GENETIC DIVERSITY IN WHEAT GERMPLASM COLLECTIONS FROM BALOCHISTAN PROVINCE OF PAKISTAN

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Abstract

Productivity of wheat varieties being bred for the last many years is stagnant in Pakistan, apparently because of the narrowed genetic base of their parental lines. As a part of the national wheat germplasm characterization programme, we examined genetic diversity among 75 accessions of wheat using RAPD markers and assessed the relationship and genetic distance between them. The accessions surveyed were comprised of landrace populations of *Triticum aestivum* L., collected from various districts of the Balochistan province of Pakistan, which is considered a reservoir of genetic diversity, particularly for wheat. The genetic similarity revealed by RAPD markers among the wheat accessions was medium to high. The accessions collected from Sibi and Pishin districts had the greatest similarity. The polymorphism revealed in the wheat accessions, appeared to be distributed with the location of collections. The high degree of similarity even among the presumably landrace material emphasises the need for the expansion of germplasm resources and development of wheat varieties with diverse genetic background, which could substantiate the wheat breeding programmes to increase its productivity.

Introduction

Assessment of genetic variability within crop accessions has important implications in breeding and conservation of genetic resources. It is useful in the characterization of individual accessions and cultivars and, therefore, it is important to have this information for germplasm collections, to determine the range of diversity in accessions and during long-term maintenance of collections. Molecular marker system is one of the most effective methods for DNA profiling of crop genotypes and assessing genetic diversity and relatedness among them. In the breeding process, parental lines are selected from the available gene pool of contemporary varieties, and in some cases wide relatives or exotic germplasm are used to introduce a new trait, which could be tracked reliably through molecular markers (Keller *et al*., 1999; Seyfarth *et al*., 1999; Martin *et al*., 2000). Thus in addition to help assess the genetic diversity, molecular markers facilitate the identification of genes responsible for target traits and effective management of segregating and back cross populations. Depending upon the lab facilities, an array of molecular markers has been used in various crop species. Among the DNA markers, Random Amplified Polymorphic DNA (RAPD) markers have been shown to demonstrate a reasonable level of polymorphism at an affordable cost even in less equipped Labs and skimpy expertise (Rahman *et al*., 2001; Zenglu *et al*., 2001; Lawson *et al*., 2006). Therefore, the technique has a reasonable merit with practical plant breeders, who can use the technique to substantiate their phenotypic observations. To cut down the field testing cost and time, RAPD markers can be used to assess diversity in the available

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germplasm, to identify genes of interest and to develop a set of markers for the screening of progenies (Karp et al., 1998). DNA marker technology is particularly valuable for analysis of crops such as wheat with relatively low levels of genetic diversity (Roder et al., 1995; Korzun et al., 1997; Chalmers et al., 2001, Bai et al., 2003). The study presented in this paper is a part of the national wheat germplasm characterization programme, carried out under Agricultural Linkage Programme in the Department of Plant Breeding and Genetics and Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Pakistan. In the project studies, RAPD markers were used for molecular characterisation and the estimation of genetic diversity in the wheat germplasm collections from the Balochistan province of Pakistan.

Materials and Methods

Collection and multiplication of germplasm: The landrace material collected from remote areas of various districts of Balochistan consisting of 75 accessions of wheat belonging to *Triticum aestivum* L. (Table 1.) was assessed for genetic diversity. The collected germplasm was multiplied under field conditions in the Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad as the seed samples collected were too small in size.

