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Molecular Genetic Characterization of Rust in Wheat Genotypes

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Abstract: The molecular characterization and genetic diversity of 10 wheat genotypes was investigated using 34 polymorphic simple sequence repeats screened primers. About thirty-one loci were found. Lr-19 gene was present in all 10 wheat genotypes that cause resistance against wheat rust. Iqbal-2000 and Ufaq showed the highest genetic diversity between each other giving a genetic similarity of 96.34% and a minimum genetic diversity was observed between Lasani-08 and Bhakar which showed the dissimilarity of about 66.64%. The current study found that all of the genotypes could distinguish and characterize by SSR makers, new screened primers could be used for study and can also be used in different saturated regions for further research. Lr-19 is a wheat rust resistant gene and present in all ten genotypes. This gene causes resistance against rust diseases.

Keywords: Genetic characterization, molecular characterization, SSR markers, wheat rust

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. Wheat contains about one half of the calories in human's food and can also fulfill the huge part of their nutritional necessities. Wheat (*Triticum* spp) belongs to the Poaceae family which is one of the most significant and diverse family of kingdom *Plantea* 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 the production of wheat grains. It can also used to improve and get better yield of grain. However, there is still consideration space for the advancement and improvement in genetics of wheat to conquer the daily problems of increasing requirements of world population. Genetic manipulation and molecular characterization is the best way to increase the production of wheat. Therefore, it is required to study and estimate the mode of inheritance and genetic variation in different parameters of plants to start the productive wheat breeding programs.

The sluggish yields of *Triticum aestivum* in developing countries and Pakistan are due to inadequate diversity in genetic resources used in breeding and reproduction programs. A large number of different alleles have been mutated and lost through selection and breeding, so that different problems have to face in modern system of agriculture for the improvement of wheat (Allard, 1996; Hoisington *et al*., 1999).

Molecular markers can show comprehensive characterization of genetic resources. Genetic diversity can directly measured by markers. Microsatellite are Simple Sequence Repeats (SSR) of about 1-6 nucleotides. Markers profusely scatter in whole genome. SSR markers show the privileged level of polymorphism than other genetic markers. Their additional advantages are their co-dominant inheritance and potential for automation when different types of molecular markers are compared with them. These features, coupled with their ease of detection and all 21 wheat chromosomes are covered with SSR markers. SSR markers have been also used in a seed bank collection of improve germplasm of wheat (Borner *et al*., 2000; Huang *et al*., 2002) and to characterize the genetic diversity in wild relatives (Hammer *et al*., 2000). The present study was conducted to estimate the molecular characterization of wheat genotypes by using SSR markers. This study addressed the molecular characterization of ten different wheat genotypes and also addressed the utilization of SSR markers to determine the molecular characterization and genetic diversity among wheat genotypes. The molecular characteristics, phylogenetic relationships and genetic diversity concluded in this study will help in breeding programs for the selection of parents and to develop high-docile wheat varieties.

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MATERIALS AND METHODS

Plant materials/wheat germplasm: Study was done in department of bioinformatics and biotechnology, International Islamic university Islamabad in 2010, Seeds of ten different genotypes of wheat were obtained; seeds of wheat genotypes were collected from Ayub Agriculture Research Institute (AARI) Faisalabad. Seeds of wheat genotypes were sown in small pots of clay in a growth chamber and normal conditions were provided for growth. After about twenty days seeding had grown. 3-4 seedlings of every genotype were cut and crammed in plastic bags. These bags were stored at -80ºC for the extraction of DNA.

Extraction of DNA and SSR analysis: Fresh leaves were used for the extraction of genomic DNA of ten different wheat genotypes according to method described by Khan *et al*. (2004). A spectrophotometer was used to check the concentration and quality of extracted DNA for the PCR amplification. Thirty screened primer pairs were used for the analysis of SSR. PCR conditions were maintained as described by Roder *et al*. (1998).

Each PCR product was carried out in about 25 μL volume of reaction containing double distilled deionizer H2O, 10 x buffers, MgCl2, dNTPs, taq polymerase and both primer pairs according to the primers profile. The amplification of PCR product of wheat genomic DNA was done by incubating the samples of DNA for 5 min at 94ºC. Forty five cycles of PCR product that comprised on 94ºC for 60 sec. Primers were annealed for 60 sec at 58-60ºC and the extension for 60 sec at 72ºC. The final extension of PCR product was carried out for 10 min at 72ºC.

The amplified PCR products were electrophori zed on 5% of agrose gel containing 8 μL ethidium bromides, at 90 volts for 60 min and UV transluminator was used for observation of gel. Bands on agrose gel were counted and the absence and presence of bands on gel were scored as 0 and 1, respectively. NTSYSPC software version 2.2 was used for cluster analysis of ten wheat genotypes to determine genetic diversity and similarity among genotypes.

RESULTS

Lr-19 translocation originally produce by Sharma and Knott (1966) when they transform leaf rust resistance genes 7e l1 chromosome of Thinopyrum ponticum a long arm of chromosome 7 D of common wheat. Heurta-Espino and Singh (1994) reported first virulence in Puccini a Triticinan to Lr-19 and it is an effective source of leaf rust resistance worldwide.

