MOLECULAR DIVERSITY OF FOUR EGYPTIAN WHEAT CULTIVARS REVEALED BY MICROSATELLITES.
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ABSTRACT

The objective of this study was to evaluate the genetic diversity of the most important Egyptian wheat cultivars grown in 2006 by using microsatellites (SSR) as molecular markers. The four varieties of wheat (Triticum aestivum L.) tested were Giza 166, Sids 1, Gemmiza 7 and Gemmiza 9) including Chinese Spring as standard. The 24 microsatellites used revealed a total of 93 alleles. The fragment size ranged from 75 bp in GWM3 to 263 bp in GWM931. The average number of alleles was 3.9 ranging from 2-9 alleles per locus. The average allele number was different for each cultivar. With 86 alleles the number was highest in Giza 166, followed by 33 alleles in Sids 1, then Gemmiza 9 with 32 alleles and the lowest allele number was found in Gemmiza 7 (30 alleles). The polymorphism information content (PIC value) reflecting the gene diversity of the 24 microsatellite loci ranged from 0.48 to 0.82 with an average of 0.66. The genetic similarity level ranged from 0.22 for Chinese Spring with the Egyptian cultivars to 0.58 for Gemmiza 7 and Gemmiza 9. The correlation coefficient between PIC and number of alleles over 24 microsatellites loci was 0.62.

INTRODUCTION

Wheat is an important crop occupying around 16% of the arable lands of the world, with increasing global demand and associated shortages of production in many countries. Although wheat production per unit area in Egypt has significantly increased during the past years, wheat production supplies only 40% of its annual domestic demand. The reasons for the lacking ability of Egypt to produce sufficient wheat for domestic consumption are that the total cultivated area represents less than one quarter of the total area; and that Egypt has one of the highest rates of wheat consumption per capita of any country in the world (200 kg per capita, compared with a world average of less than 60 to 75 kg per capita). The population growth rate (2.1% annually) increases higher than the increase of wheat production and little efforts are made for improving salt tolerance in wheat crops, e.g. only two genotypes (Sakha 8 and Sakha 93) among Egyptian wheat genotypes are tolerant to salinity. Therefore, there exists a competition for cultivated lands to grow wheat, forage and cotton crops. Most importantly, Egypt still is one of the largest countries that import wheat. Wheat imports in 2004/05 (July/June) were about 6.5 million tons, with a cost of about 986 million US $ annually (FAO; http://www.fao.org/). Therefore, the Egyptian Government needs to make a great effort to increase wheat productivity. Extending wheat growing outside
the Nile Valley is the first effort toward overcoming the described problems. However, most of the area outside the Nile Valley suffers from salinity or depends on water sources that are affected by salinity, therefore, increasing salt tolerance for wheat genotypes is one of the cheapest methods to spread growing wheat in these areas.

The power of molecular markers as powerful tools to evaluate the genetic diversity of germplasm is increasingly recognized (Melching et al., 1991 and Melchinger et al., 1994). Such markers have been used to trace the geographic origins of accessions by comparing genetic fingerprints of diverse material (Salamini et al., 2002; Beat et al 2003) and to classify germplasm resources (Almerew et al., 2004).

Microsatellites are, compared to other marker types, abundant and possess a high polymorphism information content (PIC) and are often multiallelic (Röder et al., 1995; Gupta et al., 1996). A limited number of microsatellite markers are often sufficient to detect differences even in very closely related wheat genotypes (Plaschke et al., 1995). Furthermore, a large number of wheat microsatellite markers has been developed, which are widely used in genomic mapping populations and evolutionary studies, as well as for fingerprinting and pedigree analyses (Röder et al., 1998; Röder et al., 2004).

Crop diversity studies using molecular markers have been conducted in different cereals such as barley (Hordeum vulgare L.; Macauilay et al., 2001; Mathes and Hayes, 2002; Koebrer et al., 2003), rice (Oryza sativa L.; Ishii and McCouch, 2001), maize (Zea mays L.; Mumma and Dudley, 1994 and Liu and Bernardo, 2001) and in wheat (Triticum spp.; Donini et al., 2000; Prasad et al., 2000; Röder et al., 2002; Huang et al., 2002; Eujail et al., 2002). Microsatellites have a high potential for genome analyses of self-pollinating crops because of their specific properties and their high degree of polymorphism (Plaschke et al., 1995; Röder et al., 1995).

This study was conducted to evaluate the genetic diversity of the most important Egyptian wheat cultivars grown in 2005 by using microsatellites (SSR) as molecular markers.

