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Seedling and durable resistance to stripe rust in two segregating wheat populations

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Abstract

Stripe rust is a worldwide epidemic, caused by divergent races of *Puccinia striiformis* responsible for considerable yield losses in wheat. Present study was conducted to explore the genetic resources of wheat for potential stripe rust resistance to combat with this biotic stress. Two segregating populations NIGAB-08 and NIGAB-09 have been developed in National institute for Genomics and Advanced Biotechnology (NIGAB), NARC, Islamabad as a potential genetic source for stripe rust resistance. In this study, these populations were explored for stripe rust resistance at seedling and adult plant stage. NIGAB-08 (F6 Segregating wheat Population) comprised of 48 wheat lines having genetic diversity for stripe rust. At seedling stage, 87% of this wheat population have seedling resistance with likely presence of *Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yrsp Yr2, Yr25, Yr31* and *YrA* genes whereas, 10% of population was found to have low genetic potential against stripe rust. Field data showed that the 16% of population was resistant while majority of population showed intermediate type of resistance against stripe rust. 86 wheat lines of NIGAB-09 (F6 Segregating wheat Population) showed its 83% of population resistant and 13% of population was observed to be susceptible at seedling stage, whereas, in field experiments, 79% of population was resistant while 10% of population was observed to be susceptible in field experiments. Seedling and Adult plant resistance both together will contribute to achieve durable and effective control against pathogen of stripe rust in Pakistan.

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Introduction

Stripe rust has been a center of intensive research, as it is considered to be among the current major diseases that are affecting winter cereal yield worldwide (Chen *et al.*, 2014). It results in huge economic loss as well as severe epidemics have been observed to be associated with yield and quality loss (Uauy *et al.*, 2005). *Puccinia striiformis* f. sp. *tritici*, has been observed for emergence of new races that are highly adapted to warm temperatures, with vast virulence profiles as well as more aggressive than previous races, resulting wide-scale epidemics (Bueno-Sancho *et al.*, 2017). All stage resistance or race specific resistance provides the complete protection against the disease, is considered to be non-durable when it involves one gene (Chen, 2005). Adult plant resistance that expressed at later stages in development can be race specific or race non-specific. Race non-specific resistance is generally inherited quantitatively, mostly durable, and impart as partial or slow-rusting resistance (Sthapit *et al.*, 2012).

Durable resistance provided by both major and minor genes is required to control the disease in environment favorable for development of disease. Adult plant resistance is of more interest for plant breeders to reduce yield losses caused by stripe rust at adult plant stage (Imtiaz *et al.*, 2003). In order to control the disease, it is important to identify the seedling and slow rusting genes for pyramiding of genes, gene deployment and development of slow rusting wheat varieties (Li *et al.*, 2006). Many methods have been applied to control stripe rust disease. To use fungicide is one of these methods which is cost effective and effect environment badly (Bux *et al.*, 2012). It is advantageous to use genetic control due to environmental and economic reasons and because of rust pathogens that has possibility to develop resistance against fungicides (Oliver, 2014). Genetic background of wheat cultivars in Pakistan is narrow for major biotic stresses. Most of the wheat varieties has been developed with few seedling resistance genes so, more prone to new pathotypes emerging with passage of time (Bux *et al.*, 2011). It is required to broaden the genetic base of wheat in breeding programs.

The present study has been aimed to investigate the genetic diversity present in two segregating populations of wheat, having diverse genetic background at seedling and adult plant stage.

Materials and methods

Seeds of two segregation wheat populations (F_6) have been obtained from National Institute of Genomics and Biotechnology, National Agriculture Research Centre, Islamabad (NARC), Pakistan. NIGAB-8 developed by crossing Suleman-96× Pavon-76 comprised of 50 lines and NIGAB-9 (by crossing Khyber-87× Suleman-96) has 86 lines of wheat.

Seedling Screening

5-7 seeds of each genotype were sown in 7×7 cm pot at Crop Disease Research Program (CDRP), Murree. Whole experiment was done under greenhouse conditions. 4-6 weeks old seedlings were passed through inoculation procedure. In this procedure, suspension of uridiospore made suspended in a mixture of mineral oil and petroleum (30:70) (Rizwan *et al.*, 2010). Inoculum had virulence of *Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yrsp Yr2, Yr25, Yr31* and *YrA* genes. Treated plants were placed in open air for two hours in order to evaporate the oil. Plants were transferred to dew chamber set at 10°C with photoperiod of 16h light and 8h dark for 48 hours. Then plants were shifted to greenhouse set at 6-10 °C (Rizwan *et al.*, 2010). After three weeks of inoculation, when susceptible check Morocco showing the maximum infection, infection types were recorded by using a 0-9 scale (Line and Qayum, 1992). Plant with infection types 0-3 were categorized as resistant, those having IT 7-9 considered as intermediate resistant, IT 7-9 plants as highly susceptible (Rizwan *et al.*, 2010).

