

Molecular Characterization of Wheat Genotypes Using SSR Markers

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Abstract: Wheat (*Triticum aestivum* L.) rusts are the most destructive and widespread among all other diseases of wheat because of their wide distribution, and their capacity to form new races that can attack previously resistant cultivars which result in serious yield losses. The molecular characterization and genetic diversity of 20 wheat genotypes was investigated using 34 polymorphic Simple Sequence Repeats (SSR) screened primers. About thirty-one loci were found. Lr-19 gene was present in all 20 wheat genotypes that cause resistance against wheat rust. Shalimar-86 and Chakwal-86 showed the highest genetic diversity with SH-02 and Ufaq respectively, giving a 98.94% genetic similarity and a minimum genetic diversity was observed between Chakwal-50 and Bhakar which showed that they are 74% similar. The current research found that SSR markers could distinguish and characterize all of the genotypes, more screened primers could be used for study and for saturation of different regions in further research. The identification of rust resistant genes in Pakistani wheat germplasm will help in accelerating the breeding program in future, including pyramiding of different wheat resistant genes in wheat genotypes and varieties.

Keywords: Molecular characterization, Wheat rust, SSR markers.

Introduction

Triticum aestivum, the common wheat is one of the leading edible grains of human's food. Wheat is a polyploidy and domestically cultivated grass in all over the world. It has about one half of the human's food calories and also fulfills the large part of their nutritional requirements. Wheat (*Triticum* spp) belongs to the *Poaceae* family which is the one of the most significant and diverse family of kingdom *Plantae* as the substantial increase in the population of the world demands a gradual increase in wheat production.

The research on wheat is very difficult and extensive and is used to maximize wheat grain production but also used to improve grain yield. However, there is a still consideration space for the improvement to amplify efforts for the continued genetic improvement of wheat to overcome at the growing requirement of an ever increasing world population. Molecular characterization and genetic manipulation is the best way to raise the wheat production. Therefore, it is necessary to study and guesstimate the mode of inheritance and genetic variation in different parameters of plants to start the productive programs of wheat breeding.

The stagnant yields of *Triticum aestivum* (wheat) in Pakistan and under developing countries are due to inadequate diversity in genetic resources used in breeding programs. Through selection and breeding a large number of alleles have been lost, so that more difficulties have egresses for improvement of wheat in modern agriculture systems [1, 15].

Three wheat rust diseases; leaf rust, stripe rust and stem rust are economically important cereal diseases with varying losses especially in terms of grain damage. Stripe rust caused by *Puccinia striiformis* f. sp. *tritici* is widely distributed and dangerous [7]. Rust affects wheat crop through damaging its systems, most importantly reduces grain yield by shriveling grain, respiratory makes growth of plant stunted, reducing weight and affecting its quality [7, 22].

Fungicides can be used to control leaf rust to some extent. Using rust resistant cultivars is an environmental friendly and economic way of minimizing losses caused by the disease. Fifty-six (56) leaf rust resistance genes have been designated and 51 of them have been mapped to corresponding chromosomes [31]. Lr-19 rust resistant gene is one of the few widely effective genes conferring resistance to leaf rust in wheat. Lr-19 still provides effective resistance against all leaf rust races [20]. Ayala-Navarrete et al. [2] had developed sequence-tagged-site (STS) markers for Lr-19 and Sr-25 from wheat ESTs and mapped them to chromosome 7DL [25], which was also found to be in the translocated segment of wheat [13].

Molecular markers can provide comprehensive characterization of genetic resources. Markers provide a direct measure of genetic diversity. Microsatellite are simple sequence repeats (SSR) of about 1-6 nucleotides.

They profusely scatter throughout the genome. SSR markers show the privileged level of polymorphism than other genetic markers. Their additional advantages are this potential for automation and their inheritance in co-dominant manner when compared with other types of molecular markers. These features, coupled with their ease of detection and SSR markers cover all 21 chromosomes of wheat. SSR markers have been used in a seed bank collection of improve germplasm of wheat [4, 16] and to characterize the genetic diversity in wild relatives [14].

The present study was conducted to estimate the molecular characterization of wheat genotypes of Pakistan with the help of SSR markers. The current work addressed the molecular characterization of 20 wheat genotypes and addressed the utilization of SSR markers to determine the molecular characterization and genetic diversity among 20 wheat genotypes. The molecular characteristics, genetic diversity and phylogenetic relationships concluded in this study will help in the selection of parents to develop high-docile varieties in breeding programs.