MATERIALS AND METHODS

Plant materials

Four varieties of spring wheat (Triticum aestivum L.) were used in this study (Giza 168, Sids 1, Gemmeza 7 and Gemmeza 9). They were obtained from the Wheat Department, Field Crops Institute, Agricultural Research Centre in Giza, Egypt. The variety Chinese spring was obtained from the Gene Bank, Gatersleben, Germany, and used as reference. Seeds from all cultivars were planted in the greenhouse of the Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany.

DNA extraction

Total genomic DNA was extracted from pooled leaves of five one-week-old plants. The young seedling leaves of each cultivar were harvested and frozen in liquid nitrogen 15 minutes. Approximately 2-4 g of leaf material were ground in 2 ml tubes in “Retsch-Schwingmuhlen MM 300” 2x 30 sec
and a frequency of 25/s. The extraction was performed according to the
protocol of Doyle & Doyle 1990. Polymerase Chain Reaction amplifications
were performed as described by Röder et al., (1998). The substrate
subjected to PCR contained 50-100 ng template DNA, 250 nM cy5-labelled
forward primer, 250 nM unlabelled reverse primer, 0.2 mM dNTPs, 2.5 μl
PCR buffer (10 x), 1.5 mM MgCl₂ and 1U Taq DNA polymerase in a total
volume of 25 μl.
Fragment detection was performed with an Automated Laser
Fluorescence (ALFexpress) sequencer (Amersham Biosciences Europe
GmbH, Freiburg, Germany) as described by Röder et al., (1998) and
fragment sizes were calculated using the computer programme fragment
Analyzer 1.02 (Amersham Biosciences) by comparison with internal size
standards. The cultivar Chinese spring was used as a reference to
standardize different gel runs. In the case of weak or lacking fragment
products, PCR amplifications were repeated to exclude failed PCR reaction
as the cause of null alleles.

Microsatellite markers (SSR)
A total of 24 Gatersleben Wheat Microsatellites (GWM) were used for
analysis. The GWM markers were previously described by Röder et al.

Data analysis
The presence and absence of alleles was scored as binary data
matrix. The gene diversity also called polymorphism information content (PIC)
was computed according to Nei (1973) as:

\[ \text{PIC} = 1 - \bar{P}^2 \]

Where \( P_i \) is the allele frequency of the \( j \)th allele for the \( i \)th marker summed
over the number of alleles. Anderson et al., (1993) suggested that gene
diversity is the same as the Polymorphism Information Content (PIC).
Genetic similarity was calculated according to Dice (1945). Cluster analysis
was performed using the UPGMA method.

Results:
Microsatellite diversity
The 24 wheat microsatellite markers used revealed a total of 93 alleles. The
fragment size ranged from 75 bp in GWM3 located on chromosome 3D to
285 bp in GWM931 on chromosome 5D. The average number of alleles per
locus was 3.9 and the largest number of alleles was 9 detected on locus
GWM1002. The lowest number of alleles (2 alleles) was found at GWM 291
and GWM 674 (Table 1). Average of allele numbers was different for each
cultivar. The allele number was highest (96 alleles) in the variety Giza 168
followed by 33 alleles in Sids 1, then Germiza 9 with 32 alleles and the
lowest was Gimmeza 7 with 30 alleles.

Analysis of gene diversity
The PIC value reflecting the gene diversity of the 24 microsatellite
loci ranged from 0.48 at locus GWM 291 and GWM 674 to 0.82 at locus
GWM 1016 with an average of 0.66 (Table 1).
Table 1: SSR markers and their chromosomal locations, Fragment size, polymorphism information content (PIC) and allele number in four Egyptian wheat cultivars.