Field trial to screen the adult plant resistance

Same population was characterized for adult plant resistance. The wheat populations were planted at National Agriculture Research Centre, Islamabad (NARC), Pakistan. 15-20 seeds were sown in single row plots of 1m length having 30cm row spacing. One row of susceptible check Morocco was sown at every 20th entry as well as along the border as disease spread rows.

Rust inoculations of the spreaders and check lines were carried out by the hypodermic syringe method using aqueous urediospore suspension to which 1 to 2 drops of Tween-20 was added, to break the surface tension. Inoculum of different races of rust comprised of diversified races including virulence for Yr5, Yr10, Yr15 and Yrsp was obtained from CDRI, NARC Islamabad and applied at booting stage of plants. The severity was recorded as per cent of rust infection on the plants according to the modified Cobb's scale (Peterson *et al.*, 1948).

The first disease notes were recorded when susceptible check had reached 60-70% of disease severity. It was used calculate the area under the disease progress curve by using AUDPC computer program developed at CIMMYT. By setting the AUDPC of Morocco as 100%, relative area under the disease progress curve (RAUDPC) was calculated (Ma et al., 1995). In order to categorize wheat lines in terms of stripe rust resistance, the wheat lines showed RAUDPC in the range of 0-10 were considered as resistant while, with RAUDPC 11-30 are supposed to be having intermediate type of resistance and above 30 were classified as susceptible (Bux *et al.,* 2012).

Result and discussions

NIGAB-08 (F_6 Segregating wheat Population)

This population comprised of 48 wheat lines having genetic diversity. At seedling stage, 87% of this wheat population was resistant with Infection type (IT) 0-3 and 2% of population showed intermediate type of resistance with IT value 4-6, whereas, 10% of population was found to be susceptible in this experiment.

Field data showed that the 16% of population was resistant (RAUDPC=0-10), while 47% of population showed intermediate type of resistance (RAUDPC= 11-30) and 35% of population was observed to be susceptible in field experiments (RAUDPC>30) (Fig. 1)

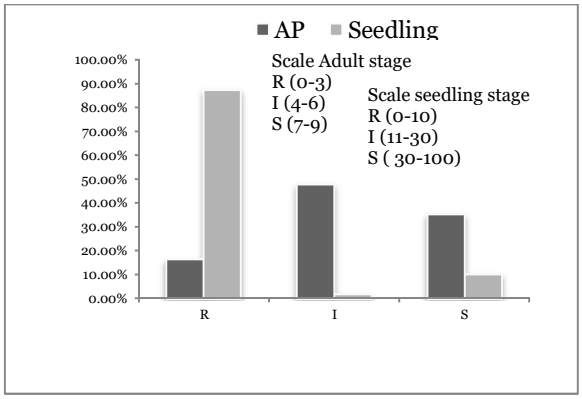

Fig 1. Frequency of segregating wheat NIGAB-08 Resistant, Intermediate and Susceptible at Adult Plant stage and seedling stage against stripe rust.

NIGAB-09 (F_6 Segregating wheat Population)

86 wheat lines have been developed as a segregating population for stripe rust resistance. Among these wheat lines, 83% of wheat population was resistant with Infection type (IT) 0-3 and 5% of population showed intermediate type of resistance with IT value 4-6, while, 13% of population was found to be susceptible in this experiment at seedling stage.

Field experiments indicate that the 79% of population was resistant (RAUDPC=0-10), while 11% of population showed intermediate type of resistance (RAUDPC= 11-30) and 10% of population was observed to be susceptible in field experiments (RAUDPC >30) (Fig.2).

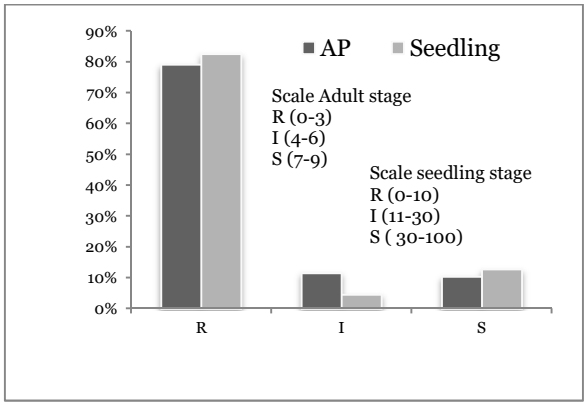

Fig. 2. Frequency of segregating wheat NIGAB-09 Resistant, Intermediate and Susceptible at Adult Plant stage and seedling stage against stripe rust.