Material and methods

Plant materials/wheat germplasm

Seeds of 20 different genotypes of wheat were obtained; all the 20 genotypes were collected from Ayub Agriculture Research Institute (AARI) Faisalabad. All wheat seeds were sown in small clay pots in a growth chamber providing normal growing conditions. After about three weeks seeding has grown 3-4 seedlings were cut and packed in plastic bags and stored at – 80°C for DNA extraction.

Extraction of DNA and SSR analysis

Genomic DNA of 20 wheat genotypes was extracted from fresh leaves. Total genomic deoxyribonucleic acid (DNA) from individual genotype was extracted from young leaves at seedling stage by CTAB (cetyl trimethyl ammonium bromide) method. Fresh leaves of each genotype were ground in mortar with a pestle with continuous addition of liquid nitrogen and transferred to a 50 ml falcon tube. The 15 ml of hot 2x CTAB was added and incubated for 30-45 min at 65°C with occasional swirling. Equal volume of chloroform: isoamyl alcohol (24:1) was added and mixed gently by inverting the tube to form an emulsion, then spun at 11 000 rpm for 10 min. The supernatant was collected into a new 50 ml tube and a nucleic acid was precipitated with 0.6 volume of chilled isopropanol and left in the refrigerator for 2 h.

Nucleic acid was pelleted at 12 000 rpm for 5 min and supernatant was discarded and the Pellet was washed with 70% ethanol and air dried before resuspending in 0.5 ml 0.1x TE buffer. The suspension was transferred to 1.5 ml Eppendorf tube, added 7 µL of RNase and incubated at 37°C for 1 h. DNA concentration was measured by flouremeter and the DNA were diluted to 10 ng/mL using sterilized distilled water and stored in microfuge tubes at 4°C for further use [19].

A spectrophotometer was used to check the concentration and quality of extracted DNA for the Polymerase Chain Reaction (PCR) amplification. Thirty screened primer pairs were used for the analysis of SSR. PCR conditions were maintained as described by Roder [27].

Each PCR was carried out in a about 25 µl reaction volume containing double distilled deionizer H₂O, 10x buffer, mgcl 2, dNTPs, taq polymerase, and both primer pairs according to the primers profile. The PCR amplification of wheat genomic DNA was done by incubating the DNA samples for 5 minutes at 94°C, then 45 cycles comprising 94°C for 60 seconds, annealing of primer for 60 seconds at 58-60°C and the extension for 60 seconds at 72°C. The final extension was carried out for 10 minutes at 72°C.

The PCR products were electrophorized on 5% of agrose gel containing 8 µl ethidium bromide, at 90 volts for 60 minutes and observed under a UV tranluminator. Bands were counted and the absence and presence of bands were scored as 0 and 1 respectively. The cluster analysis of 20 wheat genotypes was performed using the NTSYSPC software version 2.2 to determine genetic diversity and similarity among genotypes (Table 1).

