Evaluation of Advance Wheat Lines for Slow Yellow Rusting (*Puccinia striiformis* f. sp. Tritici)

Sobia Tabassum

Department of Bioinformatics and Biotechnology, International Islamic University

H-10 Campus, Islamabad, Pakistan

E-mail: sobia.tabasum@iiu.edu.pk

Abstract

Stripe rust (yellow rust), caused by *Puccinia striiformis* f. sp. *tritici* is one of the most damaging diseases of wheat in Pakistan. Lack of durable resistance in local wheat varieties is the main reason for stripe rust epidemic which could limit yields. The use of genetically resistant wheat varieties is the most economic way of controlling the disease. Evaluation of 135 advance wheat lines for slow yellow rusting was conducted during cropping season 2008-2009 under natural epidemics at field locations of Ayub Agriculture Research Institute, Faisalabad and Cereal Crops Research Institute, Pirsabak (Northwest), Pakistan. Resistance level based on final disease severity (FDS) along with other slow rusting parameters relative area under disease progress curve (rAUDPC) and relative infection rate (RIR) was assessed. A total of 25 wheat lines were identified to potentially have durable resistance with low frequency of disease severity (10-30%) and lower relative AUDPC values (2-66%). Based on the slowing rusting data, RAPD (random amplified polymorphic DNA) primers were used in order to evaluate genetic diversity among 25 lines. Of 20 OPA (Operon series) primers tested, 4 (20%) primers were polymorphic that showed amplification differences among 25 genotypes. OPA-06 and OPA-04 revealed the highest polymorphism (67% and 50%) while OPA-02 and OPA-17 exhibited the lowest polymorphism (33% and 25%) respectively. From the amplification profile, a total of five RAPD markers were obtained in this study. A similarity matrix data depicted that most of these genotypes are genetically very close (60-100%). The 25 advance lines identified from slow rusting evaluation with five RAPD markers may have partial resistance genes and can be used as slow yellow rusting lines with longer field life in Pakistan breeding program.

Keywords: RAPD, Stripe rust, Slow yellow rusting, Wheat genotypes

Abbreviations:

AUDPC: area under disease progress curve

IT: infection types, FDS: final disease severity

UPGMA: Unweighted Pair-Group Method with Arithmetic Averages

RAPD: Random Amplified Polymorphic DNA

1. Introduction

Stripe rust caused by *Puccinia striiformis* f.sp. *tritici* continue to pose a major threat to wheat production over large areas, particularly in Asia. In this continent, leaf and stripe rust could affect production on approximately 60 (63%) and 43 (46%) million hectares, respectively, if susceptible cultivars are grown (Singh et al. 2005). Developing durable resistance in wheat cultivars against rapidly evolving new pathogens is one of most sustaining way to control rust diseases. Two major types of resistance have been identified and used in breeding programs. They are; race-specific seedling resistance and non-race specific high-temperature, adult- plant resistance (HTAP) (Line and Chen, 1995). The difference between race-specific (vertical) and non-race-specific or general (horizontal) resistance has been defined by Van der Plank (1968). On this definition, a variety with specific resistance is resistant to some races, but is susceptible to others, whereas a variety with general resistance shows a degree of resistance to all known races. Mature plant resistance and environmentally determined resistance are generally considered to be non-race-specific forms of resistance. Also other terms such as 'slow-rusting' and 'horizontal' resistance are used to describe certain resistance patterns of this type. This resistance may be conditioned by groups of minor genes and may not be so easily and rapidly overcome. Wheat cultivars with slow rusting genes are often susceptible at the seedling stage, but may be moderately to highly resistant to all pathotypes at the adult plant stage in the field (Singh et al. 2000). The components of slow rusting include a longer latent period, low infection frequency, smaller uredial size, and reduced duration and quantity of

spore production (Caldwell 1968). Three currently named stripe rust resistance genes, *Yr18, Yr29,* and *Yr30,* confer slow rusting (Singh 1992, Suenaga et al. 2003, William 2003), and many more are suspected. In Pakistan, the leading cultivars like Inqilab-91 and MH97 (Attila) grown by the farmers are susceptible to stripe rust. The new stripe rust race has been reported to attack cultivars possessing *Yr27* in India, Yemen, Egypt, Ethiopia, Eritrea, Tajikistan, Uzbekistan and Kyrgyzstan during previous years (Singh et al. 2004, Afshari 2004). It has brought over 11.0 million hectare of wheat growing area of Indo-Pakistan Subcontinent alone under threat of a yellow rust epidemic, due to cultivation of three important cultivars Inqilab-91, MH-97 and PBW343 possessing *Yr27*. So there is need to develop slow yellow rusting resistant genetic stock to improve the leading high yielding cultivars of Pakistan like Inqilab-91 and MH-97. The wheat crop can be protected from rust, or at least the occurrence of epidemics in Pakistan could be reduced by enhanced information on the genetic basis of resistance in important wheat cultivars and deploying wheat cultivars with durable resistance. DNA-based molecular markers are especially useful in plant breeding since these markers provide an efficient method for scoring segregating populations and identifying the desirable genotypes without waiting for the phenotypic expression of the gene (Brown et al. 1996). As far as the ability of polymorphism detection is concerned, RAPDs are the most useful markers (Joshi and Nguyen 1993; Kuczynska et al. 2001; Weber et al. 2005). Being rapid, efficient and amenable to automation, RAPD markers have been proved to be effective for estimation of germplasm diversity (Virk et al. 1995).

