust could be achieved by accumulating from 4 to 5 minor genes in a variety (Singh *et al*., 2005). However, moderate level of resistance can be achieved by accumulating 2-3 minor genes in a line (Singh *et al*., 2005). In spite of the absence of any effective major gene, the partial resistance of varieties indicated the presence of minor genes (Hussain *et al*., 2006). Parents having partial resistance are crossed to pyramid genes for rust resistance and yield. This resulted many wheat lines that were better in yield and disease resistance as compared to their parent (Hussain *et al*., 2007). This, in addition, will result in diversification of wheat genotypes in terms of their resistance background, necessary to avoid rapid evolution of the rust pathogen to acquire new virulence. Genetic resistance of leaf, yellow and stem rust resistant varieties is being considered the only remedy to prevent the crop from diseases as the long-term strategy. The objective of this study was to develop and screen wheat germplasm against leaf and yellow rust under natural and high stress inoculation conditions, to monitor the lines that may have minor gene based resistance and transfer of this resistance to the susceptible but high yielding varieties through conventional hybridization and phenotypic markers application for the development of wheat cultivars having minor gene based resistance.

MATERIALS AND METHODS

The research work was carried out at the Wheat Research Institute, Faisalabad during the year 2011-12, for the selection and hybridization of wheat breeding material.

Selection of breeding material on the basis of parental genes for durable rust resistance in wheat: The material used for the crossing was selected on the basis of higher grain yield among wheat lines from the gene pool of WRI.Faisalabad. The 6-10 year rust history of the lines was also considered for final selection of the parents. The lines with low terminal rust reactions up to 20MRMS in slow rusting response were selected to make desirable crossing combinations (Table 1)220 advance lines were selected from 750 lines for phenotypic characterization.

Table 1. Detail of parents utilized in the gene combining crosses

Sr. No.	Name of cultivar/ line	Characteristics			
		Leaf rust resistance status	Yellow rust resistance status	Acceptable yield kg/ha	Maximum yield kg/ha
1	Shafaq-06	Partially resistant	Partially resistant	4100	6400
2	Waxwing	Slow rusting	Susceptible	4000	6150
3	MH-97	Partially resistant	Partial resistant	4500	6750
4	Kiritati	Resistant	Resistant	4050	6050
5	Juchi-F2000	Resistant	Resistant	2450	3550
6	Khawaki	Resistant	Resistant	3900	4800
7	Dollarbird	Resistant	Susceptible	4100	6700
8	Kingbird	Slow rusting	Resistant	4000	6450
9	Yecora-73	Susceptible	Susceptible	3860	6125
10	Lasani-08	Resistant	Resistant	4100	6500
11	V03007	Resistant	Resistant	4050	6450
12	V04179	Resistant	Resistant	4250	6600
13	Seher-06	Susceptible	Resistant	4500	6800
14	V04178	Resistant	Resistant	4400	6750
15	V02192	Resistant	Resistant	4050	6200
16	*Lr19*/V02192	Resistant	Resistant	3300	4900
17	Iqbal-2000	Resistant	Resistant	3750	5500
18	FRT/SA42//PRL/SA42	Resistant	Resistant	3700	4800
19	Pfau/Seri.1B//Ammad/3/Wax wing	Resistant	Resistant	3800	4900
20	Wblli2*/Brambling	Resistant	Resistant	4250	6850
21	Wattan/2*ERA/2/pak-81/2*Wattan/3/Shafaq-06	Resistant	Resistant	4300	6950

Sr. No.	Name of cultivar/ line	Characteristics			
		Leaf rust resistance status	**Yellow rust resistance status**	**Acceptable yield kg/ha**	**Maximum yield kg/ha**
22	Wattan/Fsd-08	Resistant	Resistant	4150	7000
23	Pak-81 2*/Wattan//2*Shafaq-06	Resistant	Resistant	4100	6750
24	Kambi/2*Khawaki	Resistant	Resistant	4550	6041
25	Luan/Kohistan//Pak81	Resistant	Resistant	4000	6400
26	SH88/90A204//MH97	Resistant	Resistant	3950	6300
27	PRL/2*Pastor	Resistant	Resistant	3800	6450
28	Wattan/2*ERA	Resistant	Resistant	3500	5500
29	Shafaq-06/ Luan	Resistant	Resistant	4100	6300
30	Uqab-2000/ Wattan/*Lr28*//Yecora-70	Resistant	Resistant	3900	5300
31	SH88/ Pak-81// MH97	Resistant	Resistant	4200	6750
32	Wattan/ *Lr28*//Yecora	Resistant	Resistant	4000	6200

