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NEW SOURCE OF RESISTANCE TO STRIPE RUST IN WHEAT LANDRACE PI388060 ORIGINATED FROM PUNJAB, PAKISTAN

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Abstract

Stripe rust is one of the devastating diseases with potential to affect wheat yield all over the world. It is essential to continuously identify novel causes of resistance and make them available for commercial use in wheat breeding. In current study, fifty wheat landraces were evaluated against eight stripe rust races from different areas of Pakistan at seedling stage and landrace PI 388060 was identified as resistant to all these stripe rust races. The line PI 388060 was crossed with Avocet 'S' to derive F2 and F3 progenies for analysis. Seedling and adult plant analysis of the F2 and F3 progeny revealed presence of one major gene in this line. Chi square analysis of the glasshouse data gave good fit to expected segregation ratio of 3:1. Family analysis of the randomly selected F3 lines and their field screening also revealed segregation of the lines in 3:1 ratio. Line #36 exhibited very good resistance in glass house against all the four stripe rust isolates and good resistant reaction in field with highest final rust severity of 10. PI 388060 seemed to be a significant source of resistance to stripe rust and further genetic analysis will be conducted to pinpoint new stripe rust resistant gene in this landrace. The lines from this cross are being raised to get advanced F7 population to further characterize the gene.

Key words: Final rust severity, Seedling stage, Segregation, Isolates.

Introduction

For millions of people worldwide, wheat (*Triticum aestivum* L.) is a vital portion of regular nutrition that gives near 55% carbohydrates and 20% of calories (Ahmad *et al*., 2003). From approximately 220 million hectares of land, nearly 600 million tons wheat is produced. In order to nourish 178 million individuals with high per capita use of wheat, Pakistani wheat producers have a greater challenge in country around a massive yield productivity gap (Chen, 2005; Ali *et al*., 2009). Yellow (stripe) rust, leaf (brown) rust and stem (black) rust is caused by *Puccinia striiformis*, *P. triticina* and *P. graminis* f.sp. *tritici* respectively (Bolton *et al*., 2008; Liu & Hambleton, 2010; Berlin *et al*., 2013). These three-rust species that infect wheat and are scattered worldwide. In different areas, all the three rusts have been shown to cause enormous damages supporting disease outbreaks (Beddow *et al*., 2015, Singh *et al*., 2016). Globally, among the highest disturbing diseases of wheat, stripe rust of wheat is one the top. The causative agent of this disease is Basidiomycete fungus *Puccinia striiformis* Westend. f. sp. *tritici* Eriks and is the basis of extreme damage universally (Hovmoller *et al*., 2010, Chen, 2013). Economically stripe rust is the most important infection of wheat (*Triticum aestivum* L.). The yield losses are very high as 100%, in susceptible cultivars for the reason that infection begins at the seedling phase and lasts till the growing season is ended, because of the stripe rust (Ali & Ibrahim, 2007). A series of local epidemics of stripe rust over the last decade have been described worldwide, comprising Central and West Asia and East and North Africa (www.globalrust.org). Stripe rust was largely spread causing economic damages in low-input farming system since 2010 in East Africa (Singh *et al*., 2016). In Pakistan, cultivation of wheat is done on greater than eight million hectares, 70% of which are inclined to stripe rust.

The best effective approaches for managing this disease are cultural control, use of fungicide and resistance (Roelfs, 1992), the last one being the most environment friendly and cost-effective methodology (Mclntosh *et al*., 1995). Resistance can be broadly classified into two major types, all-stage resistance and adult-plant resistance (APR) designated against wheat stripe rust (Ali & Ibrahim, 2007). The first type is recognized in the seedling period and it is all-stage resistance and it continue to be effective to provide resistance during the course of the plant's development phases. The second type is horizontal resistance also known as race non-specific resistance, by the help of minor genes it is controlled and remains operative against all races of the pathogen and is expressed at an adult plant stage, as partial and slow rusting resistance (Kolmer, 1996). Wheat germplasm of Pakistan have been evaluated by several researchers for rust resistance (Shah *et al*., 2003). Though, they concentrated primarily on vertical resistance, based on major genes, but because of the evolution of virulence by pathogen for these genes, the resistance might be overcome rapidly. Based on minor genes, the alternative way is to discover partial resistance that is measured to be extra durable (Singh *et al*., 2004). In Pakistan, the pathogen is one of the very significant yield limiting causes. The evaluation of rust resistance in wheat genotypes will be helpful in accelerating the breeding programs strategies (Parveen *et al*., 2014). The present study was carried out to find novel wheat genetic resources for stripe rust resistance to increase cultivar progress efforts.

Materials and Methods

A set of fifty landraces was screened against stripe rust races 574730, 574212, 574232, 574216, 410202, 430220, 476232 and 534202 at seedling stage under glasshouse conditions and landrace PI388060 was selected for crossing it with Australian susceptible cultivar Avocet 'S'. Seed of the land races was provided by USDA small grain collection Aberdeen and maintained at CDRI Murree seed collection. The selected line was crossed with susceptible parent to harvest F1 which was raised to F2 generation under glass house conditions. F2 were screened under glasshouse conditions in Crop Diseases Research Institute (CDRI) Murree against stripe rust race 574212. In total, 260 F3 progenies were harvested. The F3 was screened for seedling resistance at CDRI Murree glasshouse against stripe rust races 574212, 574232, 430220 and 476232 and were transplanted to NARC field for adult plant screening against stripe rust race 574212.