Table 1. SSR wheat primers profile

Sr. No	Primer name	Base pair	Incubating	Cycles	Comprising	Annealing	Extension	Final Extension
1	Lr-20, Sr-15, STS-638	355 bp	94°C at 5 mints	45	94°C at 1 mint	55°C at 1 mint	72°C at 1 mint	72°C at 10 mints
2	Yr-18, Lr-34, XBarc-352	275 bp	94°C at 4 mints	38	94°C at 30 sec	60°C at 30 sec	72°C at 30 sec	72°C at 5 mints
3	Sr-36, WMC-477	155bp	94°C at 10 mints	40	94°C at 1 mint	58°C at 1 mint	72°C at 2 mints	72°C at 10 mints
4	Sr-2, Xgwm-533	120 bp	94°C at 5 mints	45	94°C at 1 mint	60°C at 1 mint	72°C at 2 mints	72°C at 10 mints
5	Lr-19	130 bp	94°C at 5 mints	30	94°C at 1:30	55°C at 2 mint	72°C at 1:30 mint	72°C at 5 mints
6	Lr-28, Xgwm-319	378 bp	94°C at 5 mints	45	94°C at 1 mint	60°C at 1 mint	72°C at 2 mints	72°C at 10 mints
7	XWMC-44, Yr-46, Sr-29	242 bp	94°C at 10 mints	35	94°C at 1 mint	61°C at 1 mint	72°C at 2 mints	72°C at 10 mints
8	Xgwm-295	310 bp	94°C at 5 mints	45	94°C at 1 mint	60°C at 1 mint	72°C at 2 mints	72°C at 10 mints
9	Xgwm-210	245 bp	94°C at 5 mints	45	94°C at 1 mint	60°C at 1 mint	72°C at 2 mints	72°C at 10 mints
10	KSUD-14	885 bp	95°C at 8 mints	34	95°C at 30 sec	54°C at 1 mint	72°C at 1 mint	72°C at 7 mints
11	SCS-464, Lr-28	378 bp	94°C at 2 mints	40	94°C at 1 mint	60°C at 1 mint	72°C at 1 mint	72°C at 7 mints
12	SCS-253, Lr-19	736 bp	95°C at 2 mints	35	94°C at 1 mint	60°C at 1 mint	72°C at 1 mint	72°C at 7 mints
13	STS-7-8, Yr-5	139 bp	94°C at 5 mints	45	94°C at 30 sec	45°C at 30 sec	72°C at 45 sec	72°C at 7 mints
14	STS-9-10, Yr-5	139 bp	94°C at 5 mints	45	94°C at 30 sec	45°C at 30 sec	72°C at 45 sec	72°C at 7 mints
15	Xgdm-87, Lr50	747 bp	94°C at 4 mints	30	94°C at 30 sec	60°C at 30 sec	72°C at 30 sec	72°C at 5 mints
16	Lr-10	310 bp	94°C at 3 mints	35	94°C at 45 sec	57°C at 45 sec	72°C at 30 sec	72°C at 3 mints
17	Xgwm-130	245 bp	94°C at 5 mints	45	94°C at 1 mint	55°C at 1 mint	72°C at 2 mints	72°C at 10 mints
18	Lr-51, S-30-131	790 bp	94°C at 5 mints	40	94°C at 45 sec	52°C at 45 sec	72°C at 1 mint	72°C at 10 mints
19	Lr-47	380-450 bp	94°C at 4 mints	40	94°C at 30 sec	55°C at 30 sec	72°C at 30 sec	72°C at 5 mints
20	STS-638, Sr-15, Xgwm-11	202 bp	94°C at 5 mints	40	94°C at 1 mint	55°C at 1 mint	72°C at 2 mints	72°C at 10 mints
21	Xgwm-37, Lr-19	130 bp	94°C at 5 mints	45	94°C at 1 mint	50°C at 1 mint	72°C at 2 mints	72°C at 7 mints
22	WMC-44, Lr-46, Sr-29	242 bp	94°C at 5 mints	45	94°C at 1 mint	50°C at 1 mint	72°C at 2 mints	72°C at 7 mints
23	GDM-35, Lr-39	145 bp	94°C at 4 mints	30	94°C at 30 sec	55°C at 30 sec	72°C at 30 sec	72°C at 5 mints
24	Lr-25	210 bp	94°C at 3 mints	35	94°C at 1 mint	60°C at 1 mint	72°C at 2 mints	72°C at 10 mints

Results

The cut-off point of Lr-19 translocation is located in the middle of long arm of chromosome 7D and find that the distal half of 7D was replaced by *Thinopyrum* Chromatinv [14]. During meiosis *Thinopyrum* segment 7DL does not pair with homologous wheat segment, complicating attempts to study linkage relationship or to recombine its genes [18, 23].

Thirty-four screened polymorphic SSR primer pairs were used to assess the extent of molecular characterization and genetic diversity among twenty different wheat genotypes. These 34 SSR primers indicated 31 loci and all were shown to be polymorphic. All the twenty wheat genotypes showed rust resistant. These twenty wheat genotypes were closely and distinctly related. Greater numbers of alleles were observed in Genome A as compared to Genome B. The SSR markers showed a greater level of polymorphism ranging from 8.32% to 94.19%. To study the genetic relationship between 20 genotypes of wheat, a dendrogram was constructed (Fig 1). Cluster analysis showed that mutation in ancestral gene results in generation of Uqab-2000 genotype that is a distantly related to other genotypes and produces 19 genotypes after successive mutations in ancestral gene.