Data recording of leaf and yellow rust: Rusts data were recorded at 10 days interval. The rust severity and field response were recorded according to modified Cobb's scale described by Peterson *et al*. (1948). Severity was recorded on the basis of percentage of leaf area infected and field responses. Severity ratings were based upon visual observations recorded at 10 days intervals as Trace 5, 10, 20, 40, 60 and 100 percent infection, and field response as immune, resistant, moderately resistant, intermediate, moderately susceptible and susceptible by the scale given in (Table 2). At least three observations regarding rust severity were recorded before the physical maturity of the crop. Three hundred and forty five desirable lines were selected on the basis of rust reaction. The other parameters like Plant height, Grain yield assessment ha^{-1}, 1000 grain weight and protein percentage were also recorded.

Table 2. Disease rating scale used to record rust severity and level of resistance/susceptibility of wheat varieties

Field Response		Symptoms
0	Immune	No visible infection.
R	Resistant	Visible chlorosis or necrosis, no uredia are present.
MR	Moderately Resistant	Small uredia are present and surrounded by either chlorotic or necrotic areas.
M	Intermediate (Mixed)	Variable sized uredia are present some with chlorosis, necrosis or both
MS	Moderately susceptible	Medium sized uredia are present and possibly surrounded by some chlorotic areas.
S	Susceptible	Large uredia are present, generally with little or no chlorosis or necrosis.

(Peterson *et al.*, 1948).

The 345 lines selected from Kaghan Disease Screening Nursery along with Morocco as a rust spreader were planted at WRI, Faisalabad. The trial was sown by hand drill in augmented design having plot size 1.2m x 2.5m during 1st week of December, 2010-11 in order to evaluate their yield and rust resistance basis. The Morocco (used as spreader) was inoculated artificially using a hypodermic needle injection method (Rao *et al.*,1989) as well as spraying and dusting methods, twice in the month of January and February, until a heavy inoculum developed (Roelfs *et al.*,1992). During the growing period the applied rust inoculums consisted of mixture of leaf rust (TKTRN, TKTPR, KSR/JS, PGRTB and PHTTL) and Yellow rust (80E85) races collected from Faisalabad, Murree and Kaghan. This facilitated the development of epidemic rust conditions and led to reliable evaluation of rust resistance of wheat lines under study. Rust severity (percentage) and responses of plants were assessed for three consecutive observations with 10 days intervals when Morocco was about 40-50% rust intensity. The rust severity and field response were recorded according to modified Cobb's and Roelfs scale as mentioned in (Table 2). From 345 advance lines; two hundred and twenty lines were selected for high yield and rust resistance performance.

Phenotypic characterization and yield testing of 220 selected lines through markers applications: For phenotypic characterization of selected material for rust resistance was done through three phenotypic markers i.e. leaf tip necrosis-1 (Ltn-1), leaf tip necrosis-2 (Ltn-2) and pseudo black chaff. This research work was conducted at the Wheat Research Institute, Faisalabad, Ayub Agricultural Research Institute Faisalabad during the year 2011-12.

Identification of durable resistance genes through phenotypic markers: For identification of durable rust resistant genes on the basis of phenotypic expressions on adult plant stage i.e. pseudo black chaff for *Sr2*/*Yr30*, leaf tip necrosis (Ltn-1) for *Lr34*/*Yr18*. Leaf tip necrosis are closely associated with this gene (Singh, 1992) and is being used as its phenotypic marker and leaf tip necrosis (Ltn-2) for *Lr46*/*Yr29*.The planted material comprises of 220 selected lines along with Morocco as a rust spreader around each plot were planted at WRI, Faisalabad as mentioned earlier. The rust data for both types i.e. *LR* and *YR* were recorded keeping in view the phenotypic expressions. The genotypes showing symptoms of pseudo black chaff (Fig.1) and leaf tip necrosis (Ltn-1) for *Lr34*/*Yr18* as well as leaf tip necrosis (Ltn-2) for *Lr46*/*Yr29*