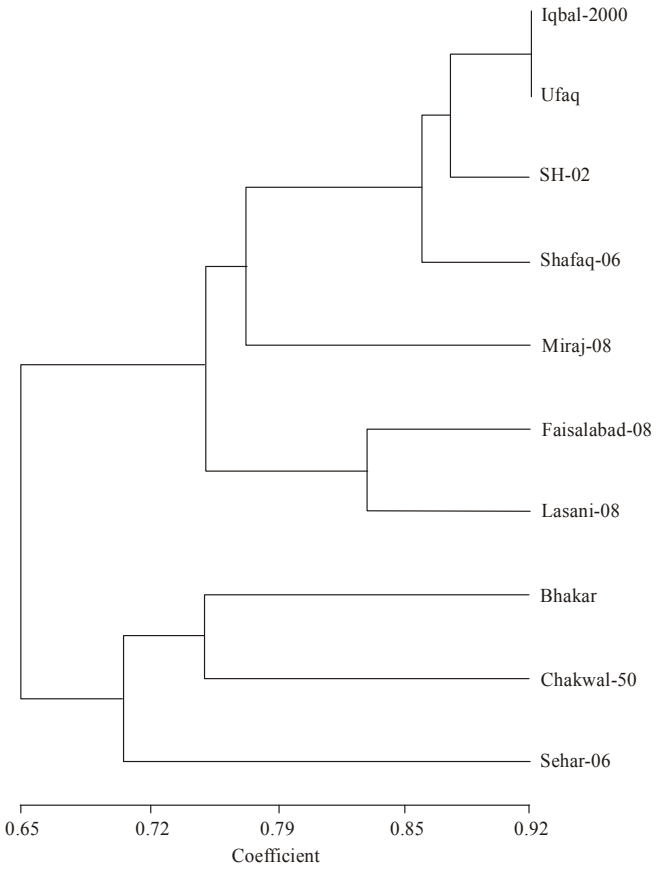

Fig. 1: Ten genotypes showed in dendogram that based on Nei's original measures (1972)

The cut-of point of Lr-19 translocation is located in the middle of long arm of chromosome 7D and find that the distal half of 7 D was replaced by Thinopyrum Chromatinv (Knott, 1980). During meiosis Thinopyrum segment 7DL does not pair with homologous wheat segment, complicating attempts to study linkage relationship or to recombine its genes (Kim *et al*., 1993; Marais and Marais, 1990).

Thirty-four SSR screened and polymorphic primer pairs were used to assess the extent of molecular characterization and genetic diversity among ten different genotypes of wheat. These 34 SSR primers were indicated 31 polymorphic loci. All the ten wheat genotypes were showed rust resistant. These ten wheat genotypes were closely and distinctly related. High level of polymorphism was shown by SSR markers ranging from 8.32 to 94.19%. A dendogram (Fig. 1) was constructed to study the genetic relationship between ten wheat genotypes. Cluster analysis of ten wheat genotypes showed 2 groups. Cluster I showed seven wheat genotypes and cluster II indicated remaining 3 genotypes. Iqbal-2000 and Ufaq were closely related each other and show 96.34% similarity. Genotypes of cluster I are more closely related to each other. A minimum similarity was observed between Lasani-08 and Bhakar which showed that they are 66.64% dissimilarity.

DISCUSSION

The wheat cultivars are of different types and become susceptible to different types of rust because it has narrow genetic bases for resistance. The evolution rates of pathogens are very fast and rapid. So, it is necessary to find out new and better sources for resistance. The genetic resistance is important to control many phytopathogenic epidemics. The wheat production has been dependent on the use and development of rust resistance genotypes having well characterized and diverse genes. It is also concluded that, in wheat certain and different combinations of genes give long lasting and better resistance for rust diseases than given by any individual genes (Dyck and Sambroski, 1982).

The molecular markers have prospective to detect the molecular characterization and genetic diversity (Ford-Lloyd *et al*., 1997; Song *et al*., 2003; Virk *et al*., 1995). A variety of SSR or molecular markers are often selected as the preferred markers for a diversity of significance and applications in breeding because the SSR markers have extensive genome coverage, relative abundance, co-dominant inheritance and multi-allelic nature (Gupta and Varshei, 2000). Microsatellite markers or SSR markers are becoming the markers of best choice due to the higher reliability and as well as high level of polymorphism (Fu *et al*., 2005; Plaschke *et al*., 1995).

In wheat, there are many SSR markers have been mapped for wheat genome and now also commercially available (Roder *et al*., 1998). SSR markers are functional and becoming famous for various applications in breeding of wheat due to their easy handling and high level of polymorphism (Bryan *et al*., 1997; Lelley *et al*., 2000; Roder *et al*., 1995; Roy *et al*., 1999). Microsatellite markers are used to assess the molecular characterization and genetic diversity of hexaploid wheat landraces in associated with their geographical origin (Al-Khanjari *et al*., 2007). In this study, one of the polymerase chain reaction based system (SSR) has been used and compared for studying the genetic diversity and molecular characterization between ten different genotypes of wheat. The SSR system is different in principle type and amount of polymorphism detected. 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.

CONCLUSION

The current study showed the efficacy of SSR markers in revealing assessment molecular characteristics and genetic variability among ten different wheat genotypes, wherein 34 SSR screened polymorphic markers were used. SSR markers loci generated by 34 primers pairs were used to study the molecular characterization and genetic diversity among ten wheat genotypes. This study also showed that a maximum number of bands were generated by SCS-253 primer pair. All the ten wheat genotypes were showed rust resistant. These ten wheat genotypes were closely and distinctly related. High level of polymorphism was shown by SSR markers ranging from 8.32 to 94.19%. A dendogram was constructed to study the genetic relationship between ten wheat genotypes. Cluster analysis of ten wheat genotypes showed 2 groups. Cluster I showed seven wheat genotypes and cluster II indicated remaining 3 genotypes. Iqbal-2000 and Ufaq were closely related each other and show 96.34% similarity. Genotypes of cluster I are more closely related to each other. A minimum similarity was observed between Lasani-08 and Bhakar which showed that they are 66.64% dissimilarity. Lr-19 is a wheat rust resistant gene and present in all ten genotypes. This gene causes resistance against rust diseases.

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