DNA extraction: The multiplied seed of the wheat accessions was harvested and stored separately for molecular marker studies. The seed of the 75 accessions was sown in plastic pots (250 ml) and after two weeks of growth, DNA was extracted from them following the modified DNA extraction procedure developed in our laboratory (Khan et al., 2004). Briefly, after cutting into small pieces the leaf tissues were weighed and transferred immediately into zipper plastic bags containing 1.5ml CTAB solution. The leaf material in the bags was completely homogenized with a hand roller. After incubation, at 65°C for 30 minutes the homogenized leaf tissues (0.75 ml) were transferred into two 1.5 ml micro tubes. Equal volume (0.75ml) of chloroform: isoamylalcohol (24:1) was added and the tubes were inverted vertically 5-10 times followed by spinning at 13000rpm for 10 minutes. After centrifugation, 800ul of supernatant was transferred from both tubes into another 1.5ml micro tube. Then, approximately 700μl (0.9 volume) of isopropanol was added in the supernatant and mixed by inverting the tube about 10 times. The DNA was pelleted, washed and resuspended in 150ul of 0.1X TE. Finally the concentration of DNA was measured at 260nm in a spectrophotometer. The quality of DNA was checked by running 5μl DNA on 0.8% agarose gel prepared in 0.5X TBE buffer. The DNA samples giving smear in the gel were rejected and extracted again.

Amplification and analysis: The PCR amplifications were performed in 25 μl reactions containing 15 ng genomic DNA, 1 unit *Taq* polymerase, 3 mM MgCl₂, 100 mM dNTPs and 0.2 mM decamer RAPD primers. The PCR was carried out on a DNA thermocycler (Eppendorf) programmed as (95°C/5 min)1, (95°C/1 min, 36°C/1 min, 72°C/2 min)40, (72°C/10 min)1. The PCR products were separated on 1.2% agarose gel in TBE buffer with added ethidium bromide (10ng/100 ml) and agarose gels were photographed with UV light. All amplifications were repeated and only reproducible bands were scored for analysis. The wheat accessions screened for RAPD primers were scored for presence (1) and absence (0) of bands from top to the bottom of each lane. The RAPD data on the number of bands for the 75 wheat accessions thus collected was subjected to Popgene software (Version 1.44) using Unweighted Paired Group of Arithmetic Means (UPGMA) and similarity matrix Nei and Li’s (1979).
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<td>PARC/JICA</td>
<td>Kalat</td>
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<tr>
<td>75.</td>
<td>012187</td>
<td>Triticum</td>
<td>aestivum</td>
<td>KHOLAM</td>
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<td>Kalat</td>
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</table>
Results and Discussions

Detection of polymorphism in single seedlings: To determine the reproducibility of the RAPD bands, three replications of the same DNA sample were screened with 23 primers, which yielded consistent amplifications. Additionally, the banding patterns of 10 individual plants within each of the five wheat accessions also revealed consistent results. DNA samples of 10 individual plants from five wheat accessions were bulked and the banding pattern revealed from the bulked samples was also consistent with that of the pattern revealed from individual plants. Ten primers screened as polymorphic in a previous study (Khan et al., 2005) were tested on the present material. With these primers, a total of 76 DNA fragments were observed among the 10 individual plants of the five different accessions when tested separately. Three polymorphic loci were detected which showed the genetic differentiation within a cultivar. These types of genetic changes are, however, absent when a variety is developed by tissue culturing (Fu et al., 2002). This methodology of detection of polymorphism in single seedling thus increased confidence for the reproducibility and the consistency of the RAPD markers (Fu et al., 2002). Therefore, further characterization of the accessions was carried out following the procedure described previously (Khan et al., 2005).

Characterisation of wheat germplasm with RAPD markers: For affecting genetic improvement through selection and breedings the presence and estimation of genetic diversity in a crop species is a prerequisite. The results reported here pertain to the estimation of genetic diversity among 75 accessions of wheat using RAPD markers. RAPD technique has been extensively used for genetic characterization of wheat (Wang et al., 1995; Rashed et al., 2008). A total of 177 DNA fragments were amplified in the 75 accessions of wheat with the 20 RAPD primers with an average of about 8.85 bands per primer (Table 2). The number of bands that a primer yielded ranged from 4 (GLA-17 and GLB-9) to 16 (GLA-20). Of the total, 142 fragments were polymorphic among the wheat accessions, which indicated ~ 80.22% polymorphism. Rest of the bands (35) were monomorphic in the wheat accessions. The maximum polymorphism was revealed by the primers GLB-9 and GLB-16, while minimum polymorphism was produced by the primer GLC-9. The wheat accessions could be distinguished with the RAPD primers. The use of abundant and high polymorphic DNA markers eliminates the limitations associated with morphological and biochemical characterization, especially for closely related varieties (Asif et al., 2005; Schulman, 2007).

