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Molecular characterization of wheat germplasm using microsatellite markers

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ABSTRACT. We investigated the genetic diversity of 63 wheat genotypes, composed of 48 accessions and 15 varieties, using 56 polymorphic simple sequence repeat primers. One hundred and eighty-six loci were found, with a mean of 131.26 alleles per locus. Cluster analysis based on microsatellite allelic diversity discriminated the accessions and varieties into different clusters; genetic diversity was the highest between variety Kohistan-97 and accession number 011512, giving a genetic similarity value of 0.4198. Accession numbers 011484 and 011356 gave a genetic similarity value of 0.9589, indicating that these accessions were 95.89% similar. We found that microsatellite markers could characterize and discriminate all of the genotypes; more primers could be used for saturation of different regions in further studies.

Key words: *Triticum aestivum*; Microsatellite; Molecular markers; Genome; Genetic distance; Population genetics

INTRODUCTION

Common wheat *Triticum aestivum* (2n = 6x = 42), belonging to the family Poaceae, which is the most diverse and important family of the plant kingdom, produces large edible grains and provides about one-half of humans' food calories and a large part of their nutrient requirements. Wheat (*Triticum* spp) is a worldwide cultivated and domesticated grass. The gradual increase in population demands a substantial increase in its productivity. Wheat has always been subjected to extensive and ceaseless research so as to maximize grain production but also to improve grain yield per unit area. However, there is still considerable room for improvement, especially to amplify efforts for continued genetic improvement of wheat to meet the growing requirements of an ever increasing population. Genetic manipulation is the best way to boost up wheat production. Therefore, it is necessary to estimate and study the genetic variation and mode of inheritance in different plant parameters to initiate productive wheat breeding programs. The stagnant yield of wheat in Pakistan is due to limited diversity in the germplasm used in breeding programs.

Through breeding and selection, great numbers of alleles have been lost, so that more difficulties have emerged for wheat improvement in modern agriculture systems (Allard, 1996; Hoisington et al., 1999). Molecular markers can provide detailed characterization of genetic resources. Molecular markers provide a direct measure of genetic diversity and go beyond the indirect diversity measures based on agronomic traits or geographic origin. Microsatellites are simple sequence repeats (SSR) of 1-6 nucleotides. They are abundant, dispersed throughout the genome and show higher levels of polymorphism than other genetic markers. These feature, coupled with their ease of detection, have made them useful markers. Their potential for automation and their inheritance in a co-dominant manner are additional advantages when compared with other types of molecular markers, and they cover all 21 wheat chromosomes. SSR markers have been used to characterize genetic diversity in wild relatives (Hammer et al., 2000) and in a seed bank collection of improved wheat germplasm (Börner et al., 2000; Huang et al., 2002). This study was conducted to estimate the genetic divergence among wheat accessions as well as cultivated varieties of Pakistan with the help of SSR markers. The present study addressed the utilization of microsatellite markers, to determine genetic diversity and relationships at the molecular level, among 63 genotypes of wheat. The genetic diversity and phylogenetic relationships determined in this study will help in the selection of parents to develop high-yielding varieties in breeding programs.

MATERIAL AND METHODS

Plant materials/wheat germplasm

Seeds of 63 genotypes (48 accessions and 15 varieties) were obtained: forty-eight wheat accessions collected from the Plant Genetic Resources Institute (PGRI), Islamabad, and 15 varieties of wheat, collected from Ayub Agricultural Research Institute (AARI), Faisalabad. All wheat accessions/cultivars were sown in small plastic pots in a growth chamber providing normal growing conditions. After two weeks, seedlings had grown. A

total of 4-5 seedlings were cut and packed in plastic bags and stored at -70°C for DNA extraction.

DNA extraction and SSR analysis

Genomic DNA of 63 wheat genotypes was extracted from fresh leaves, according to the method described by Khan et al. (2004). The quality and concentration of extracted DNA were estimated with a spectrophotometer. For SSR analysis, a total of 56 screened primer pairs were used for polymerase chain reaction (PCR) amplification. PCR conditions were maintained as described by Roder et al. (1998).

Each PCR was carried out in a 25-µL reaction volume, containing 11.3 µL double-distilled deionized H_2O, 2.5 µL 10X buffer, 2 µL $MgCl_2$, 2 µL dNTPs, 0.2 µL Taq polymerase, 1 µL of each primer of a primer pair, and 5 µL DNA.

The SSR (PCR) amplification of genomic DNA was done by incubating the DNA samples at 94°C for 4 min, then 45 cycles comprising 94°C for 1 min, annealing of primer at 58-60°C for 1 min and then extension at 72°C for 1 min. The final extension was carried out at 72°C for 10 min.