The aim of this study was to evaluate the 135 advance wheat lines for slow yellow rusting under different field conditions. Further the molecular diagnostic in these lines was carried out using RAPD markers for evaluation of genetic diversity in these lines. Based on the slow rusting trait, the tested lines may have genes for varying degree of slow yellow rusting and can be used for further manipulation in wheat breeding program.

2. Materials and methods

2.1 Wheat germplasm

The wheat germplasm comprised of 135 advance wheat lines derived from three crosses: Inqilab91 X SA42, MH97 X SA42 and Parula X SA42, received from AARI (Ayub Agriculture Research Institute Faisalabad). Two breeding methods i.e., double haploid production (DH) and single seed descent methods (SSD) were used to develop homozygosity in these F_6 advanced wheat lines.

2.2 Field testing

A total of 135 advance wheat lines along with their parents were evaluated for slow yellow rusting at adult plant stage under natural infection of *P. striiformis* f. sp. *tritici*. These lines along with parents were planted during cropping season 2008-2009 at the experimental farms of the Ayub Agriculture Research Institute Faisalabad and Cereal Crops Research Institute, Pirsabak, (34°N Latitude, 72°E Longitude and 288m Altitude) NWFP, Pakistan. The Pirsabak station is located at sea level and it was selected for the reason that it is known as the hot spot due to favorable environmental conditions for stripe rust. Each plot was 1 m^2 and contained six rows which were 27 cm apart. Susceptible check Morocco was planted all around the experimental field for creating stripe rust epidemic. Rust severity (percentage) and responses of plants were assessed for three consecutive observations with 10 days intervals according to modified Cobb scale method (Peterson et al. 1948) and infection types were scored using a 0 to 4 scale (Bariana and McIntosh 1993). The disease severity data were used to calculate the area under the disease progress curve (AUDPC).

$$\text{AUDPC} = \sum i\ [(xi + x\ i+1)/2]ti,$$

where xi is the severity value on date i, ti the time in days between dates i and $i + 1$ (Chen and Line 1995a). Relative AUDPC (rAUDPC), calculated using actual AUDPC divided by the AUDPC of the check (Morocco) and final disease severity (FDS), when the susceptible check (Morocco) displayed maximum disease severity, were used (Ma and Singh 1996). Infection rate was estimated as the unit leaf area damaged by disease per day when Morocco showed > 50% severity, following Vanderplank (1968). Relative infection rate was (RIR) was calculated by comparing infection rate value with susceptible check.

2.3 DNA extraction and RAPD analysis

DNA extraction from fresh leaves (2g) of 135 advance lines along with their parents (at two leaf seedling stage) was carried out according to the the cetyltrimethylammonium bromide (CTAB) procedure described by Murray and Thompson (1980). RAPD analysis was carried out with 10 decamer primers of Operon technologies, Inc. A total of 20 random decamer primers of Operon series were used (Table 2). PCR amplifications were performed in 25µl reaction volume containing 20 ng genomic DNA, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl2, 50 mM

KCl, 0.2 mM dNTPs (Sigma Chemical Co., St. Louis, MO), 1 U T*aq*-polymerase (Promega, Madison, WI), 0.2 µM primer. Amplifications were performed in Perkin Elmer Thermal Cycler, after 5 min of denaturation at 94°C, amplifications were programmed for 45 cycles each consisting of 1 min at 92°C, 2 min 36°C and 2 min at 72°C followed by 7 min extension step at 72°C. PCR-products were mixed w loading buffer and analysed by electrophoresis in 1.2% agarose gels in 0.5 X TBE buffer (0.089 M Tris-borate, 0.089M boric acid, and 0.002M EDTA). A 1-kb DNA ladder was used to estimate the size of each amplified DNA fragment. The gel was run for 90 min at 100 V, stained with ethidium bromide (0.5 µg/ml) and photographed under UV light.

2.4 Data analysis

To determine the genetic relationships among wheat advance wheat lines, dendrogram based on the RAPD banding patterns were constructed using the Numerical Taxonomy System (NTSYS-pc), version 2.1 (Rohlf 1993). All the amplified bands were treated as dominant genetic markers. The presence of a band in a polyacrylamide gel was coded as 1 and its absence was coded as 0. A similarity matrix based on simple matching was generated by the SIMQUAL program, and cluster analysis was performed with the unweighted pair group arithmetic mean method (UPGMA) in the SAHN program.