Seedling resistance is a race specific resistance, so it is short lived due to mutation in virulence in the

pathogen population (Line *at al.*, 1995) while resistance at the adult plant stage including high temperature adult plant resistance or may be called as slow rusting is considered to be more durable than seedling resistance (Mclntosh, 1992: Chen and Line, 1995). Race specific genes in wheat germplasm can be identified by comparing their resistance to various pathotypes of *Puccinia graminis tritici* with lines having known resistant gene (Singh *et al.*, 2000). In the present study, pathotype's race having virulent genes *Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yrsp Yr2, Yr25, Yr31* and *YrA* have been used at CDRI, Murree. 87% of first wheat segregating wheat population. (NIGAB-08) showed resistance to these races while 10% are found to be susceptible at seedling stage. Second wheat population (NIGAB-09) showed that 82% of population are resistant at seedling stage and 10% are susceptible to stripe rust. It indicate the likely presence of race specific genes *Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yrsp Yr2, Yr25, Yr31* and *YrA* in segregating wheat population which was used as inoculum in this experiment while susceptible ones lack these genes. Only 2% and 5% of population showed intermediate type of resistance in NIGAB-08 and NIGAB-09 respectively.

Wheat cultivars having slow rusting gene become sometime susceptible at the seedling stage but showed moderate to high form of resistance in field screening. Field experiment was conducted in Islamabad as it is reported to be a hot spot region for stripe rust (Bux *et al.*, 2011). Cool and wet weather is suitable for development of yellow rust. Light yellow color pustules present on leaves in straight stripes are yellow to orange color. Disease severity also affects the yield attributing traits (Wellings, 2011). Disease data is used to calculate RAUDPC at adult plant stage by comparing with severity of susceptible check Morocco. RAUDPC was used by various plant pathologists for data analysis of stripe rust (Ma *et al.*, 1995). Field observation indicate that 16% population of NIGAB-08 population is resistant to stripe rust with RAUDPC=0-10 while 35% of population is found to be susceptible in field experiment (RAUDP=10-30) and rest of population showed intermediate type of resistance RAUDPC >30. Whereas 79% of NIGAB-09 population showed its resistance against stripe rust in field experiment and 10% showed their susceptibility to yellow rust.

If we look at whole segregating wheat population in individual perspective, some wheat lines showed very strong resistance against yellow rust both in field as well as in greenhouse trials. These wheat lines are line no. 23, 36 and 40 among NIGAB-08 and line no. 2, 5, 6, 10, 16, 17, 19, 20, 21, 22, 24, 26, 27, 28, 29, 30, 31, 32, 33, 34, 34, 35, 36, 37,39, 40, 42, 44, 45, 46, 49, 52, 53, 54, 57, 60, 61, 62, 68, 70, 71, 73, 82, 83, 84, 86, 89, 94, 95, 97, 98, 105, 108, 109, 110 of NIGAB-09 as mentioned in table 1 and 2. These wheat lines have been supposed to be enriched with major and minor genes of stripe rust and may perform better in commercial application. Inoculum which is used in field experiment comprised of mixed diversified races including *Yr5, Yr10, Yr15* and *Yrsp* and in greenhouse experiment is *Yr6, Yr7, Yr8, Yr9, Yr17, Yr27, Yrsp Yr2, Yr25, Yr31* and *YrA*. Results revealed that resistant wheat lines, having strong resistance at seedling and adult plant stage may have likely presence of these yellow rust resistance genes.

Table 1. Rust notes, CI, AUDPC, seedling data and RAUDPC data of segregating population NIGAB-08.

Sr. No.	NIGAB-08	CI	AUDPC	RAUDPC	Yr seedling score
1	1	15	355	16.14	0
2	3	27	785	35.68	9
3	4	3	160	7.27	3
4	6	45	1080	49.09	9
5	8	30	860	39.09	0
6	9	18	530	24.09	0
7	10	4.5	320	14.55	0
8	11	9	320	14.55	4
9	15	18	530	24.09	8
10	16	9	320	14.55	0
11	19	36	750	34.09	0
12	20	27	630	28.64	1
13	21	63	1180	53.64	0
14	22	30	665	30.23	3
15	23	0	0	0.00	0
16	24	36	630	28.64	0
17	25	18	715	32.50	0
18	26	4.5	160	7.27	0
19	27	9	320	14.55	1
20	29	15	495	22.50	0
21	30	28	530	24.09	0
22	32	9	320	14.55	1
23	34	6	320	14.55	1
24	35	6	100	4.55	3

Sr. No.	NIGAB-08	CI	AUDPC	RAUDPC	Yr seedling score
25	36	0	0	0.00	0
26	37	52	995	45.23	0
27	38	10	355	16.14	1
28	39	4.5	320	14.55	1
29	40	0	0	0.00	2
30	41	9	320	14.55	1
31	42	45	665	30.23	0
32	44	40	710	32.27	0
33	45	58.5	1030	46.82	3
34	46	18	530	24.09	0
35	47	36	885	40.23	1
36	48	30	785	35.68	0
37	49	18	530	24.09	0
38	51	18	455	20.68	0
39	53	12	355	16.14	0
40	54	39	1180	53.64	8
41	56	49.5	1005	45.68	0
42	57	4.5	197.5	8.98	0
43	60	3	160	7.27	1
44	61	12	320	14.55	0
45	62	58.5	1060	48.18	0
46	63	6	440	20.00	8
47	66	12	320	14.55	0
48	67	65	1180	53.64	0

Table 2. Rust notes, CI, AUDPC, seedling data and RAUDPC data of segregating population NIGAB-9.

