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Molecular Profiling of Egyptian Rice Varieties Using DNA Markers

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ABSTRACT

Genetic variability and phylogenic relationships among ten Egyptian commercial rice varieties namely Giza 175, Giza 177, Giza 178, Giza 181, Giza 182, Sakha 101, Sakha 102, Sakha 103, Sakha 104 and Egyptian Yasmine, were established by using fifty two STMS (Sequence Tagged Microsatellites) Markers. A high level of polymorphism (84.6%) revealed on agarose gel by SSRs among the tested genotypes. The varieties were clustered based on their banding patterns using UPGMA method largely on their genetic background with few exceptions. Molecular profile was established for each variety as a fingerprint using clear and distinct patterns of 28 SSR markers. The profile will be used as a reference for the breeder>s rights and Intellectual Property Rights (IPR) and any other related issues. It will also help in assessment of seed purity for specific varieties. Molecular profiling is also an essential requirement for registering the new rice varieties. The profile could bas used for detection of essentially derived varieties.

Key Words: Molecular, profiling, rice, DNA markers.

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INTRODUCTION

Rice *(Oryza sativa L.)* is one of the most important crops that provide food for about half of the world population. In areas such as Asia, Africa and Latin America where the demand for rice is a top priority, the world population is expected to increase 1.5 fold by 2025 *(Sasaki, 2002)*. Egypt is one of the few countries, which produce high yielding rice varieties and succeeded to achieve one of the highest productivities per unit area through the last decade. From the commercial point of view, DNA fingerprinting is a useful tool for varietal protection to prove ownership or derivation of plant lines. Moreover, the analysis of genetic diversity and relatedness between or within different species, populations and individuals is a prerequisite towards effective utilization and protection of plant genetic resources *(Weising et al., 1995)* With DNA being the only basis of genetic differences between distinct organisms; DNA fingerprinting is presently the ultimate method of biological individualization. Unlike the morphological and biochemical markers, which may be affected by environmental factors and growth practices *(Xiao et al., 1996)*.

The choice of the marker system to be used is the most important decision and that depends mainly on the application. Using PCR based SSR markers to detect genetic variability in rice cultivars are reported here. The objectives of this study were to determine the genetic relationships among several Egyptian rice genotypes and the suitability of the different approaches for developing unique molecular markers profile for the ten rice varieties and therefore, developing unique fingerprint for each variety.

MATERIALS AND METHODS

The seeds of ten Egyptian rice cultivars, namely Giza 175, Giza 177, Giza 178, Giza 181, Giza 182, Sakha 101, Sakha 102, Sakha 103, Sakha 104 and Egyptian Yasmine were grown in the green house of Rice Research and Training Center (RRTC). The list of the tested varieties is presented in (Table 1). Total genomic DNA were isolated from young leaves grown in the green house for 15 days using CTAB method *(Muray and Tompson, 1980)*. quantification was carried out by gel based assay using different concentrations of λ un-cut DNA. PCR reactions for SSR markers were carried out in 10 ul volume containing 1.0 µl total genomic DNA 4.9 ul H_2O, 1.0 µl 10 X PCR buffer, 0.8 µl Mg Cl2, 0.4 µl dNTPs, 0.3 µl Taq polymerase and 0.3 µl from SSR markers (forward and reverse primers). Amplification was performed in Berkin Elemar Gene Amp PCR system 2400 and DNA Engine Peltier Thermal Circler with following the profile, 95°C for 5 min (initial denaturation), 95°C for 1 min, 55°C for 1 min, 72°C for 2 min and for 35 cycles with final extension 72°C for 7 min. The PCR products were analyzed directly on 1.5 % agarose gels in 0.5x TAE buffer, visualized by staining with ethidium bromide and Transillumination under ultra violet light. Comparison of genotypes, based on the presence (1) or absence (0) of unique and shared polymorphic products was used to

generate similarity coefficients using UPGMA method with statistical software package NTSYSpc2.1 *(Rohlf, 2001)*.