Glasshouse seedling screening for selection of resistant line: The stripe rust races used for crossing were maintained at CDRI Murree's rust culture collection. Screening of the landrace was done by the inoculums preserved at -80°C, which was taken out of freezer and immediately dipped in water bath set at 65°C for 15 minutes to break dormancy of the spores. The inoculum was then suspended in the mixture of petroleum ether and mineral oil (80:20v/v) and sprayed on the 10 days old plants with the help of fine atomizer at two leaf stage (Rizwan *et al*., 2010). The plants were then left in open air for two hours before moving them to growth room set at 18°C temperature and 100% relative humidity. The plants were left in growth room for 12 hours after which they were moved to glasshouse set at 18-20°C temperature. Data was recorded after 15 days using 0-9 scale (pictorial scale). Plants with infection type 0-6 were considered as resistant while those with 7-9 were regarded as susceptible.

Crossing to develop F2 & F3 progenies: Landrace PI 388060 was selected as female parent crossed with Avocet 'S' as male parent to obtain F1 progeny. The F1s were grown under glasshouse conditions to raise F2s which were screened against race 574212 under glasshouse conditions at seedling stage. After screening the plants were transplanted in field to harvest F3 for further analysis.

Adult plant screening: F3 population consist of 260 lines and two parents along with susceptible check Morocco was screened at adult plant stage in field. Every entry was planted in a pair of 1-meter rows and was bordered by susceptible check Morocco. Stripe rust epidemic was created at pre-booting stage by inoculating spreader rows with stripe rust race 574212. Uredio-spore suspension in mixture of petroleum ether and mineral oil (v/v 80:20) was sprayed in the evening using ULV sprayer. The field was watered a day before inoculation to ensure desired relative humidity. Percent severity along with infection type recorded based on Modified Cobb's scale (Peterson *et al*., 1948) when the susceptible check showed 90S reaction (McIntosh *et al*., 1995). Coefficient of infection (CI) which is the product of disease severity (DS) and constant values of infection type (IT), was calculated for the population and parents. Constant values for IT were considered, which were based on; Resistant (R)=0.1, Moderately Resistant (MR)=0.25, Moderate (M)=0.5, Moderately Susceptible (MS)=0.75, Susceptible (S)=1 (Pathan & Park, 2006). The area under disease progress curve also represented as AUDPC was calculated by the formula AUDPC $=\sum i[(xi+xi+1)/2]ti$, for each of the population's line along with the parents, where xi is used to calculate the DS on date (i), ti is the period in days amongst days i and i+1. rAUDPC which is the abbreviation of relative area under disease progress curve was calculated for each plot, it was reliant on DS, was measured relative to the mean AUDPC values of susceptible check AvS.

F3 family screening: It was proposed that, dependent on the results of the seedling tests, PI388060 x AVOCET S could have carried at least one major gene. To check this hypothesis, 56 selected F3 families of the cross PI388060 x AVOCET S were grown in the greenhouse, to the flag leaf stage and were inoculated with the similar 574212 race, and incubated as mentioned earlier. Based on the consequences of earlier assessments, the F3 families were selected whereas, at the adult stage of plant development in the field conditions, these families were regarded as resistant however later on, in the seedling stage, parallel families were considered to be susceptible. Because of greenhouse space limitations; the number of these F3 families was restricted to only 56. The plants were required to grow in separate pots (2-3 plants in every 20-cm-diameter pot) using 10 plants of each F3 family. By using 0-9 scale, the adult plant's ITs were recorded as described before, for the seedling tests.

Results and Discussion

Landrace PI388060 showed 0-4 reaction at seedling stage where as Avocet 'S' showed 8-9 reaction when screened against eight stripe rust races (Table 1).

Seedling response at F2 stage: The F2 analysis was done at seedling stage by using the most dominant race 574212. 257 F2 plants derived from cross PI 388060 x AVOCET S along with parents were evaluated at seedling stage against stripe rust race 574212. Infection types of the progeny is given in (Fig. 1). Susceptible check morocco gave IT 9 while AVOCET S gave type of reaction susceptible. PI 388060 was resistant having IT 4. Chi-square ($\chi 2$) test was used to assess the goodness of fit of observed to expected segregation ratios to evaluate the number of resistance genes conditioning resistance. The frequencies of seedling infection types were a good fit to the expected segregation ratio for one gene ($\chi 2$ 3:1= 0.19, $p<0.5$).

Table 1. Seedling stage characterization of 50 landraces for stripe rust resistance.