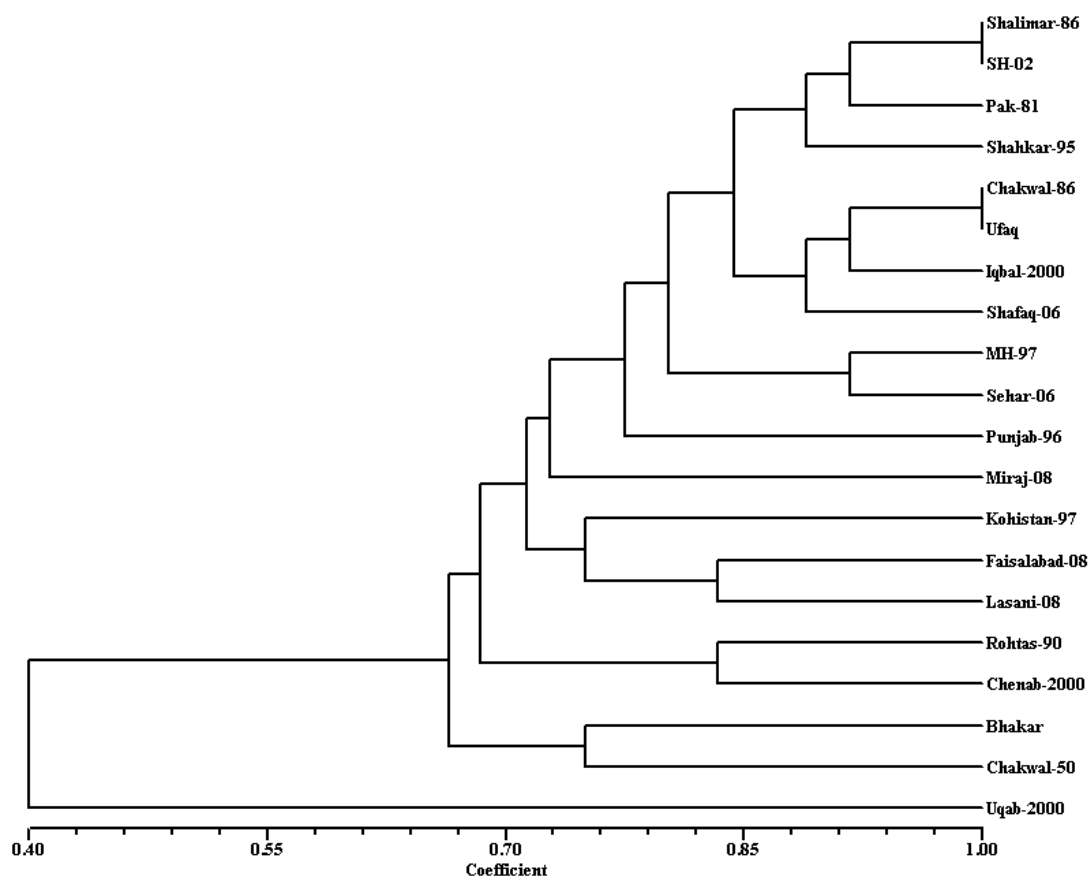


Fig. 1 Dendrogram constructed for 20 wheat genotypes using data obtained from 34 SSR primer sets

A maximum similarity of 98.94% was observed between Shalimar-86 and SH-02, and between Chakwal-86 and Ufaq genotypes. Shalimar-86 and SH-02 are genetically more divergent while Chakwal-50 and Bhakar showing minimum 74% similarity are less divergent. All 20 genotypes are involved in rust resistant, showing genetic diversity and similarities.

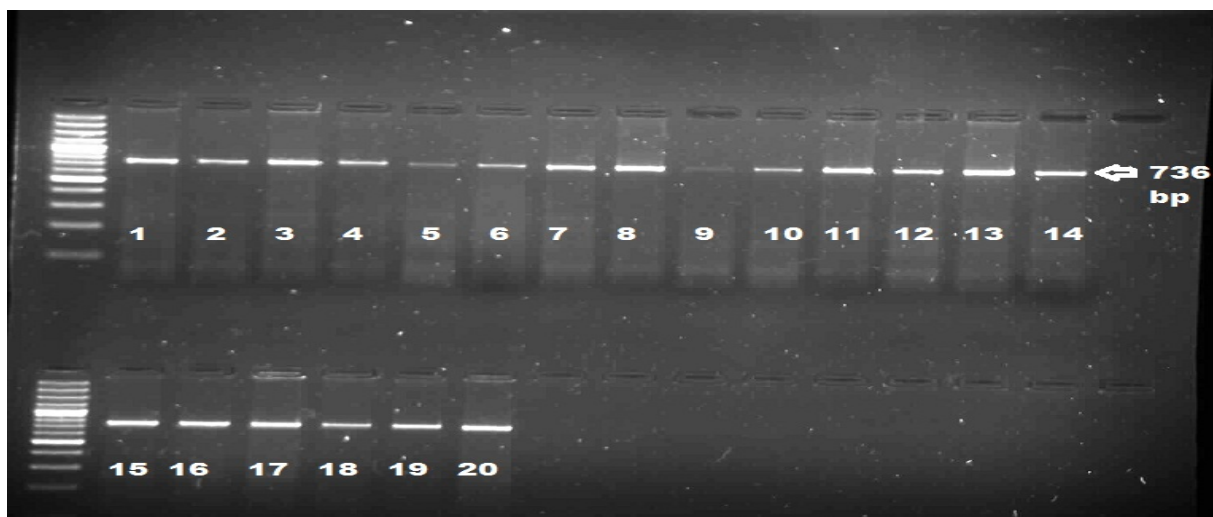


Fig. 2 Banding patterns showing the presence Lr-19 gene in wheat genotypes amplified 736 bp size fragments. Arrow is showing amplified gene (736 bp).