3. Results

3.1 Evaluation of advance wheat lines for slow yellow rusting at adult plant stage

A total of 135 advance wheat lines were evaluated for slow yellow rusting resistance at adult plant stage under natural infection at Ayub Agriculture Research Institute, Faisalabad and Cereal Crops Research Institute, Pirsabak (Northwest), Pakistan. Following parameters were used to assess slow yellow rusting in advance wheat lines at both locations.

3.2 Final Disease Severity (FDS)

Of 135 lines, 47 (35%) genotypes showed no visible symptoms (FDS 0%), 60 (44%) were resistant (Disease Severities 5-30%), 24 (17%) were intermediates (FDS 30-70%), and 4 (3%) genotypes were susceptible (FDS90-100%). The same set of 135 advance wheat breeding lines was also evaluated for stripe rusting at and Pirsabak (Northwest) location of Pakistan. At the Pirsabak location, 81 (60%) genotypes showed no visible symptoms (FDS 0), 24 (18%) were resistant (FDS 5-30%), 29 (21%) intermediate (FDS 30-70%), and 1 (0.74%) susceptible (FDS 100%) as shown in Figure 2. FSD of susceptible check Morocco was 100 in Faisalabad and Pirsabak (Figure 2), indicating that disease developed well at both locations.

3.3 Relative Area under Disease Curve (rAUDPC)

Based on the AUDPC values, 135 advance lines were categorized in to two distinct groups. The first group comprised lines exhibiting relative AUDPC values up to 30% of check, while lines showing relative AUDPC values up to 70% of check were placed in second group. This second group was ranked as moderately resistant or moderately susceptible. At this stage rust develops at slower rate. All those lines of group 1 were ranked as better slow rusting and that of group 2 were marked as moderately slow rusting because rust develops slowly at this stage exhibiting high infection types, but low FDS and AUDPC in the field tests.

At the Faisalabad location of the 135 lines, 60 (44%) lines showed relative AUDPC values less than 30% of susceptible check and these were marked as better slow rusting lines. 24 (17%) lines showed relative AUDPC values up to 70% and these were regarded as moderately slow rusting 4 (3%) lines exhibited high susceptible reaction as their relative AUDPC values were higher than 90% (Figure 2). 47 (35%) lines showed no symptoms at this location (rAUDPC 0). At the Pirsabak location, 24 (18%) lines showed relative AUDPC values less than 30% and these were regarded as better slow rusting, 29 (21%) lines showed relative AUDPC values up to 70% and these were moderately slow rusting lines, 1 (0.74%) line was found highly susceptible as it exhibited relative AUDPC value up to 100% of the susceptible check and remaining 81 (60%) lines showed no visible symptoms as shown in Figure 2. Twenty five lines showed low frequency of disease severity (10-30%) with lower rAUDPC values (2-66%) at two locations and these lines are considered as better slow rusting lines (Table 1). The correlations of FDS and relative AUDPC at two locations were 0.99 and 0.90, respectively, indicating a high consistency of the test in two locations for slow rusting resistance.

3.4 Relative Infection Rate (RIR)

Relative infection rate ranged as 0 for 39 lines at two locations. These lines showed no visible symptoms. At two locations 14 lines exhibited relative infection rate values 25-300 while 11 lines showed moderately slow rusting behavior as they showed relative infection rate values 325-600 at two locations. Maximum frequency of relative

infection rate (1000-1050) was present in five lines at two locations while it ranged as 700-900 for the remaining lines.

3.5 Molecular characterization of advance breeding lines using RAPD markers

RAPD primers were used to evaluate genetic diversity among 25 advance wheat lines which were selected as better and moderately slow rusting lines along with their parents. Of 20 RAPD primers tested, 4 (20%) primer that generated strong and repeatable polymorphic bands were selected (Table 3). The total number of amplification products produced was 100 with an average of 3 loci per primer. All the RAPD primers screened produced a number of well resolved bands in the 100-2500 bp range. A total of five RAPD markers were obtained in this study. The highest polymorphisms detected with RAPD primers were 67% and 50 with the mean polymorphism of 42%. OPA-06 and OPA-04 revealed the highest polymorphism (67% and 50%) while OPA-02 and OPA-17 exhibited the lowest polymorphism (25%) as shown in Table 3.

Figure 3 illustrates the agarose gel electrophoresis pattern detected among 19 (65%) genotypes using the primer OPA-02. A band of 510 bp that was present in 16 genotypes (P1, P2, P4, 1, 2, 3, 4, 5, 8, 9, 10, 11, 12, 13, 14 and 15) but it was absent in 3 genotypes (P3, 6 and 7). Figure 4 shows amplification of 18 (62%) genotypes with primer OPA-04. A band of fragment size 300 bp was present in 17 genotypes (P1, P2, P3, P4, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12 and 13). Figure 5 illustrates amplification of 19 (65%) genotypes with primer OPA-06. This primer exhibited the highest polymorphism (67%). A band of 400 bp was absent in 7 genotypes (P2, P3, 1, 3, 4, 6 and 7) while another band of 290 bp was absent in 8 genotypes (P3, 1, 3, 4, 6, 7, 10 and 12). Figure 6 shows amplification of 12 (41%) genotypes with primer OPA-18 which exhibited polymorphism in 4 genotypes (P2, P3, 1 and 4).