Plant identification (PI)	Race-534202	Race-574730	Race-574212	Race-574232	Race-574216	Race-410202	Race-430220	Race-476232
7739	6	7	5	7	4	7	4	4
40945	M	7	3	3	3	4	3	3
40954	6	7	6	2	5	3	4	3
40956	6	7	9	7	6	6	3	4
40959	5	M	3	5	4	7	3	3
40962	7	7	7	7	7	7	5	8
40965	7	7	5	9	7	6	6	7
40966	6	M	5	7	7	7	4	M
40968	7	8	5	7	6	7	6	5
40969	7	8	6	7	7	M	7	5
182125	M	7	5	5	7	6	3	6
189743	8	7	5	7	6	6	6	7
189744	8	7	5	7	7	6	6	7
189745	8	9	5	7	6	7	6	7
189746	7	7	5	6	6	4	6	5
193383	5	7	5	5	6	3	5	6
193384	7	7	5	7	5	6	5	6
193387	6	7	5	3	6	7	6	4
193389	6	6	4	6	5	3	5	4
217544	6	7	5	3	6	5	3	3
218119	6	7	7	7	6	6	5	5
218121	7	6	6	4	6	5	5	5
219742	7	8	8	7	7	6	5	6
219744	6	7	5	7	7	6	6	6
219745	8	8	7	7	7	7	4	7
219747	8	7	8	7	7	6	4	4
219748	5	6	4	6	5	5	3	4
219749	8	7	5	7	6	6	4	4
219752	4	4	1	2	5	4	4	3
250236	M	0	4	3	5	4	6	3
250237	6	6	4	5	7	5	4	4
250408	4	6	5	6	6	6	4	4
250409	6	6	5	7	6	4	4	4
250412	7	5	4	6	6	4	M	5
250413	7	4	5	6	7	4	5	4
250414	7	4	6	7	6	6	6	4
250629	4	4	3	3	5	5	3	3
250630	4	M	3	7	4	3	3	3
250632	4	M	1	3	6	3	4	2
250633	4	M	2	1	6	3	3	M
270003	5	4	3	1	7	2	4	2
270004	4	4	3	3	7	3	3	3
270050	6	5	5	5	7	4	4	4
388059	6	4	5	7	7	6	4	3
388060	**3**	**2**	**4**	**3**	**2**	**2**	**3**	**0**
388061	7	4	5	7	6	6	4	M
388064	7	6	5	6	6	6	4	3
388065	7	5	5	3	6	3	6	3
388070	7	4	5	3	6	3	5	3
388074	7	7	4	7	7	6	6	4
AVOCET "S"	**9**	**8**	**9**	**9**	**9**	**8**	**9**	**9**

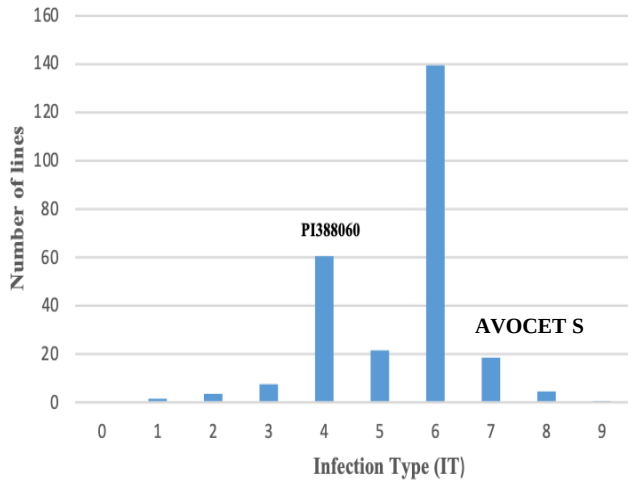

Fig. 1. Histograms for PI380660 x AVOCET S population for the distribution of seedling infection type IT for *Puccinia striiformis f. sp. Tritici* race 574212 on F2 stage.

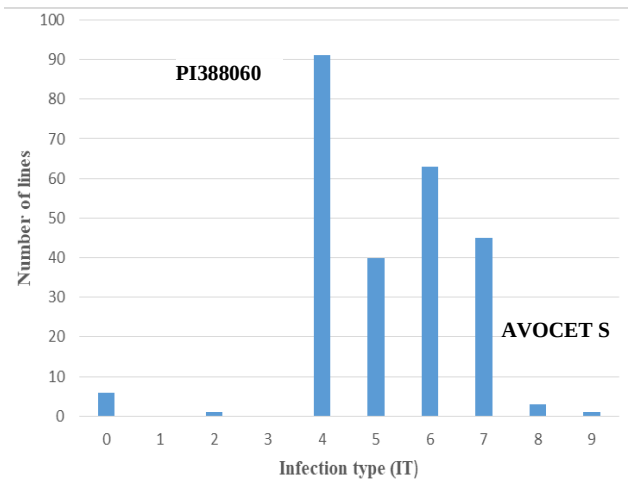

Fig. 2. Histograms for PI380660 x AVOCET S population for the distribution of seedling infection type IT for *Puccinia striiformis* f. sp. *Tritici* race 574212 on F3 stage.

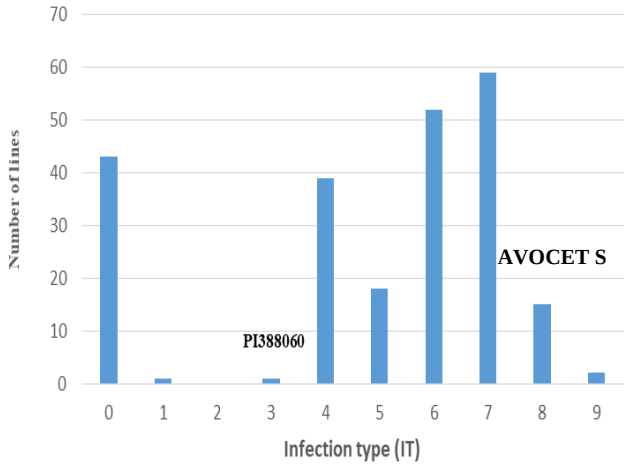

Fig. 3. Histograms for PI380660 x AVOCET S population for the distribution of seedling infection type IT for *Puccinia striiformis f.* sp. *Tritici* race 574232 on F3 stage.