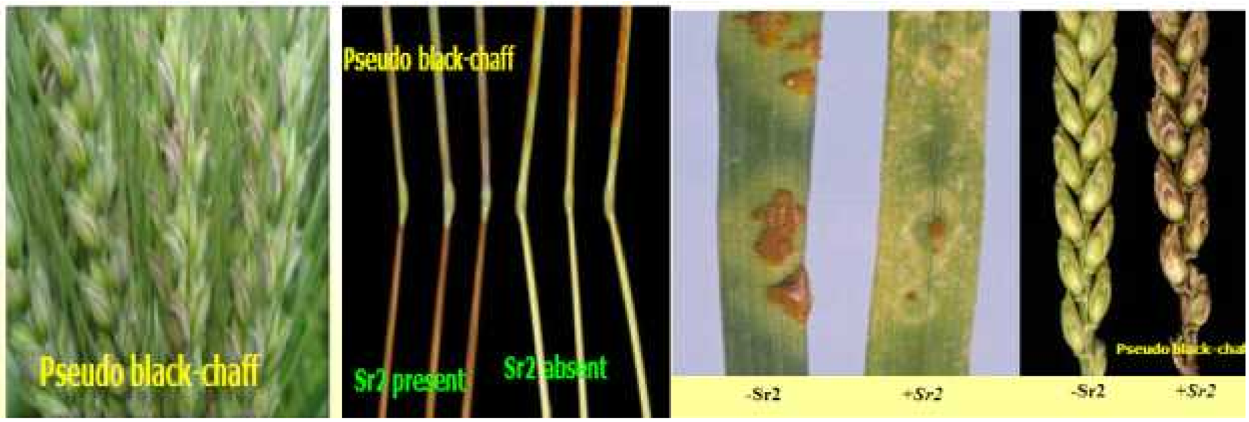

Fig. 1. Pseudo-black chaff morphological markers for *Sr*2/*Yr*30

Fig. 2. Leaf tip necrosis (Ltn-1) for *Lr34*/*Yr*18

Fig. 3. Leaf tip necrosis (Ltn-2) for *Lr46*/*Yr29* + *Lr*46 −*Lr46*

RESULTS

The present study was planned to achieve durable rust resistance through combining designated slow rusting genes with high yield potential of wheat genotypes. The material was selected from 750 heads rows of 25 crosses planted at Kaghan during June, 2010, only 345 wheat lines were selected on the basis of rust reactions and grain characteristics as shown in (Table 3).

Phenotypic characterization and yield testing of 220 selected lines through markers applications: The outstanding 345 lines were evaluated for their rust response on the basis of rust reactions, phenotypic uniformity and high yield under the field conditions at experimental area of Wheat Research Institute, Faisalabad 220 lines were selected. In further testing, it was found that among 220 entries under yield, 99 entries were found to be high yielding ranging from 3973-4786 kg/ha, 71 entries showed height ranging from 100 to 120 cm, 34 entries showed higher 1000 grain weight ranging from 42 to 47 grams and 50 entries had higher protein % which ranged from 12-14 percent and their rust response on the basis of AUDPC as compared to check varieties. These genotypes would be a good source for future wheat hybridization program in the country to achieve higher yield and high resistance. Such type of work has been reported by research workers (Singh *et al*., 1991; McIntosh, 1992). Among tested entries, 67 entries showed *Lr34/Yr18*, 23 entries showed *Lr46/Yr29* and 62 entries showed *Sr2/Yr30* the presence. The results for identification of durable rust resistant genes were inferred on the basis of phenotypic expressions (Table 4).

Phenotypic characterization of 220 selected lines through markers applications: Among tested entries, 67 entries showed leaf tip necrosis-1 (Ltn-1), 23 entries showed leaf tip necrosis-2 (Ltn-2) and 62 entries showed pseudo black chaff (Table 4).