Sr. No.	NIGAB-09 lines	CI	AUDPC	RAUDPC	Yr Score
1	2	3	68	3.09	0
2	3	60	960	43.64	0
3	5	2	50	2.27	0
4	6	8	177	8.05	0
5	8	4	142	6.45	8
6	10	4	142	6.45	0
7	11	4	142	6.45	0
8	12	6	177	8.05	9
9	13	24	715	32.50	0
10	15	36	715	32.50	0
11	16	3	92	4.18	0
12	17	2	50	2.27	0
13	18	3	110	5.00	8
14	19	4	138	6.27	0
15	20	9	138	6.27	0
16	21	9	160	7.27	0
17	22	3	110	5.00	9
18	24	3	110	5.00	0
19	25	2	50	2.27	0
20	26	0	0	0.00	0
21	27	2	50	2.27	7
22	28	0	0	0.00	0
23	29	3	110	5.00	0
24	30	2	18	0.82	0
25	31	0	0	0.00	0
26	32	0	0	0.00	0
27	33	3	110	5.00	0
28	34	3	110	5.00	0
29	35	4	160	7.27	0
30	36	0	0	0.00	1
31	37	0	0	0.00	0
32	38	6	195	8.86	0
33	39	3	110	5.00	0
34	40	2	50	2.27	0
35	42	6	195	8.86	0
36	43	2	50	2.27	9
37	44	3	110	5.00	0
38	45	4	160	7.27	0
39	46	2	50	2.27	0
40	47	6	233	10.59	0
41	49	2	18	0.82	0
42	51	4	160	7.27	0
43	52	0	0	0.00	0
44	53	0	0	0.00	0
45	54	3	128	5.82	0
46	56	0	0	0.00	4
47	57	4	160	7.27	0
48	60	4	160	7.27	0
49	61	0	0	0.00	0
50	62	4	160	7.27	0
51	64	6	233	10.59	0
52	65	4	160	7.27	4
53	66	6	160	7.27	1
54	68	2	62	2.82	0
55	69	4	160	7.27	4
56	70	4	160	7.27	7
57	71	2	62	2.82	0
58	73	0	0	0.00	0
59	74	6	233	10.59	0
60	75	8	298	13.55	0
61	76	55	1,125	51.14	7
62	79	50	1,375	62.50	0
63	82	6	195	8.86	0
64	83	0	0	0.00	0
65	84	4	160	7.27	0
66	85	6	233	10.59	0
67	86	6	177	8.05	0
68	87	24	715	32.50	7
69	88	50	1,190	54.09	0
70	89	2	62	2.82	0
71	91	24	715	32.50	0
72	92	0	0	0.00	8
73	93	0	0	0.00	5
74	94	0	0	0.00	0
75	95	6	160	7.27	0
76	96	6	160	7.27	8
77	97	2	62	2.82	0
78	98	2	18	0.82	0
79	101	24	433	19.68	0
80	105	0	0	0.00	0
81	106	21	530	24.09	0
82	107	50	1,190	54.09	0
83	108	4	160	7.27	9
84	109	0	0	0.00	0
85	110	0	0	0.00	0
86	113	15	355	16.14	0

There is a need to explore wheat germplasm, further in molecular analysis with available yellow rust markers. As Bux *et al.*, 2012 reported the genetic potential in synthetics, advanced and Chinese

cultivars for stripe rust and concluded that both types of seedling and adult plant resistance offer promising genetic enrichment to acquire long lasting durable control against yellow rust in Pakistan. Genetic erosion in wheat germplasm demands to broaden the genetic background and need diversification for developing stripe rust resistance in wheat varieties.

Conclusion

It is necessary to broaden the gene pool (comprised of stripe rust gene) of future wheat varieties of Pakistan by introducing the multiple rust resistant genes to overcome stripe rust disease. Developing segregating populations having wide genetic background of stripe rust resistance genes is a contribution to cope with stripe rust stress, when the pathogen of disease is highly adapted to environment and variable in its genetic makeup. This segregating population which has been developed in years is enriched with stripe rust resistance major and minor genes which can be an effective control measure against stripe rust disease.

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