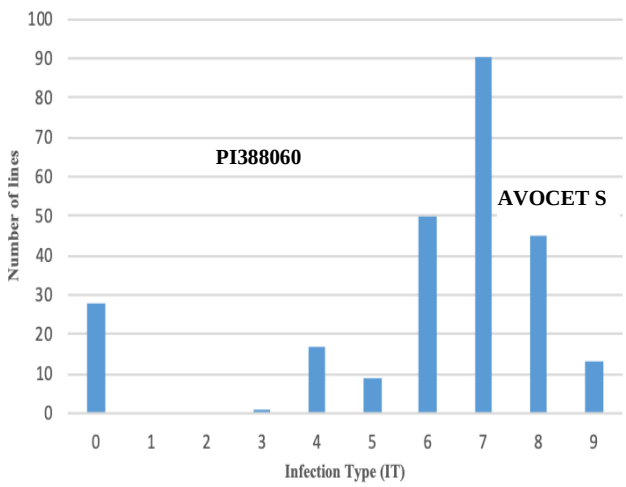

Fig. 4. Histograms for PI380660 x AVOCET S population for the distribution of seedling infection type IT for *Puccinia striiformis* f. sp. *Tritici* race 430220 on F3 stage.

Seedling response at F3 stage: A significant variation was observed for major gene evaluation at F3 by using four most virulent races (574212, 574232, 430220 and 476232).

In total 200 F3s showed resistant type of reaction when population was screened with 574212 and 47 F3s showed susceptible reaction (Fig. 2). The parents PI 388060 show resistant (IT 4) and second parent AVOCET S showed susceptible type (IT 8) reaction. In the same way when population was screened with 574232, 153 F3s showed resistant type of reaction, while 75 F3s showed susceptible reaction (Fig. 3). The parental screening with the race 574232 shows infection type 3 and 9 for PI 388060 and AVOCET S respectively. The screening of the F3 population with the race 430220 gives 104 R and 146 S F3s (Fig. 4), while the parents (PI 388060 and AVOCET S) showed Infection type 3 and 9 respectively. PI 388060 showed resistant reaction having infection type 0 while AVOCET S showed susceptible reaction having infection type 9 when screened with the race 476232.When the population was screened 188 F3s were resistant and 62 F3s were susceptible (Fig. 5). The comparison of all these four Pst races are given in Fig. 7. As the susceptible check showed maximum infection as 9 for all the four races. F3 # 36 exhibit very good resistance against all the four stripe rust races. It shows Infection type 1, 2 and 4 against all used stripe rust isolates in glass house. As well as it also exhibited a good resistance reaction having infection type 2 in the field. As the homozygosity increased in F3 the virulence pattern is also diverse. Among all the four races the F3 population was most resistant to the race 574212 in which 200 out of 260 F3s were resistant. On contrary to that the most virulent race was 430220. By screening the population with the race 430220, 146 F3s out of 260 F3s were susceptible. A comparison of all four races on F3 stage is also represented by graph (Fig. 5). Based on virulence/avirulence outline of the pathogen, seedling resistant genotypes might have genes Yr5, Yr10, Yr15, Yr24, Yr32, Yr44, YrSP YrTr1 and YrTye. The virulence pattern of the isolates used is shown in Table 2. In Pakistan, in different wheat growing regions, these seedling resistance genes have been tested to carry resistance under field conditions (Ali *et al*., 2007; Rizwan *et al*., 2010). Yet, in studied germplasm, still authentication of these genes is necessary, by means of multi pathotype testing, genetic studies and molecular diagnostic markers.

Table 2. Virulence pattern of the Yr isolates used for screening at F3.

Yr genes	Race-574212	Race-574232	Race-430220	Race-476232
Yr1	+	+	-	-
Yr5	-	-	-	-
Yr6	+	+	+	+
Yr7	+	+	+	+
Yr8	+	+	+	+
Yr9	+	+	-	+
Yr10	-	-	-	-
Yr15	-	-	-	+
Yr17	+	+	-	+
Yr24	-	-	-	-
Yr27	+	+	+	+
Yr32	-	-	-	-
Yr43	+	+	-	+
Yr44	-	+	+	+
YrSP	-	-	-	-
YrTr1	-	-	-	-
YrExp2	+	+	-	+
YrTye	-	-	-	-

Field assessment at F3 stage: The parents PI 388060 and AVOCET S exhibit high and low value of CI respectively in field. The high disease pressure was significantly observed by the high CI value of susceptible check. For adult plant resistance, CI values 0-20, 21-40, 41-60 for the lines were considered as having high, moderate and low levels respectively (Ali *et al*., 2007). Maximum value of CI was observed by 6 F3s (i.e., line # 7, 31, 35, 89, 195 and 36). Minimum value of CI was observed by 7 F3s (i.e. line # 14, 18, 97, 222, 272 and 289). Table 1 shows final rust severity data of F3 population comprising of 260 F3s along with two parents PI388060, AVOCET S and the susceptible check (Morocco). For Morocco, a great disease pressure was observed at the testing site as maximum Final rust severity (FRS) up to 100%. Likewise, based on FRS, the lines which were tested were clustered in to three groups of partial resistance, i.e. high (1-30% FRS), moderate (31-50% FRS) and low levels of partial resistance (51-70% FRS). The first group having highest FRS comprises of 88 F3s. This group also includes one of the parent (PI 388060) having FRS value thirty. The group having moderate level of FRS comprises of 150F3s. The third group that showed low levels of partial resistance have 12 F3s. This group includes the second Parent AVOCET S having FRS value of 80. In the same way, Ali *et al.*, (2009) and Safavi *et al.*, (2012), similarly carried out field evaluation for classification of lines, used for quantitative resistance to stripe rust. Line # 36 that have a good resistant reaction in glass house against all the four stripe rust isolates and good resistant reaction in field had a good highest FRS value of 10 that confirms its high level of resistance (Table 3). Centered on the results of other investigators (Johnson, 1988; Ali *et al.*, 2007), lines which had resistance reaction at both stages could probably carry major gene or combination of major genes-based resistance effective against all virulences used. However usually, the lines/ cultivars with race specific resistance turn into susceptible in few years after their release because of the rapid evolution of novel virulent races of the pathogens (Wan & Chen, 2012). The rates of adult plant infection types were a good fit to the expected segregation ratio for one gene ($\chi 2$ 3:1= 0.2, $p<0.5$).