It was clear from the dendrogram (Fig. 1) that the accession 011226 was more dissimilar genetically from the other accessions and made a separate cluster. The accessions 011226 and 012184 were found genetically most dissimilar than the other accessions and had 60% similarity.

It may be concluded from this part of the studies that a good degree of polymorphism existed in the material examined, yet the genetic similarity among the accessions was medium to high (95.51% to 56.74%). The accession 011242 collected from Sibi district and 011286 collected from Pishin district had the greatest similarity 95.51%. The accessions 011226 and 012122 both collected from Lorali district were least similar. The accession 012173 collected from Mastung district also showed least similarity with accession number 011226. Thus, the polymorphism revealed in the wheat accessions appeared to be distributed with the collection site. However, the level of genetic diversity expected in the landrace material did not appear in the collections examined.
Table 2. Selected primers and their sequence and level of polymorphism.

<table>
<thead>
<tr>
<th>Sr. no.</th>
<th>Primer</th>
<th>Sequence</th>
<th>Total No. of bands</th>
<th>No. of polymorphic bands</th>
<th>Percentage of polymorphic bands</th>
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<td>1</td>
<td>GL DecamerA-01</td>
<td>CAGGCCCTTC</td>
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<td>2</td>
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<td>GAAACGGGTG</td>
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<tr>
<td>4</td>
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<tr>
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<td>5</td>
<td>71.42</td>
</tr>
<tr>
<td>6</td>
<td>GL DecamerA-15</td>
<td>TCCGAACCC</td>
<td>7</td>
<td>6</td>
<td>85.71</td>
</tr>
<tr>
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</tr>
<tr>
<td>8</td>
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<td>CAAACGTCCG</td>
<td>7</td>
<td>5</td>
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</tr>
<tr>
<td>9</td>
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<td>15</td>
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</tr>
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<tr>
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<tr>
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<td>GL DecamerB-19</td>
<td>ACCCCCGAAG</td>
<td>7</td>
<td>6</td>
<td>85.71</td>
</tr>
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<td>14</td>
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</tbody>
</table>

Total 20 --- 177 142 ---

Average --- --- 8.85 7.1 80.22

Fig. 1. Dendrogram of 75 wheat accessions collected from various districts of the Balochistan Province of Pakistan based on genetic similarities calculated from the data using 20 RAPD primers.
Conclusions

Food security through sustainable wheat production in the country is absolutely crucial for the sustenance of the people of Pakistan. However, in spite of concerted efforts by the wheat breeders, wheat yield and production is facing stagnancy in Pakistan for the last many years. The present assessment of the ‘presumably’ landrace material does not show the expected level of genetic diversity in the wheat germplasm even from those areas of Balochistan, which are still away from the access of the modern agriculture. It appears that the indigenous landrace material being grown in those centers of genetic diversity has been replaced by the short stature, fertilizer responsive high yielding varieties of wheat. This also appears that the genetic diversity has been drastically narrowed down after Green Revolution and same parental source has been used consistently for breeding the fertilizer responsive wheat varieties and eroded the natural variability existed in the form of landraces even in the centers of genetic diversity of wheat. Therefore, there is a need to develop wheat varieties with a diverse genetic background and augment variability into the existing wheat gene pool (Sud et al., 2005). Furthermore, the situation demands finding new solutions, which could substantiate the breeding programmes to increase wheat productivity. Enrichment of wheat germplasm resources through the creation of synthetic hexaploid wheat, development of high efficiency TILLING (Targeted Induced Local Lesions IN Genomes) populations and development of transgenics seems to offer hope to revamp the eroded genetic variability and generate sources of genetic variation for the development of commercial wheat cultivars.

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References


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