3.6 Cluster analysis based on RAPD data

Cluster analysis based on polymorphic RAPD bands separated the 25 wheat genotypes into two main clusters, A and B according to their grouping at the 80% similarity level approximately. Cluster A comprised of 10 genotypes where 8 genotypes (QA-1, QA-6, QA-18, JINNAH-4, JINNAH-29, AF-1, QA-26 and QA-25) grouped together with 2 genotypes (QA-23 and QA-35). The second cluster B is further divided into three sub clusters. The first sub-cluster (b1) is composed of 5 genotypes (QA-2, QA-10, JINNAH-3, AF-20 and QA-24). The second sub-cluster (b2) is smallest one grouped with 3 genotypes (QA-32 and JINNAH-6 and JINNAH-15). The third sub-cluster (b3) is the largest sub-cluster of MAIN cluster B where genotypes QA-4 is grouped with 6 genotypes (JINNAH-1, JINNAH-26, JINNAH-9, JINNAH-16, JINNAH-25 and JINNAH-7) as shown in Figure 7. The cophenetic correlation for the resulting RAPD dandrogram was 0.79. A similarity matrix data depicted that most of these genotypes are genetically very close (80-100%) as shown in Table 4.

4. Discussion

In the present study fast yellow rusting wheat varieties, Inqilab 91and MH 97 showed higher values of relative AUDPC (80 % and 79%) while the varieties classified as resistant to moderately resistant were SA 42 and Parula with lower values of relative AUDPC (2 %and 50%), respectively. Hong and Singh (1996) studied field resistance to stripe rust and classified wheat varieties as highly resistant with relative AUDPC less than or equal to 5% of Morocco, acceptable resistant (relative AUDPC less than or equal to 20%) and moderately resistant (relative AUDPC 30-50%). Similar classification of wheat varieties were also made by Khan et al. (2001). A total of 135 were planted at two different locations to evaluate their slow yellow rusting responses to stripe rust. Five lines (QA-9, QA-12, QA-28, JINNAH-31, JINNAH-32) showed maximum relative final disease severity 80-100% of susceptible check with higher values of relative infection rates (1000-1050) during present evaluation based on relative infection rate (RIR). These lines were found highly susceptible and were considered very poor slow rusting lines based on their relative infection rate values. Similar results were found by Ali et al. (2007) during evaluation of 20 wheat breeding lines for slow yellow rusting. Previous studies showed that final disease severity is one of the parameter which can be used to measure the resistance levels along with other slow rusting parameters (Parlevliet and van Omeran 1975; Li et al. 2006).

Slow rusting behavior was also assessed through AUDPC which is another parameter to evaluate slow rusting resistance level during present study. At two locations, 14 lines exhibited relative AUDPC values less than 30% of Morocco and were marked as better slow rusting. 11 lines showed moderately slow rusting behavior as they showed relative AUDPC values up to 70% at two locations. These twenty five lines showed low frequency of final disease severity (10-30%) with lower rAUDPC values (2-66%) with relative infection rate values 25-600 were considered as better slow rusting lines. These lines showed better slow yellow rusting behavior because in these lines rust development was slower as compared to the remaining lines. Breeding lines with such slow rusting traits are expected to possess genes that confer partial resistance which is more durable type of resistance

(Parlevliet 1988) and it has been emphasized recently (Singh et al 2004). In the present study a high correlation (0.99) was found between FDS and AUDPC which is consistent with previous studies made by Li et al. (2006) during the evaluation of 135 wheat lines for slow rusting responses to stripe rust at two locations in China.

Genetic diversity was determined in advance wheat lines using RAPD primers. The 25 advance lines were selected based on slow rusting resistance at two locations. Of 20 OPA (Operon series) primers tested, 4 (20%) primers were polymorphic that showed amplification differences. The total number of amplification products produced was 100 with an average of 3 loci per primer. OPA-06 and OPA-04 revealed the highest polymorphism (67% and 50%) while OPA-02 and OPA-17 exhibited the lowest polymorphism (33 % and 25%), respectively. A total of five RAPD markers were obtained in this study. RAPDs were successfully used to analyze genetic variability between wheat varieties (Devos and Gale 1992; He et al. 1992) and for the development of closely linked markers to important characters. RAPD markers linked to important resistance genes in wheat were described by Qi et al. (1996) and Demeke et al. (1996).