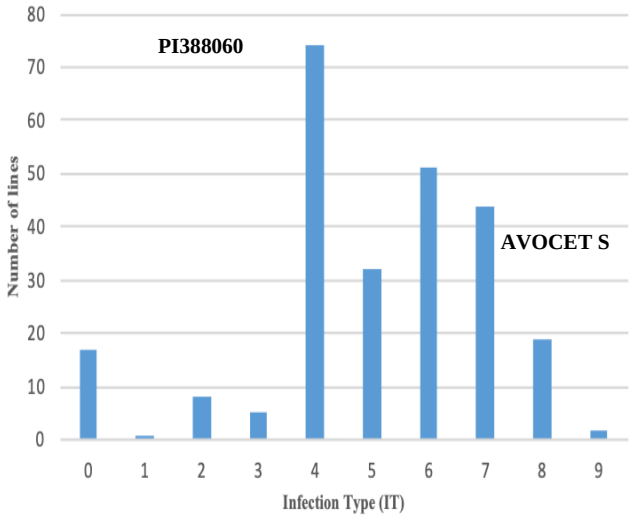

Fig. 5. Histograms for PI380660 x AVOCET S population for the distribution of seedling infection type IT for *Puccinia striiformis* f. sp. *Tritici* race 476232 on F3 stage.

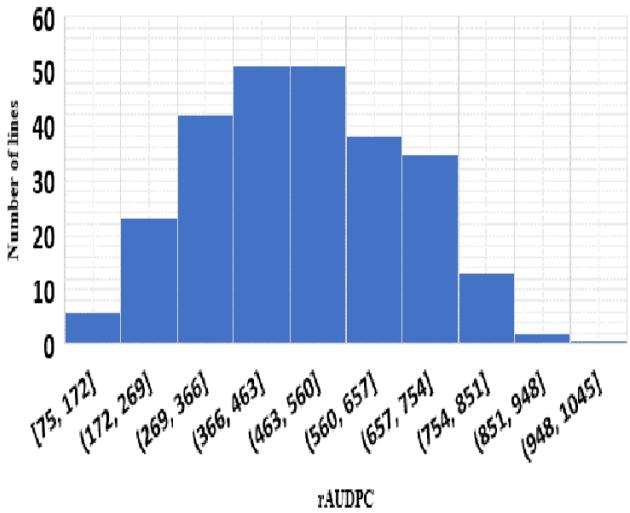

Fig. 6. Histogram for PI388060 x AVOCET S population for relative area under disease progress.

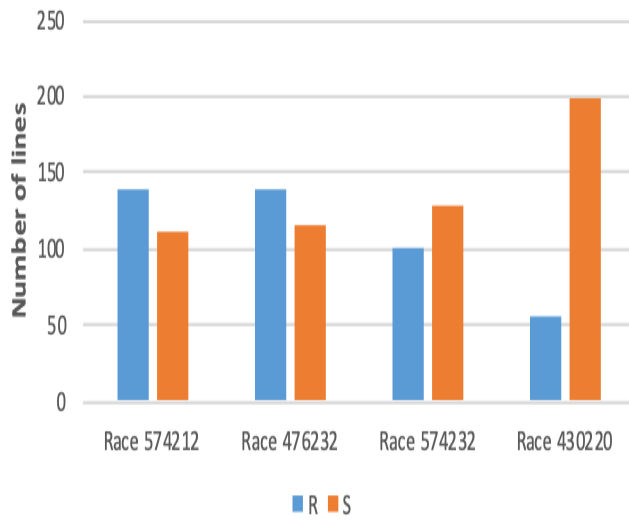

Fig. 7. Reaction distribution of 260 F3s against *Puccinia striiformis* f.sp. *Tritici* races 574212, 476232, 574232 and 430220.

Table 3. Adult plant infection type, seedling reaction, coefficient of infection and final rust severity to stripe rust in F3 Population PI388060 x AVOCET S.