<table>
<thead>
<tr>
<th>SSR markers</th>
<th>Chromosomal locations</th>
<th>Fragment size range</th>
<th>PIC</th>
<th>Allele no</th>
</tr>
</thead>
<tbody>
<tr>
<td>gwm234</td>
<td>5B</td>
<td>202-258</td>
<td>0.64</td>
<td>4</td>
</tr>
<tr>
<td>gwm294</td>
<td>2A</td>
<td>89-118</td>
<td>0.77</td>
<td>5</td>
</tr>
<tr>
<td>gwm497</td>
<td>1A, 2A, 3D</td>
<td>156-159</td>
<td>0.84</td>
<td>3</td>
</tr>
<tr>
<td>gwm113</td>
<td>4B</td>
<td>Null, 147-156</td>
<td>0.72</td>
<td>4</td>
</tr>
<tr>
<td>gwm1016</td>
<td>5B, 6B</td>
<td>111-155</td>
<td>0.82</td>
<td>6</td>
</tr>
<tr>
<td>gwm1002</td>
<td>7D</td>
<td>null, 140-185</td>
<td>0.78</td>
<td>9</td>
</tr>
<tr>
<td>gwm291</td>
<td>5A</td>
<td>null, 163</td>
<td>0.48</td>
<td>2</td>
</tr>
<tr>
<td>gwm540</td>
<td>5B</td>
<td>124-132</td>
<td>0.64</td>
<td>3</td>
</tr>
<tr>
<td>gwm931</td>
<td>5D</td>
<td>null, 270-284</td>
<td>0.72</td>
<td>4</td>
</tr>
<tr>
<td>gwm1078</td>
<td>1B</td>
<td>139-141</td>
<td>0.64</td>
<td>3</td>
</tr>
<tr>
<td>gwm1184</td>
<td>7B</td>
<td>139-143</td>
<td>0.64</td>
<td>3</td>
</tr>
</tbody>
</table>

The correlation coefficient between Polymorphism Information Content (PIC) and number of alleles over 24 microsatellites loci was 0.62. (Figure 1).

Cluster analysis

The genetic similarity values between the cultivars were used to produce a dendogramme. The analysis was derived from a UPGMA cluster analysis which helps to explain the relationship between wheat cultivars. The genetic similarity level ranged from 0.22 for Chinese Spring with the Egyptian cultivars and 0.53 for Gimmeza 7 and Gimmeza 9.

Cluster analysis allowed to discriminate two groups. The first cluster consisted only of cultivars Gimmeza 7 and Gimmeza 9, while the second cluster comprised the other two cultivars Sids 1 and Giza 168 (Figure 2).
Figure 1. Correlation between gene diversity and number of alleles over 24 microsatellites loci in 4 Egyptian wheat cultivars.

Figure 2: Dendrogram of 4 Egyptian wheat cultivars clustered according to UPGMA using Dice's similarity coefficients.
DISCUSSION

In this study 24 microsatellites revealed 93 alleles from 5 wheat cultivars were sufficient to discriminate the germplasm for these cultivars. The average number of alleles was 3.9 and genome B was more polymorphic than A. These results were similar to previous studies on wheat (Figueroado and Pernino, 2004) they noted that 15 markers produced 63 bands with an average of 7.7 alleles. Moraovar, Teklu et al. (2005) reported a higher number of alleles per locus for T. durum than for T. turgidum. He noted that 29 microsatellite markers revealed 320 and 271 alleles in T. durum and T. turgidum, respectively, with average number of alleles per locus of 11.0 in T. durum and 9.3 in T. turgidum. On the other hand, Bertin et al., (2001) found an average number of 5.2 alleles per locus in spelt wheat. While, Eujail et al., (2001) detected an average of 5.5 alleles per locus with 64 genotypes and Ben Amer et al. (2001) found an average off 4.5 alleles per locus from 24 wheat microsatellite markers with 15 Libyan wheat genotypes. The results also were confirmed by Khilekina et al., (2004) reported an average PIC-value of 0.7 in 54 Siberian wheat plants. Huang et al., (2002) reported a gene diversity of 0.77 in 998 European wheat varieties. While Prasad et al., (2000) found a PIC-value of 0.71 in 55 wheat genotypes.

The cluster study discriminated the investigated four Egyptian wheat cultivars. Similar results were found by Kihlekina (2004) for old and modern Siberian spring wheat varieties. Huang (2002) found that not all accessions originating from the same region clustered in the same group, indicating that the genetic diversity of T. aestivum is not completely related to geographic distribution. In contrast, Alamere et al., (2004) found that all of the used accession in his study could be separated, clustering in two large groups.

Acknowledgements

The authors grateful to the professional and personal support from inWent Organization also to Dr. Röder and her group staff of the Gene and Genome Mapping, IPK Gatersleben.

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التنوع في الجزيئات الإحائية لأربعة أصناف فحم مصرية باستخدام المعملات الإحائية

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تعتبر هذه الدراسة تقييم التَّوَّر الوَرَاثي في أربعة أصناف فحم مصرية مكونة من 312 سمه، 115 سمة، و 89 سمة، و 103 سمة، وذلك باستخدام المعملات الإحائية. تم استخدام 40 مصلحه و 34 مصلحه، و 36 مصلحه، و 28 مصلحه، و 22 مصلحه، و 18 مصلحه، و 14 مصلحه، و 11 مصلحه، و 7 مصلحه، و 5 مصلحه، و 3 مصلحه، و 2 مصلحه، و 1 مصلحه.

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