In this present study DNA fingerprinting profiles were found useful to confirm the genetic relationships among closely related lines. RAPD assay showed that most of the advance lines share common amplification bands confirming the genetic similarities among these lines. The lack of genetic variations in most of these lines could be attributed to similar parentage. Genetic similarity matrix based on simple matching (SM) coefficient ranging from 60 to 100%. The cophenetic correlation for the resulting RAPD dandrogram was 0.79. A similarity matrix data depicted that 10 (40%) genotypes (QA-1, QA-6, QA-18, JINNAH-4, JINNAH-29, AF-1, QA-26, QA-25, QA-23 and QA-35) form a distinct group indicated that these genotypes were most distant from the remaining genotypes with the lowest similarity index (0.32). Cluster analysis based on molecular and slow yellow rusting data is useful in identifying genetic relationships among 25 advance lines. Similarly Mukhtar et al. (2002) studied genetic diversity in Pakistan wheat using random amplified polymorphic DNA (RAPD) markers.

It is concluded that the identified 25 advance wheat lines from slow rusting data with five RAPD markers may possess partial resistance genes and these are proved to be durable stripe rust resistant lines with longer field life. These lines may be exploited for slow rusting resistance in Pakistan wheat breeding program.

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Table 1. Advance wheat lines showing slow rusting parameters at Faisalabad and Pirsabak locations during 2007-2008

Sr.No	Genotypes	Parentage / Cross	Relative Infection Rate	Final Disease Severity	Relative AUDPC Value
P1	INQILAB 91	WL711/CROW 'S'	950	80	80
P2	MH 97	ATTILA=ND/VG9144//KAL/BB/3/YACO/4/VEE#5	950	80	79
P3	PARULA	FRN3/2*FR//KAD/GB/4/BB/CHA	600	40	50
P4	SA42	C271/LR64//SON64	25	5	2
1	QA-1	INQ.91 X SA42	100	20	10
2	QA-6	INQ.91 X SA42	100	20	10
3	QA-18	INQ.91 X SA42	25	5	2
4	JINNAH--4	MH97 X SA42	25	5	2
5	JINNAH--29	MH97 X SA42	25	5	2
6	AF-1	PARULA X SA42	225	25	19
7	QA-26	INQ.91 X SA42	225	25	19
8	QA-25	INQ.91 X SA42	200	20	19
9	QA-23	INQ.91 X SA42	225	20	19
10	QA35	INQ.91 X SA42	200	25	19
11	QA-2	INQ.91 X SA42	225	25	19
12	QA-10	INQ.91 X SA42	25	5	2
13	JINNAH-3	MH97 X SA42	50	10	5
14	AF-20	PARULA X SA42	125	20	12
15	QA-24	INQ.91 X SA42	400	40	38
16	QA-32	INQ.91 X SA42	450	40	38
17	JINNAH-6	MH97 X SA42	475	50	40
18	JINNAH-15	MH97 X SA42	600	60	50
19	QA-4	INQ.91 X SA42	350	40	33
20	JINNAH-1	MH97 X SA42	375	40	36
21	JINNAH-26	MH97 X SA42	650	70	62
22	JINNAH-9	MH97 X SA42	450	50	43
23	JINNAH-16	MH97 X SA42	650	70	62
24	JINNAH-25	MH97 X SA42	475	50	40
25	JINNAH-7	MH97 X SA42	600	70	57
	MOROCCO	Susceptible Check	100	100	100

Table 2. Sequences of decamer RAPD primers used in the molecular analysis of slow rusting resistance among advance wheat lines

Sr. No	Primer	Sequence 5' to 3'	Molecular weight
1	OPA-01	CAGGCCCTTC	2955
2	OPA-02	TGCCGAGCTG	3035
3	OPA-03	AGTCAGCCAC	2988
4	OPA-04	AATCGGGCTG	3059
5	OPA-05	AGGGGTCTTG	3090
6	OPA-06	GGTCCCTGAC	2995
7	OPA-07	GAAACGGGTG	3108
8	OPA-08	GTGACGTAGG	3099
9	OPA-09	GGGTAACGCC	3044
10	OPA-10	GTGATCGCAG	3059
11	OPA-11	CAATCGCCGT	2979
12	OPA-12	TCGGCGATAG	3059
13	OPA-13	CAGCACCCAC	2933
14	OPA-14	TCTGTGCTGG	3041
15	OPA-15	TTCCGAACCC	2939
16	OPA-16	AGCCAGCGAA	3037
17	OPA-17	GACCGCTTGT	3010
18	OPA-18	AGGTGACCGT	3059
19	OPA-19	CAAACGTCGG	3028
20	OPA-20	GTTGCGATCC	3010

Table 3. RAPD Primers showing Polymorphism for slow yellow rusting in advance wheat genotypes

Sr. No	RAPD Primers	Total loci	Polymorphic loci	%Polymorphism
1	OPA-02	3	1	33%
2	OPA-04	2	1	50%
3	OPA-06	3	2	67%
4	OPA-17	4	1	25%

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Table 4. RAPD similarity matrix (based on Simple Matching (SM) coefficient) of 25 wheat genotypes