Table 3. Selection of single headlines from F_6 generation crosses

Detail of Selected entries from 750 progeny lines of 25 crosses planted at Kaghan during 2010			
Sr. No	Name of the cross	Tested entries	Selected entries
1	FRT/SA42//PRL/SA42/4/Pfau/SERI.1B//Ammad/3/Waxwing	20	12
2	FRT/SA42//PRL/SA42/3/Wblli2*/Brambling	22	13
3	FRT/SA42//PRL/SA42/3/Kiritati	24	16
4	Wattan/2*ERA/2/Pak-81/2*Wattan/3/Shafaq-06/4/Brambling	37	24
5	Wattan/2*ERA/2/Pak-81/2* Wattan /3/Shafaq-06/3/Wblli/ Brambling	21	12
6	Wattan /Fsd-08//Kiritati	29	12
7	Pak-81 2*/Wattan//2*Shafaq-06/3/Kiritati	35	16
8	Pak-81 2*/Wattan//2*Shafaq-06/3/Juchi F2000	21	7
9	Pak-81 2*/Wattan//2*Shafaq-06/3/Dollarbird	24	9
10	Pak-81 2*/Wattan//2*Shafaq-06/3/Kambi/2*Khawaki	23	6
11	Luan/Kohistan/Pak81/3/Kiritati	42	24
12	Wattan/2*ERA/2/Pak-81/2*Wattan/3/Shafaq-06/4/Kiritati	26	14
13	Wattan/2*ERA/2/Pak-81/2*Wattan/3/Shafaq-06/4/Kingbird	22	6
14	SH88/90A204//MH-97/3//PRL/2*Pastor	20	9
15	Wattan/2*ERA//Lasani-08	22	12
16	Shafaq-06/ Luan// MH-97	34	19
17	Uqab-2000/ Wattan/*Lr28*//Yecora-70	37	22
18	SH88/ Pak-81// MH97/3/ Shafaq-06	27	16
19	Wattan/ 2*ERA// V04178	35	17
20	Wattan/ 2*ERA// V03007	42	14
21	Wattan/ 2*ERA// V04179	25	6
22	Wattan/ 2*ERA// Wattan/ *Lr28*//Yecora-70	28	6
23	Lasani-08/Seher-06	38	10
24	Lasani-08/Iqbal-2000	27	14
25	*Lr19*/V02192// Shafaq-06	56	27
	Total	750	345

From the study, it was concluded that out of 220 genotypes, only nine genotypes V-11211,V-11227, V-11288, V-11296, 11304, V-11308, V-11319, V-11353 and V-11396 showed the combination of three designated slow rusting genes, along with high yield , 1000-grain weight, protein % and plant height ranging from (100 to120cm). This is very important combination, as it provides protection against all three types of rusts (*LR*, *YR* and *SR*), while 15 genotypes including V-11203, V11212, V-11218, V-11223, V-11245, V-11248, V-11250, V-11262, V-11267, V-11289, V-11321, V-11232, V-11338, V-11365 and V-11359 showed the combination of *Sr*2/*Yr*30 and *Lr34*/*Yr18*. Similarly, the combination of *Sr2/Yr30* and *Lr46*/*Yr29* was found in 2 genotypes including V-11190, V-11193 and the combination of *Lr46*/*Yr29* and *Lr34*/*Yr18* was found in 6 genotypes including V-11276, V-11247, V-11313, V-11345, V-11376 & V-11380 (Table 5).

Table 4.Genotypes showing slow rusting linkage.

Genotypes		Phenotypic Markers			Units of AUDPC ranging	
V-Codes	**Total**	***Lr34*/*Yr18* (Ltn-1)**	***Lr46*/*Yr29* (Ltn-2)**	***Sr2*/*Yr30* (Pbc)**	***Lr***	***Yr***
V-11195, V-11196, V11211, V11222, V-11227, V-11230, V-11231,V-11288, V-11296, 11304, V-11308, V-11319 V-11353&V-11396	14	+	+	+	0-200	0-175
V-11194, V11198, V11200, V-11203, V11207 ,V-11208,V11209, V11212, V11215, V11216, V-11218, V-11219, V-11221,V-11223,V11224, V11225, V-11226, V11228,V-11229,V-11232,V-11244, V -11245, V-11248, V-11250, V-11262, V-11263, V-11267,V-11270,V-11280,V11282, V-11289,V-11307, V-11321,V-11328, V-11232,V-11329, V-11333, V-11337,V-11338,V-11340,V-11356,V-11359,V-11367,V-11375, V-11390, & V-11392	46	+	-	+	0-325	0-225
V-11190 & V-11193	2	-	+	+	25-100	25-200
V-11276, V-11247,V-11290,V-11313, V-11345,V-11376 & V-11380	7	+	+	-	0-325	0-275

Table 5. Elite lines with combination of three designated slow rusting/durable resistant genes, high in yield, grain weight, proteins percentage and height