S. No.	SR	APR	FRS	CI	S. No.	SR	APR	FRS	CI	S. No.	SR	APR	FRS	CI
RIL1	4	MR	30	8	RIL37	7	MS	60	45	RIL73	4	MR	40	10
RIL2	M	M	M	M	RIL38	4	R	40	4	RIL74	4	R	20	2
RIL3	0	MR	40	10	RIL39	M	M	M	M	RIL75	6	MR	50	12.5
RIL4	4	MR	30	30	RIL40	4	R	30	3	RIL76	6	MR	60	15
RIL5	4	MR	20	5	RIL41	6	MR	60	15	RIL77	6	MR	50	12.5
RIL6	4	MR	30	8	RIL42	6	MR	50	12.5	RIL78	6	MR	50	12.5
RIL7	0	R	5	1	RIL43	4	R	40	4	RIL79	4	R	30	3
RIL8	4	MR	40	10	RIL44	4	R	40	4	RIL80	6	MR	50	12.5
RIL9	6	MS	60	45	RIL45	4	R	30	3	RIL81	4	R	20	2
RIL10	4	R	30	3	RIL46	7	MS	70	52.5	RIL82	4	R	30	3
RIL11	6	MR	40	10	RIL47	5	MR	40	10	RIL83	7	MS	70	52.5
RIL12	6	MR	50	13	RIL48	7	MR	60	15	RIL84	6	MR	50	12.5
RIL13	4	R	30	3	RIL49	6	MS	70	52.5	RIL85	4	R	30	3
RIL14	8	S	90	90	RIL50	4	R	30	3	RIL86	M	M	M	M
RIL15	4	R	40	4	RIL51	4	R	40	4	RIL87	M	M	M	M
RIL16	4	R	30	3	RIL52	5	MR	40	10	RIL88	4	R	20	2
RIL17	7	MS	70	53	RIL53	6	MR	60	15	RIL89	0	R	5	0.5
RIL18	7	S	90	90	RIL54	4	R	40	4	RIL90	4	R	20	2
RIL19	7	MS	60	45	RIL55	6	MR	50	12.5	RIL91	4	R	30	3
RIL20	4	R	30	3	RIL56	4	R	30	3	RIL92	6	MR	50	12.5
RIL21	5	MR	40	10	RIL57	4	R	40	4	RIL93	6	MR	60	15
RIL22	6	MR	50	13	RIL58	4	R	40	4	RIL94	7	MS	60	45
RIL23	4	R	40	4	RIL59	5	MR	40	10	RIL95	6	MR	50	12.5
RIL24	6	MR	50	13	RIL60	4	R	20	2	RIL96	4	R	30	3
RIL25	6	MR	60	15	RIL61	6	MR	50	12.5	RIL97	7	S	90	90
RIL26	4	MR	50	13	RIL62	M	M	M	M	RIL98	5	MR	40	10
RIL27	4	R	40	4	RIL63	7	MS	70	52.5	RIL99	6	MR	50	12.5
RIL28	6	MR	50	13	RIL64	5	MR	50	12.5	RIL100	5	MR	30	7.5
RIL29	6	MR	50	13	RIL65	4	R	40	4	RIL101	4	R	20	2
RIL30	4	R	40	4	RIL66	4	MR	40	10	RIL102	4	R	20	2
RIL31	0	R	5	1	RIL67	4	R	30	3	RIL103	4	R	30	3
RIL32	4	R	30	3	RIL68	7	MS	60	45	RIL104	4	R	30	3
RIL33	5	MR	40	10	RIL69	5	MR	40	10	RIL105	5	MR	40	10
RIL34	4	MR	30	8	RIL70	M	M	M	M	RIL106	6	MR	50	12.5
RIL35	0	R	5	1	RIL71	7	MS	70	52.5	RIL107	M	M	M	M
RIL36	2	R	10	1	RIL72	4	R	30	3	RIL108	5	MR	40	10
RIL109	4	R	20	2	RIL145	4	R	30	3	RIL181	6	MR	50	12.5
RIL110	4	R	20	2	RIL146	6	MR	50	12.5	RIL182	7	MS	70	52.5
RIL111	6	MR	50	13	RIL147	4	R	20	2	RIL183	4	R	20	2
RIL112	4	R	30	3	RIL148	6	MR	50	12.5	RIL184	6	MR	60	15
RIL113	7	MS	70	53	RIL149	4	R	30	3	RIL185	5	MR	40	10
RIL114	5	MS	70	53	RIL150	5	MR	40	10	RIL186	6	MR	60	15
RIL115	7	MS	60	45	RIL151	5	MR	50	12.5	RIL187	6	MR	50	12.5
RIL116	6	MR	50	13	RIL152	5	MR	40	10	RIL188	4	MR	40	10
RIL117	5	MR	40	10	RIL153	7	MS	70	52.5	RIL189	4	R	30	3

Table 3. (Cont'd.).