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	1																								
2	0.6	1																							
3	0.2	0.6	1																						
4	1	0.6	0.2	1																					
5	0.4	0.8	0.8	0.4	1																				
6	1	0.6	0.2	1	0.4	1																			
7	0.6	0.6	0.6	0.6	0.8	0.6	1																		
8	0.4	0.8	0.8	0.4	1	0.4	0.8	1																	
9	1	0.6	0.2	1	0.4	1	0.6	0.4	1																
10	0.4	0.4	0.8	0.4	0.6	0.4	0.8	0.6	0.4	1															
11	0.4	0.4	0.8	0.4	0.6	0.4	0.8	0.6	0.4	1	1														
12	1	0.6	0.2	1	0.4	1	0.6	0.4	1	0.4	0.4	1													
13	0.8	0.8	0.4	0.8	0.6	0.8	0.4	0.6	0.8	0.2	0.2	0.8	1												
14	0.4	0.8	0.8	0.4	1	0.4	0.8	1	0.4	0.6	0.6	0.4	0.6	1											
15	0.6	0.6	0.2	0.6	0.4	0.6	0.2	0.4	0.6	0	0	0.6	0.8	0.4	1										
16	0.6	0.6	0.6	0.6	0.8	0.6	0.6	0.8	0.6	0.4	0.4	0.6	0.8	0.8	0.6	1									
17	0.8	0.8	0.4	0.8	0.6	0.8	0.4	0.6	0.8	0.2	0.2	0.8	1	0.6	0.8	0.8	1								
18	0.8	0.8	0.4	0.8	0.6	0.8	0.4	0.6	0.8	0.2	0.2	0.8	1	0.6	0.8	0.8	1	1							
19	0.6	0.6	0.2	0.6	0.4	0.6	0.2	0.4	0.6	0	0	0.6	0.8	0.4	1	0.6	0.8	0.8	1						
20	0	0.4	0.8	0	0.6	0	0.4	0.6	0	0.6	0.6	0	0.2	0.6	0.4	0.4	0.2	0.2	0.4	1					
21	0	0.4	0.8	0	0.6	0	0.4	0.6	0	0.6	0.6	0	0.2	0.6	0.4	0.4	0.2	0.2	0.4	1	1				
22	0	0.4	0.8	0	0.6	0	0.4	0.6	0	0.6	0.6	0	0.2	0.6	0.4	0.4	0.2	0.2	0.4	1	1	1			
23	0	0.4	0.8	0	0.6	0	0.4	0.6	0	0.6	0.6	0	0.2	0.6	0.4	0.4	0.2	0.2	0.4	1	1	1	1		
24	0	0.4	0.8	0	0.6	0	0.4	0.6	0	0.6	0.6	0	0.2	0.6	0.4	0.4	0.2	0.2	0.4	1	1	1	1	1	
25	0	0.4	0.8	0	0.6	0	0.4	0.6	0	0.6	0.6	0	0.2	0.6	0.4	0.4	0.2	0.2	0.4	1	1	1	1	1	1

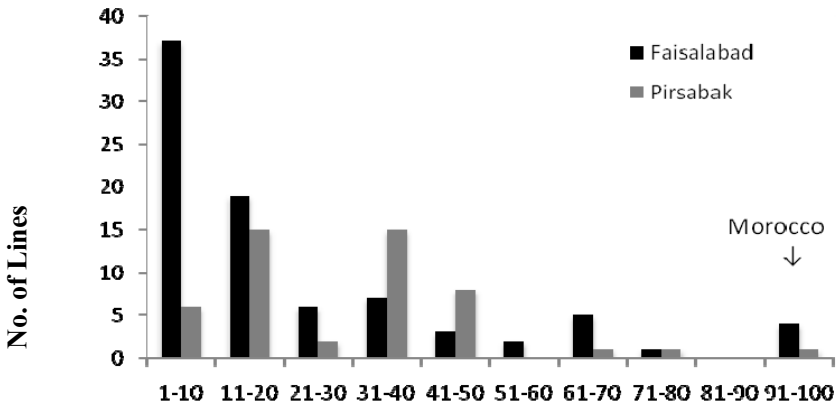


Figure 1. Realtive AUDPCs for advance wheat lines along with susceptible check, at Faisalabad and Pirsabak

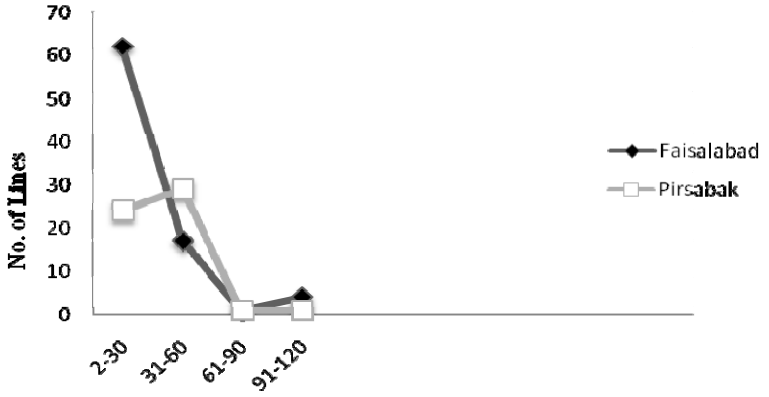


Figure 2. Frequency distributions of relative disease severity for advance wheat lines at Faisalabad and Pirsabak