Sr. No	Selection	V-Code	*Lr34*/*Yr18* (Ltn-1)	*Lr46*/*Yr29* (Ltn-2)	*Sr2*/*Yr30* (Pbc)	Kg/ha	1000-grain wt	height in cm	Protein %
1	119	11308	+	+	+	4786	44	109	10
2	23	11212	+	-	+	4780	45	119	10
3	73	11262	+	-	+	4749	34	113	10
4	130	11319	+	+	+	4737	47	115	11
5	115	11304	+	+	+	4663	42	114	10
6	78	11267	+	-	+	4577	37	117	10
7	31	11220	-	-	+	4035	39	113	11
8	38	11227	+	+	+	4558	34	118	13
9	34	11223	+	-	+	4558	37	119	10
10	100	11289	+	-	+	4552	36	124	10
11	176	11365	+	-	+	4550	41	112	10
12	61	11250	+	-	+	4539	38	110	11
13	149	11338	+	-	+	4517	35	119	12
14	107	11296	+	+	+	4515	33	107	10
15	56	11245	+	-	+	4502	40	120	12
16	59	11248	+	-	+	4490	33	115	12
17	207	11396	+	+	+	4488	41	118	11
18	29	11218	+	-	+	4484	37	120	12
19	164	11353	+	+	+	4467	34	110	11
20	58	11247	+	+	-	4465	36	114	10
21	99	11288	+	+	+	4453	37	124	12
22	132	11321	+	-	+	4416	40	137	12
23	211	11400	-	-	-	4414	37	129	12
24	131	11320	+	-	-	4391	37	122	12

25	32	11221	+	-	+	4360	44	113	10
26	43	11232	+	-	+	4360	41	136	12
27	87	11276	+	+	-	4317	46	118	11
28	140	11329	+	-	+	4270	35	120	13
29	170	11359	+	-	+	4270	38	111	13
30	14	11203	+	-	+	4262	38	107	12
31	136	11325	+	-	-	4245	43	119	11
32	201	11390	+	-	+	4241	41	109	10
33	2	11191	+	-	+	4237	33	133	14
34	124	11313	+	+	-	4231	47	129	11
35	37	11226	+	-	+	4225	44	120	12
36	70	11259	-	-	+	4218	33	103	9
37	218	11407	-	+	-	4216	36	101	11
38	28	11217	+	-	+	4212	46	106	9
39	166	11355	-	-	-	4208	43	112	9
40	90	11279	-	+	-	4194	45	120	12
41	93	11282	+	-	+	4194	45	120	12
42	4	11193	-	+	+	4175	31	110	12
43	220	11409	-	-	-	4167	30	108	10
44	167	11356	+	-	+	4159	41	122	12
45	72	11261	-	-	+	4157	34	109	9
46	88	11277	-	-	-	4157	36	110	12
47	186	11375	+	-	+	4118	42	104	12
48	156	11345	+	+	-	4085	40	110	11
49	163	11352	-	-	-	4085	44	100	10
50	135	11324	+	-	-	4072	36	117	12
51	96	11285	-	-	-	4070	39	121	13
52	184	11373	-	-	-	4068	42	112	13
53	122	11311	-	-	-	4058	42	121	11
54	92	11281	-	-	+	4046	44	137	11
55	191	11380	+	+	-	4031	38	114	10
56	1	11190	-	+	+	4027	42	113	12
57	161	11350	-	+	-	4023	42	113	11
58	169	11358	-		-	4023	36	102	10
59	187	11376	+	+	-	4019	38	102	11
60	152	11341	-	+	-	3986	43	111	10
61	39	11228	+	-	+	4311	36	116	10
62	139	11328	+	-	+	4295	35	19	10
63	140	11329	+	-	+	4270	34	120	13
64	22	11211	+	+	+	4484	37	120	11

+ Sign shows the presence of rust resistance genes in wheat genotypes while - Sign shows absence of rust resistance genes in wheat genotypes.