S. No.	SR	APR	FRS	CI	S. No.	SR	APR	FRS	CI	S. No.	SR	APR	FRS	CI
RIL118	4	R	20	2	RIL154	6	MR	60	15	RIL190	5	MR	50	12.5
RIL119	4	R	30	3	RIL155	6	MR	60	15	RIL191	5	MR	40	10
RIL120	6	MR	50	13	RIL156	6	MR	50	12.5	RIL192	4	R	20	2
RIL121	4	R	20	2	RIL157	7	MS	80	60	RIL193	4	R	30	3
RIL122	7	MS	60	45	RIL158	7	MS	70	52.5	RIL194	4	R	40	4
RIL123	7	MS	70	53	RIL159	7	MS	50	37.5	RIL195	0	R	5	0.5
RIL124	7	MS	70	53	RIL160	5	MR	30	7.5	RIL196	4	R	20	2
RIL125	7	S	80	80	RIL161	5	MS	60	45	RIL197	M	M	M	M
RIL126	4	R	20	2	RIL162	7	MS	60	45	RIL198	5	MR	50	12.5
RIL127	6	MR	50	13	RIL163	4	R	20	2	RIL199	4	R	40	4
RIL128	6	MR	60	15	RIL164	6	MR	50	12.5	RIL200	5	MR	50	12.5
RIL129	4	R	20	2	RIL165	7	MS	70	52.5	RIL201	4	R	30	3
RIL130	7	MS	70	53	RIL166	7	MS	70	52.5	RIL202	4	R	40	4
RIL131	7	MS	60	45	RIL167	6	MR	60	15	RIL203	6	MR	60	15
RIL132	6	MR	50	13	RIL168	7	MS	60	45	RIL204	4	R	30	3
RIL133	6	MR	60	15	RIL169	6	MR	50	12.5	RIL205	6	MS	70	52.5
RIL134	5	MR	40	10	RIL170	5	MR	40	10	RIL206	4	R	20	2
RIL135	6	MR	60	15	RIL171	5	MR	40	10	RIL207	4	R	30	3
RIL136	6	MR	60	15	RIL172	8	S	80	80	RIL208	5	R	50	5
RIL137	7	MS	70	53	RIL173	6	MR	50	12.5	RIL209	6	R	60	6
RIL138	6	MR	50	13	RIL174	4	R	30	3	RIL210	M	M	M	M
RIL139	5	MR	40	10	RIL175	7	MS	60	45	RIL211	4	R	20	2
RIL140	6	S	80	80	RIL176	M	M	M	M	RIL212	4	R	30	3
RIL141	6	MR	60	15	RIL177	4	R	20	2	RIL213	7	MS	70	52.5
RIL142	7	MS	70	53	RIL178	4	R	30	3	RIL214	6	MR	60	15
RIL143	5	MR	40	10	RIL179	4	MR	50	12.5	RIL215	7	MS	70	52.5
RIL144	4	R	30	3	RIL180	4	R	40	4	RIL216	5	MR	50	12.5
RIL217	4	R	40	4	RIL233	7	MS	60	45	RIL249	M	M	M	M
RIL218	7	MS	80	60	RIL234	6	MR	50	12.5	RIL250	5	MR	50	12.5
RIL219	6	MR	50	13	RIL235	5	MS	70	52.5	RIL251	4	R	40	4
RIL220	7	MS	70	53	RIL236	5	MR	40	10	RIL252	7	S	70	70
RIL221	5	MR	40	10	RIL237	5	MR	50	12.5	RIL253	5	MS	70	52.5
RIL222	7	S	90	90	RIL238	6	MS	50	37.5	RIL254	7	MS	70	52.5
RIL223	4	R	40	4	RIL239	7	MS	60	45	RIL255	6	MR	60	15
RIL224	6	MR	50	13	RIL240	7	MS	70	52.5	RIL256	5	MS	70	52.5
RIL225	6	MR	60	15	RIL241	M	M	M	M	RIL257	7	S	60	60
RIL226	4	R	30	3	RIL242	4	R	20	2	RIL258	7	S	70	70
RIL227	7	MS	70	53	RIL243	6	MR	50	12.5	RIL259	4	R	30	3
RIL228	4	R	40	4	RIL244	4	R	30	3	RIL260	5	MR	50	12.5
RIL229	5	MR	50	13	RIL245	4	R	40	4	**PI388060**	4	R	30	3
RIL230	6	MR	60	15	RIL246	5	MR	40	10	**AVOCET S**	8	S	80	80
RIL231	M	M	M	M	RIL247	6	MR	50	12.5	**Morrorco**	9	S	100	100
RIL232	4	R	40	4	RIL248	4	R	30	3					

R-Resistant, MR-Moderately resistant, MS-Moderately susceptible, S-Susceptible

SR-Seedling reaction, APR-Adult plant resistance, FRS-Final rust severity, 1-30% Highly resistant, 31-50%, Low resistance 51-70%

CI-Co-efficient of infection, by multiplying disease severity with adult plant reaction

Table 4. χ2 value for randomly selected F3 Families against the race 574212.