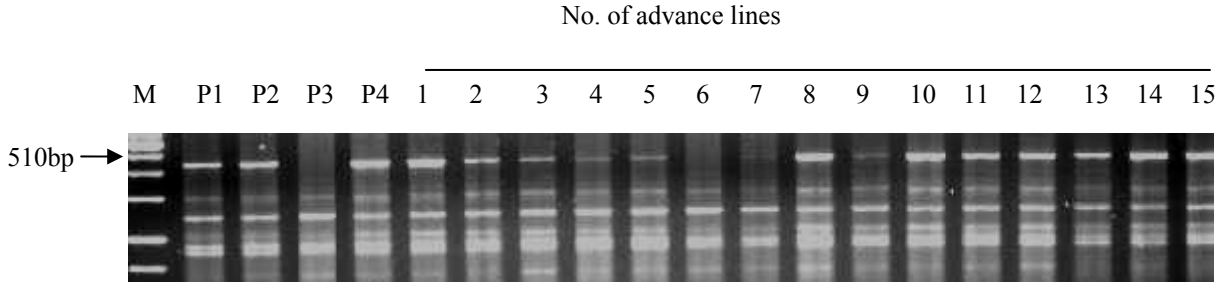


Figure 3. Ethidium bromide stained Agrose gel showing amplification by OPA-02 primer

M: 1 kb ladder, P1: Inqilab 91, P2: MH 97, P3: PARULA, P4: SA 42

1: QA-1, 2: QA-6, 3: QA-18, 4: JINNAH—4, 5: JINNAH—29, 6: AF-1, 7: QA-26, 8: QA-25, 9: QA-23

10: QA35, 11: QA-2, 12: QA-10, 13: JINNAH-3, 14: AF-20, 15: QA-24

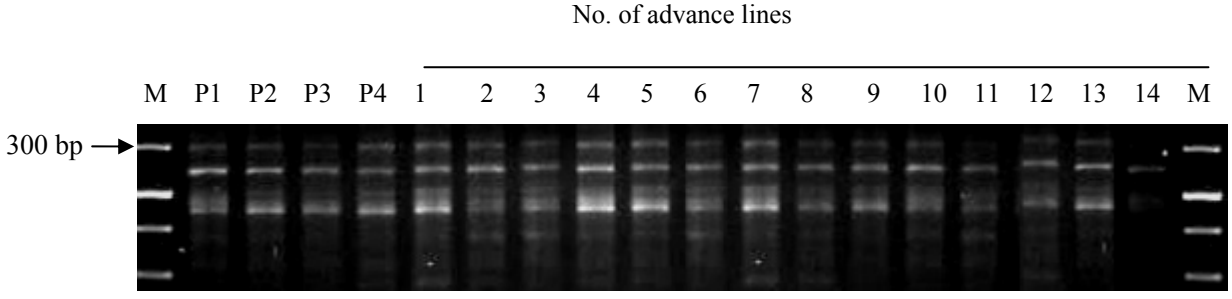


Figure 4. Ethidium bromide stained Agrose gel showing amplification by OPA-04 primer

M: 1 kb ladder, P1: Inqilab 91, P2: MH 97, P3: PARULA, P4: SA 42

1: QA-1, 2: QA-6, 3: QA-18, 4: JINNAH—4, 5: JINNAH—29, 6: AF-1, 7: QA-26, 8: QA-25, 9: QA-23

10: QA35, 11: QA-2, 12: QA-10, 13: JINNAH-3, 14: AF-20

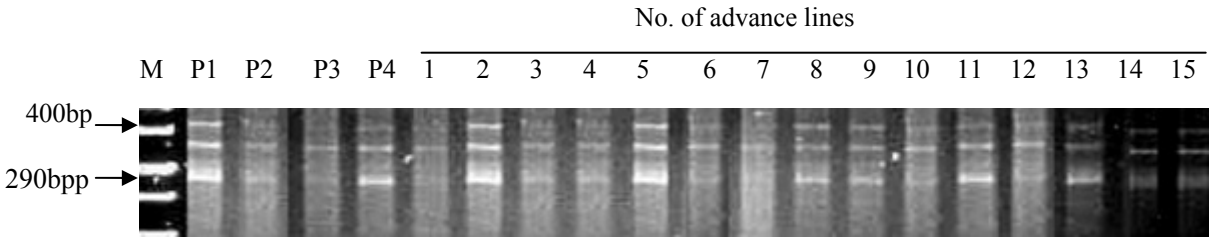


Figure 5. Ethidium bromide stained Agrose gel showing amplification by OPA-06 primer