The dendrogram (Fig. 1) shows the evolutionary history and changes in genome of wheat according to wheat rust resistant genes. The result in figure 1 shows the occurring evolution in wheat rust resistant genes. Rust resistant genes are found in this experiment and Lr-19 is a rust resistant genes present in all twenty wheat genotypes. SSR markers are used for the identification of wheat rust resistant genes. These markers found the rust resistant genes from these twenty wheat genotypes. The banding pattern shown (Fig. 2) the presence of Lr-19 gene on all of the wheat genotypes and the bands are amplified at 736 base pair.

Discussion

Genetic base of Pakistan wheat cultivars has been reported as narrow for major biotic stresses. Most of the national wheat varieties in Pakistan have been established around few seedling resistance genes against rust, therefore is vulnerable to its new pathotypes emerging from time to time [6].

The different types of wheat cultivars become susceptible to different rusts due to their narrow genetic base for resistance, and the rapid rate of evolution of the pathogen, it is necessary to find out new sources for resistance. The genetic resistance is important to control many phytopathogenic epidemics. The production of the wheat has been dependent on the use and development of resistance genotypes having diverse and well characterized genes. It is also believed that, in wheat certain and different gene combinations give long lasting and better resistance to different rust disease than given by any of the genes individually [9].

The characterization of genetic diversity with a related and closely similar crop germplasm is an important tool for the use of genetic resources [8]. The molecular markers have the potential to detect the molecular characterization and genetic diversity [10, 29, 30]. The various molecular markers or SSR markers are often chosen as the preferred markers for a variety of significance and applications in breeding because the SSR markers have multi-allelic nature, relative abundance, co-dominant inheritance and extensive genome coverage [12].

In this study one of the PCR based system (SSR) has been used and compared for studying the genetic diversity and molecular characterization between twenty different genotypes of wheat. The SSR system is different in principle type and amount of polymorphism detected.

SSR markers or microsatellite markers are becoming the markers of choice due to the higher reliability and as well as high level of polymorphism [11, 24].

In wheat, there are many SSR markers now available for wheat and have been mapped [27] SSR markers are useful and becoming famous for different applications in wheat breeding due to their high level of polymorphism and easy handling [5, 21, 26, 28]. Microsatellite markers are used to evaluate the genetic diversity of hexaploid wheat (*Triticum aestivum.L*) landraces in relation to their geographic origin [3].

In view of the massive information about the close genetic kinship of common wheat, it is suggested that mission oriented breeding programs with the help of DNA fingerprinting technology and molecular characterization will be helpful to produce distinct cultivars and genotypes that will remain maintain steady genetic improvement.

The current study showed the utility of SSR markers in revealing assessment genetic variability and molecular characteristics among twenty genotypes of wheat, wherein 34 screened polymorphic SSR markers were used .SSR markers loci generated by 34 primers pairs were used to study the genetic diversity among ten genotypes of wheat. This research also showed that primer pair SCS-253 generated a maximum number of bands. The twenty genotypes of wheat were collected by AARI Faisalabad. Cluster analysis showed that mutation in ancestral gene results in generation of Uqab-2000 genotype that is a distantly related to other genotypes and produces 19 genotypes after successive mutations in ancestral gene. A maximum similarity of 98.94% was observed between Shalimar-86 and SH-02, and between Chakwal-86 and Ufaq genotypes. Shalimar-86 and SH-02 are genetically more divergent while Chakwal-50 and Bhakar showing minimum 74% similarity are less divergent. All 20 genotypes are involved in rust resistant, showing genetic diversity and similarities. During present study 34 Simple Sequence Repeat (SSR) primer sets were used to characterize 20 Pakistani wheat genotypes to know about the diverse varieties for future breeding programs to enhance wheat production. Microstallites displayed a high level of polymorphism in the present study. It was assumed that such a high level of genetic similarity may be the result of biased selection of the material in the previous breeding programs, which ultimately narrowed the genetic base of the wheat germplasm in the country. It is further suggested that more polymorphic wheat microsatellites could be used for efficient screening of the germplasm by saturating more regions of the wheat genome.

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