DISCUSSION

The current study was conducted to characterize selected wheat genotypes of wheat research Institute, AARI, Faisalabad, Pakistan for combining rust resistance genes with high yield potential. The characterization of current study indicates that among the tested genotypes, the advance lines that are V-11211, V-11212, V-11218, V-11227, V-11262, V-11288, V-11296, V-11304, V-11308, V-11319, V-11338, V-11353, V-11365 and V-11396 showed the combination of three designated slow rusting genes. *Sr2/Yr30*, *Lr46/Yr29* and *Lr34/Yr18*, along with high yield, 1000 grain weight, protein % and plant height (ranging from 100 to 120cm) carried resistance near immunity under the severe leaf and yellow rust severity conditions. A high level of resistance to yellow rust could be achieved by accumulating from 4 to 5 minor genes in a variety (Singh *et al*., 2005). However, moderate level of resistance can be achieved by accumulating 2-3 minor genes in a line (Singh *et al*., 2005). These lines may be a valuable source of rust resistance with amber grain color. The resistance in the derived lines seems to be race non-specific and durable nature. The major genes possessed by the parents were susceptible as the individual line V-87094 had high terminal rust rating up to 80% in the rust screening nurseries and Era exhibited 10-20% rust rating. Combinations form these parents against the prevalent leaf and yellow rust races showed fairly very low rust intensity in the country (Hussain *et al*., 2006). As the lines which possessed major genes individually are susceptible in most rust virulences in Pakistan. Although the rust development was slow in case of *Lr34*, but alone this gene did not give desired protection and terminal rust rating was more than 60%. The better resistance in the derivatives from these crosses was most probably through the pyramiding of additional minor genes in their ancestors. Most of the lines were resistant to moderately resistant to leaf and yellow rust under high leaf and yellow rust inoculums pressure, developed artificially at the WRI, Faisalabad. The spreader rows of susceptible Morocco were full of rust rating 80-100SN and there was no chance of escape. The year 2012 was the worst epidemic year for yellow rust, wiped out most of the wheat cultivars of the country including Seher-2006, MH-97 and Bakhar-2002. Only Faisalabad-2008, Lasani-2008, AARI-11, Millat-11 & Pb-11 were found relatively resistant.Therefore, a mechanism based on the additive effects of partial resistance/ minor genes and probably differentfrom all the existing wheat varieties of the country Pakistan. This kind of resistance is desirable, as it is long lasting, more durable against changing rust virulence patterns. This is evidently supported by the consistent resistance behavior of Frontana and Era in Pakistan for the last twenty years (Hussain *et al*., 1999). Hence high economic returns may be achieved from such kind of resistance. Similar findings and ideas have been emphasized, entrusted and floated by Sing *et al*., (1998). Some new forms of rust virulence are generated as the result of mutations in the nature. New rust virulence is appearing with the introduction of new wheat varieties and many wheat varieties have been banned for commercial cultivation only due to rust susceptibility against new rust virulence (Khan *et al*., 2002). Incorporation of more than one gene to cultivars for durable leaf rust resistance has remained the focus of the breeders to cope with the dynamic nature of the pathogen (Roelfs, 1988). To address this issue, gene postulation as well as phenotypic marker approach is being utilized for enhancing rust resistance mainly through identification of durable rust resistance gene. In present investigation, the phenotypic characterization through the application of phenotypic markers i.e pseudo black chaff (Pbc), for *Sr2/Yr30*, leaf tip necrosis (Ltn-1) for *Lr34/Yr18* and leaf tip necrosis (Ltn-2) for *Lr46/Yr29* revealed that 67 entries showed presence of *Lr34*/*Yr18*; 23 entries showed *Lr46*/*Yr*29 while 62 entries showed *Sr2*/*Yr30* linkage therefore it remains successful for longer time, even if the pathogen under goes mutations. Hence, as per our findings, lines showing low frequency of disease severity with lower AUDPC values could be considered as slow rusting lines carrying durable rust resistance against *Lr34*, *Lr46* and *Sr2* virulences, which can be utilized in breeding programs. Determining the presence of *Lr*34, *Lr46* and *Sr2* in current cultivars can be helpful to predict the field resistance. The durability of these cultivars aid decisions in selecting parents for future breeding and development of new improved cultivars with improved leaf rust resistance. Therefore, the strategy of incorporating partial resistant minor gene in wheat genotypes through hybridization is the best way to achieve long lasting resistance in the wheat cultivars under the changing pattern of rust races/ virulence in the country.

Conclusion: Among the tested genotypes nine lines including V-11211, V-11227, V-11288, V-11296, V-11304, V-11308, V-11319, V-11353 and V-11396 showed the combination of three designated slow rusting/durable resistant genes (*Sr2/Yr30*, *Lr46/Yr29 and Lr34/Yr18*). Fifteen lines including V-11203, V11212, V-11218, V-11223, V-11245, V-11248, V-11250, V-11262, V-11267, V-11289, V-11321, V-11232, V-11338, V-11365, & V-11359 showed the combination of *Lr34/Yr18 and Sr2/Yr30*. Two lines including V-11190, as well as V-11193 showed the combination of *Lr46/Yr29* and *Sr2/Yr30*. Six genotypes including V-11276, V-11247, V-11313, V-11345, and V-11376 & V-11380 showed combination of *Lr46*/*Yr29* and *Lr34*/*Yr18*. The outstanding lines having highly better level of partial resistance along with lower AUDPC may be used in breeding program to transfer its better partial/ durable resistance character to the adapted wheat varieties of Pakistan (Inqilab-91, MH-97, Wattan, Pb-96, Seher-2006 and Shafaq-2006 etc).

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