S #	Line #	IT	R	S	N=Total	Exp Ratio	Obs	Exp	(Ob-Exp)	(Ob-Exp)^2	χ2
1	6	6,6,7,5,5	4	1	5	3:01	0.75	3.75	0.25	0.0625	0.016667
2	7	7,5,4,6,6	4	1	5	3:01	0.75	3.75	0.25	0.0625	0.016667
3	8	5,4,7,6,6,5,6	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143
4	19	6,5,7,6,6	4	1	5	3:01	0.75	3.75	0.25	0.0625	0.016667
5	22	4,4,4,7,6,5,6	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143
6	25	6,4,5,5,7,6	5	1	6	3:01	0.75	4.5	0.5	0.25	0.055556
7	27	7,6,6,6,5,5,5	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143
8	31	6,6,7,5,5,6,5	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143
9	33	5,6,7,4,5	4	1	5	3:01	0.75	3.75	0.25	0.0625	0.016667
10	36	6,4,7,8,7,6,4	4	3	7	1:01	0.5	3.5	0.5	0.25	0.071429
11	38	4,4,5,5,4,4,7	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143
12	39	5,4,7,5,4,5,5	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143
13	50	6,5,6,4,5,7,6	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143
14	55	6,7,4,6,6,6	5	1	6	3:01	0.75	4.5	0.5	0.25	0.055556
15	60	4,7,1,4,7,4	4	2	6	3:01	0.75	4.5	-0.5	0.25	0.055556
16	61	5,6,6,5,7,6,5	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143
17	65	7,6,7,5,6	3	2	5	3:01	0.75	3.75	-0.75	0.5625	0.15
18	67	6,4,4,7,7,4,6	5	2	7	3:01	0.75	5.25	-0.25	0.0625	0.011905
19	70	6,6,8,7,6,6	4	2	6	3:01	0.75	4.5	-0.5	0.25	0.055556
20	71	7,4,4,5,5,4	5	1	6	3:01	0.75	4.5	0.5	0.25	0.055556
21	72	5,6,6,6,7,7	4	2	6	3:01	0.75	4.5	-0.5	0.25	0.055556
22	73	6,6,7,4,7,5,4	5	2	7	3:01	0.75	5.25	-0.25	0.0625	0.011905
23	78	4,7,5,7,0,1,0	5	2	7	3:01	0.75	5.25	-0.25	0.0625	0.011905
24	82	7,6,4,6,4,5,4	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143
25	88	4,7,0,7,5,4	4	2	6	3:01	0.75	4.5	-0.5	0.25	0.055556
26	101	7,6,5,5,6,7	4	2	6	3:01	0.75	4.5	-0.5	0.25	0.055556
27	103	8,7,4,6,4,4	4	2	6	3:01	0.75	4.5	-0.5	0.25	0.055556
28	107	4,5,6,7,4,5	5	1	6	3:01	0.75	4.5	0.5	0.25	0.055556
29	123	4,6,5,7,6,0,6	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143
30	125	6,5,7,7,4	3	2	5	3:01	0.75	3.75	-0.75	0.5625	0.15
31	130	5,4,7,7,5,5	4	2	6	3:01	0.75	4.5	-0.5	0.25	0.055556
32	135	7,7,7,5,3	2	3	5	3:01	0.75	3.75	-1.75	3.0625	0.816667
33	140	4,6,5,7,6,5	5	1	6	3:01	0.75	4.5	0.5	0.25	0.055556
34	146	4,6,5,4,4,7,6	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143
35	151	5,5,5,5,6,7,7	5	2	7	3:01	0.75	5.25	-0.25	0.0625	0.011905
36	158	0,1,0,7,0,0,6	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143
37	163	5,7,6,6,6,5,0,6	7	1	8	3:01	0.75	6	1	1	0.166667
38	185	6,6,6,6,7,6,6	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143
39	190	5,5,4,6,7,4,7	5	2	7	3:01	0.75	5.25	-0.25	0.0625	0.011905
40	193	4,7,6,6,5,7	4	2	6	3:01	0.75	4.5	-0.5	0.25	0.055556
41	194	6,5,7,6,8,6,7	4	3	7	3:01	0.75	5.25	-1.25	1.5625	0.297619
42	199	7,7,6,7,6,5,6	4	3	7	3:01	0.75	5.25	-1.25	1.5625	0.297619
43	202	6,5,5,7,8,5,6	5	2	7	3:01	0.75	5.25	-0.25	0.0625	0.011905
44	232	7,5,5,4,6,6,5	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143
45	233	4,7,4,7,4	3	2	5	3:01	0.75	3.75	-0.75	0.5625	0.15
46	236	6,7,5	2	1	3	3:01	0.75	2.25	-0.25	0.0625	0.027778
47	237	4,7,4,7,7,4,4	4	3	7	3:01	0.75	5.25	-1.25	1.5625	0.297619
48	240	6,7,5,4,4	4	1	5	3:01	0.75	3.75	0.25	0.0625	0.016667
49	241	5,5,6,6,8,7,6	5	2	7	3:01	0.75	5.25	-0.25	0.0625	0.011905
50	245	6,6,6,4,7,7	4	2	6	3:01	0.75	4.5	-0.5	0.25	0.055556
51	249	5,6,6,7,6,5	5	1	6	3:01	0.75	4.5	0.5	0.25	0.055556
52	253	7,7,6,4	2	2	4	3:01	0.75	3	-1	1	0.333333
53	254	5,8,7,6,8,4	3	3	6	3:01	0.75	4.5	-1.5	2.25	0.5
54	256	5,7,6,6,7	3	2	5	3:01	0.75	3.75	-0.75	0.5625	0.15
55	258	7,6,6,6,7,6,5	5	2	7	3:01	0.75	5.25	-0.25	0.0625	0.011905
56	259	5,6,7,5,5,5,6	6	1	7	3:01	0.75	5.25	0.75	0.5625	0.107143

At adult stage, disease data was used to calculate rAUDPC of each genotype associating with the severity of susceptible check Morocco. This measure indicates the advancement of the disease at adult stage in specified time and gives a sign of level of resistance against the disease in host germplasm. This measure therefore has been extensively used to categorize slow rusting or durable resistance in test germplasm. In related studies conducted previously, rAUDPC have been used by many wheat pathologists for the analysis of stripe rust data (Shah *et al*., 2010). The current field data shown that in 2017, at Islamabad location, 6 (2%) lines were resistant (rAUDPC 0-10), while 72 (27.5%) were intermediate (rAUDPC 11-30), and 183 (70%) were susceptible (rAUDPC >30) (Fig. 6). The previous analysis conducted for stripe rust data evaluation shows that various pathologist used rAUDPC for the disease analysis. (Rizwan *et al*., 2010).

Family analysis: The randomly selected F3 family analysis revealed the confirmation of one gene. The resistance gene's number can be assessed by using Chi-square ($\chi 2$) test to estimate the goodness of fit of observed to expected segregation. The frequencies of seedling infection types were a good fit to the expected segregation ratio for one gene. ($\chi 2$ 3:1, $p<0.5$) (Table 4). The value of P for all the randomly selected fifty-six F3s were less than 0.5 confirming the presence of single major gene.

Conclusion

The present study results indicated that the landraces have a range concerning resistance reaction, from moderate resistance to susceptible. Final rust severity data revealed that 69 percent among the assessed lines displayed moderate or good performance under high disease pressure. Whereas these progenies showed resistance to both seedling and adult plant stage indicating presence of major genes. Line #36 exhibit very good resistance against all the four stripe rust races. Therefore, it can be suggested that that there will be some important resistant gene in it that can be identified by further genetic analysis like QTL analysis to find stripe rust resistant gene.

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