The PCR products were electrophoresed on 3% agarose gels containing 7 µL ethidium bromide, at 80 V for 1 h, and observed under a UV transilluminator.

Bands were counted and the presence and absence of bands were scored as 1 and 0, respectively. The data were collected and aligned for the construction of cluster analysis. The cluster analysis of 63 genotypes was performed using the POP Gen software version 1.32 (Yeh et al., 2000) to determine genetic diversity and similarity among accessions.

RESULTS

Fifty-six screened polymorphic SSR primer pairs were used to assess the extent of genetic diversity among 63 wheat genotypes. These 56 SSR primer pairs indicated 186 loci and all were shown to be polymorphic. A total of 7351 alleles were identified and the number of alleles detected at a single locus ranged from 0 (Xgwm 112-3B) to 368 (Xgwm 63-7A) with a mean of 131.26 alleles per locus. More alleles were observed in B genome as compared to A and D genomes. The SSR markers showed a high level of polymorphism ranging from 10.52% (Xgwm 162-3A) to 98.42% (Xgwm 111-7D). To examine the genetic relationship between 63 wheat genotypes, a dendogram was constructed (Figure 1). Cluster analysis indicated that 63 genotypes could be divided into three major clusters. Cluster I included all 15 varieties, cluster II was the largest and included 25 accessions of 48 and cluster III comprised the remaining 20 accessions. Three accessions were distinct, namely 011350, 011351, and 011352, and of these three, accessions 011351 and 011352 are closely related, 011350 is separate, and these three accessions may be used in a breeding program as distinct parents. A maximum genetic similarity value of 0.9589 was observed between accession numbers 011484 and 011356, which revealed a high degree of similarity to the extent of 95.89% existing between them. Whereas, a minimum genetic similarity value of 0.4198 was observed between the variety Kohistan-97 and accession number 011512, which showed that they are 41.98% dissimilar.