M: 1 kb ladder, P1: Inqilab 91, P2: MH 97, P3: PARULA, P4: SA 42
1: QA-1, 2: QA-6, 3: QA-18, 4: JINNAH—4, 5: JINNAH—29, 6: AF-1, 7: QA-26, 8: QA-25, 9: QA-23
10: QA35, 11: QA-2, 12: QA-10, 13: JINNAH-3, 14: AF-20, 15: QA-24

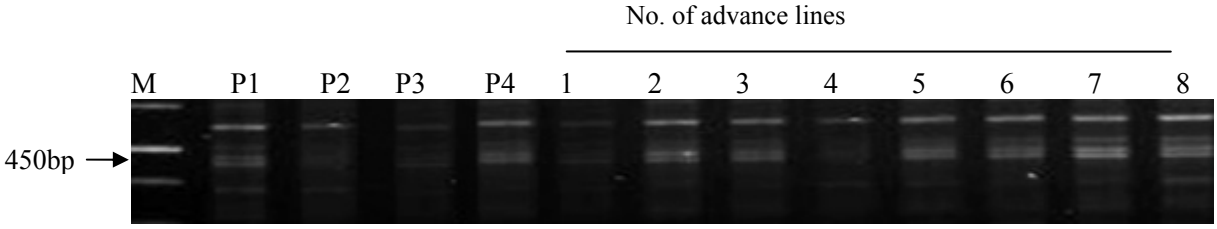


Figure 6. Ethidium bromide stained Agrose gel showing amplification by OPA-17 primer

M: 1 kb ladder, P1: Inqilab 91, P2: MH 97, P3: PARULA, P4: SA 42
1: QA-1, 2: QA-6, 3: QA-18, 4: JINNAH—4, 5: JINNAH—29, 6: AF-1, 7: QA-26, 8: QA-25

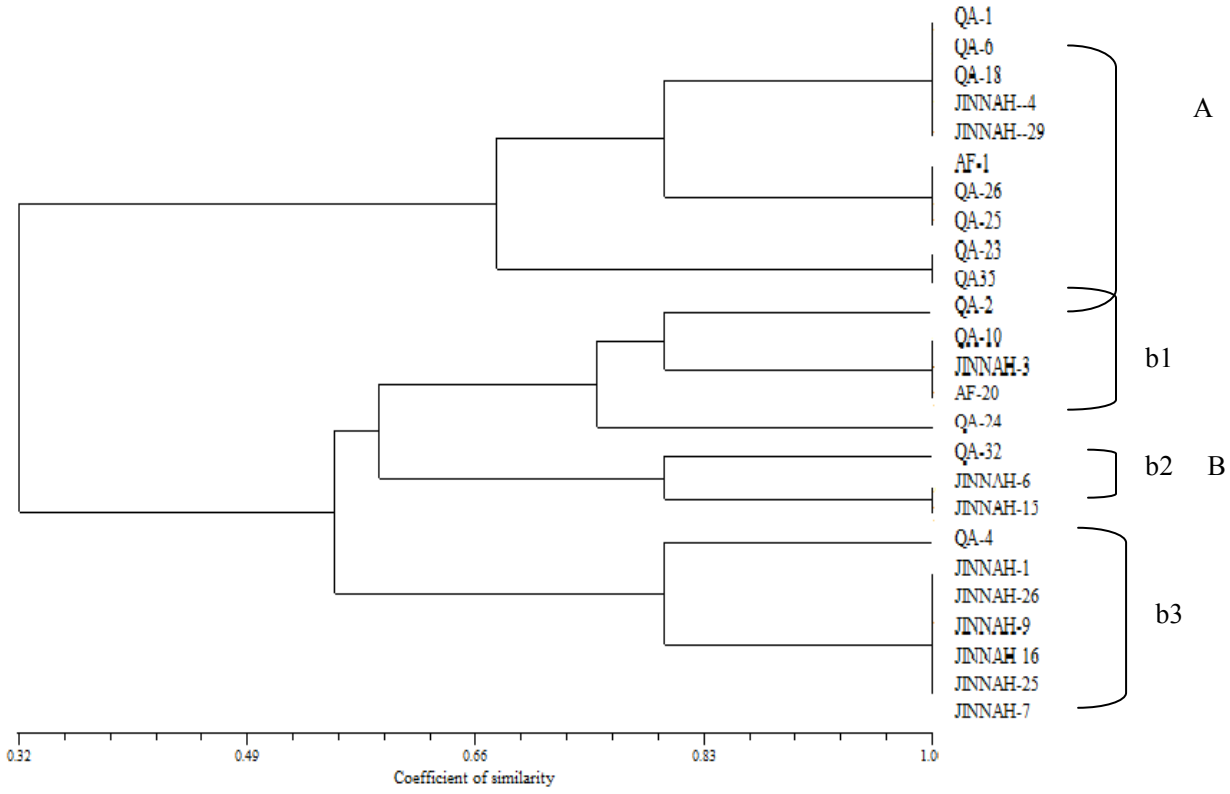


Figure 7. Dendrogram of 25 wheat genotypes based on RAPD data analysis