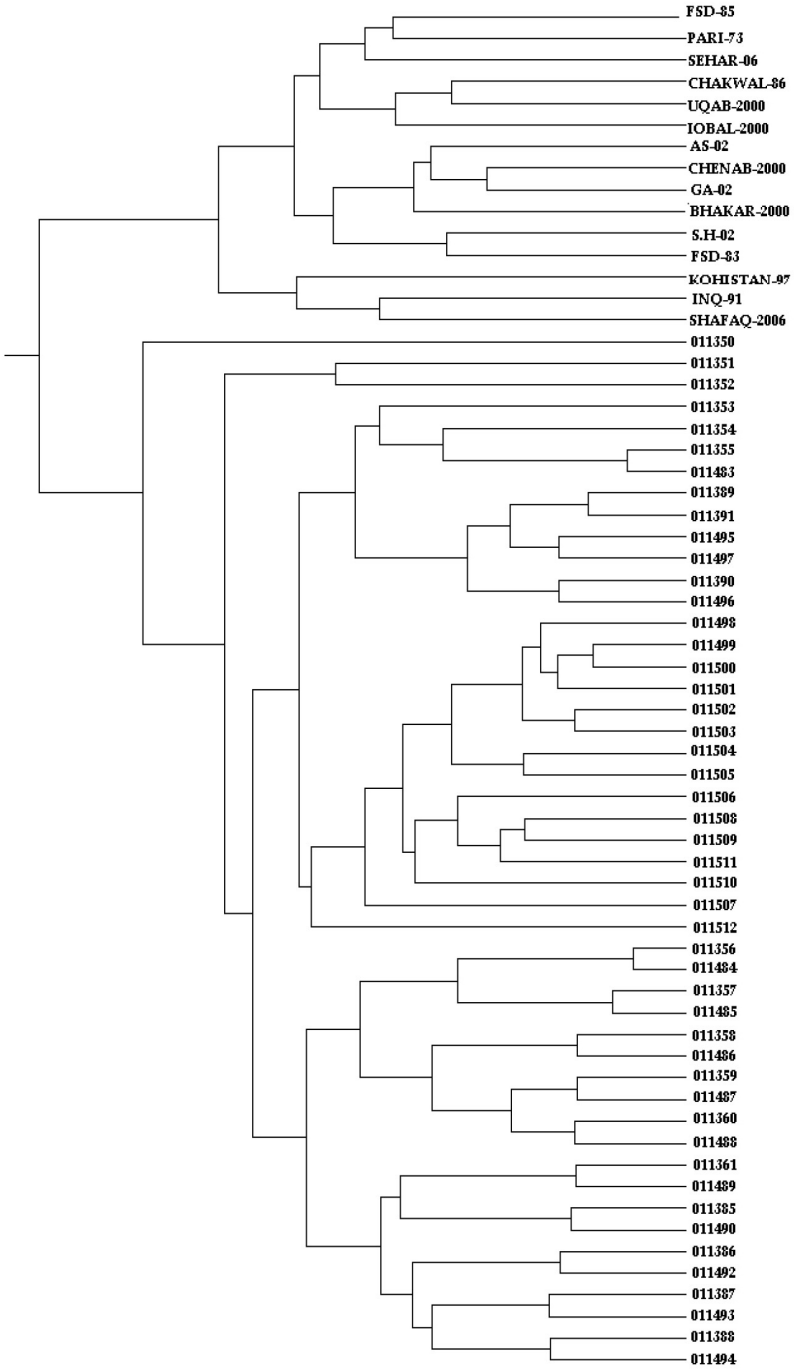

Figure 1. Overall dendogram showing 63 genotypes, based on Nei's original measures (1972).

DISCUSSION

The characterization of genetic diversity within a closely related crop germplasm is an essential tool for rational use of genetic resources. The analysis of genetic variation in breeding materials is of fundamental interest to plant breeders, as it contributes to selection, monitoring of germplasm and prediction of potential genetic gain (Chakravarthy and Naravaneni, 2006). Molecular markers have the potential to detect genetic diversity and to aid in the management of plant genetic resources (Ford-Lloyd et al., 1997; Virk et al., 1995; Song et al., 2003). Among various molecular markers currently available, SSR or microsatellite markers are often chosen as the preferred markers for a variety of applications in breeding because of their multi-allelic nature, co-dominant inheritance, relative abundance, and extensive genome coverage (Gupta and Varshney, 2000). Microsatellite markers are becoming the markers of choice due to the level of polymorphism, as well as higher reliability (Plaschke et al., 1995; Fu et al., 2005). In wheat, abundant wheat genomic SSR markers are now available and have been mapped (Roder et al., 1998), making them a useful resource for further studies. Microsatellite markers are useful and becoming popular for different applications in wheat breeding due to their high level of polymorphism and easy handling (Devos et al., 1995; Roder et al., 1995; Bryan et al., 1997; Roy et al., 1999; Lelley et al., 2000) and are used to evaluate genetic diversity of hexaploid wheat (*Triticum aestivum* L.) landraces in relation to their geographic origin (Al Khanjari et al., 2007). The present study addressed the utility of SSR markers in revealing assessment of genetic variability and diversity at the molecular level among 63 wheat genotypes wherein 56 screened polymorphic SSR primers were used, which were earlier identified in the genomic regions of A, B, and D genomes of wheat. The SSR marker loci generated by the 56 primer pairs were used to assess the genetic diversity among 63 wheat genotypes. The microsatellite or SSR primers generated 7351 alleles with the number of alleles per locus varying from 0 to 368. This study also showed that primer pair Xgwm 63-7A generated a maximum number of 368 bands while primer pair Xgwm 112-3B produced a minimum number of bands (0). This revealed significant differences in allelic diversity among various microsatellite loci. Many studies have also reported remarkable differences in allelic diversity among various microsatellite loci (Akagi et al., 1997; McCouch et al., 2001; Ravi et al., 2003; Ram et al., 2007). The alleles revealed by markers showed a higher degree of polymorphism. More alleles were identified in genome B (a total of 3069 alleles) as compared to A and D genomes (genome A contained a total of 2457 alleles and genome D contained a total of 1825 alleles). This confirmed that there was a higher polymorphism level in the B genome. Similar observation for higher polymorphism level of genome B was also reported by Wang et al. (2007). The present observations also agree with the results of studies by Cho et al. (2000) in rice, Scott et al. (2000) in grapes and Eujayl et al. (2002) in wheat.

The 63 genotypes, which consisted of 48 accessions collected from PGRI, Islamabad, and 15 varieties of wheat from AARI, Faisalabad, were further subjected to cluster analysis. Cluster analysis of the SSR-based genetic similarity matrix resulted in the classification of accessions and varieties into separate clusters. The dendogram resulting from cluster analysis revealed 3 clusters. Cluster I included all 15 varieties, cluster II was the largest and included 25 accessions of 48, and cluster III comprised the remaining 20 accessions. Three accessions were distinct, namely 011350, 011351, and 011352, and of these three, two accessions, 011351 and 011352, are closely related, 011350 is separate, and these three accessions may be used in breeding programs as distinct parents.

A maximum genetic similarity value of 0.9589 was observed between the accession numbers 011484 and 011356, which revealed a high degree of similarity to the extent of 95.89% existing between them. A minimum genetic similarity value of 0.4198 was observed between the variety Kohistan-97 and accession number 011512, which showed that they are 41.98% dissimilar. Similar studies were conducted by different investigators using SSR markers (Panaud et al., 1996; Chakravarthy and Naravaneni, 2006; Ram et al., 2007). 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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