Table 1: List, Type and Parentage of the Egyptian tested varieties.

No	Genotypes	Type	Parentage
1	Giza 175	Indica/ Japonica	22 / IR 1541 IR / / 18014 / Giza
2	Giza177	Japonica	Giza 171 / Yomji no.1 // Pi no.4
3	Giza178	Indica/ Japonica	Giza175 / Milyang 49
4	Sakha101	Japonica	176 Giza79 / Milyang
5	Sakha 102	Japonica	GZ4096-7-1 /Giza 177
6	Sakha103	Japonica	Giza 177 / Suweon 349
7	Sakha104	Japonica	GZ 4096-8-1 / GZ 4100-9-1
8	Giza 182	Indica	181 /IR 39422-163-1-2/ Giza 181
9	Giza 181	Indica	IR 24 /IR 22
10	Egyptian Yasmine	Indica	Basmati 370 / IR line

REAULTS AND DISCUSSIOBN

Genetic relationship among the ten Egyptian rice genotypes has been carried out using SSR markers. Fifty two primer pairs flanking simple sequence repeats were used to investigate the level of polymorphism among the ten rice cultivars. Forty four primers showed different levels of polymorphism and eight primers were monomorphic. The percent of polymorphism was 84.6 %. The size of detected alleles produced from using SSR primer sets ranged from 50–500 bp. The number of alleles detected using SSR markers ranged from one (in case of RM 36) to 3 (in case of RM214) (Figure 1). The polymorphism detected reflects the amount of diversity among the tested genotypes and thus the possibility of genetic improvement using such a set of genotypes in the breeding program since genetic diversity is the prerequisite for successful breeding programs.

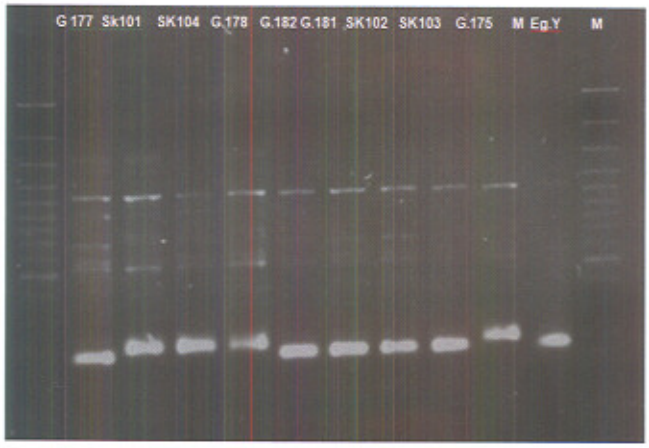

Figure 1: Banding patterns of the ten commercial varieties as revealed by RM214 on 1.5% agarose gel. M 100 bp ladder

Based on the banding patterns, a dendrogram explaining the genetic relationships among the tested varieties were constructed using UPGMA method. The dendrogram is shown in (Figure 2). The ten varieties were clustered in three main groups A, B and C. The first group included four pure Japonica genotypes Giza 177, Sakha 101, Sakha 104 and Sakha 103. The second group B included four varieties Giza 175, Sakha 102, Giza 181 and Giza 182. While the third group C included the Indica Japonica variety Giza 178 and the Indica variety Egyptian yasmine. The clustering succeeded to separate most of the pure Japonica varieties in one cluster (A), while the two other groups included Indica /Japonica and pure Indica varieties with one exception, Sakha 102, which abnormally clustered with the second group. The results obtained here concluded that clustering were largely depending on the varietal genetic background. Similar results were reported by many authors similar trends were also reported by many authors. Ammar *(2004)* reported that, 250 STMS primers, detected considerable amount of genetic diversity among a set of eight genotypes differing in salt tolerance. Results showed that genotypes were clustered based on genetic background rather than salt tolerance levels. *Hammoud, et al. (2007)* used 26 STMS and ISJ markers to assess the genetic diversity among four Sakha 101 derived lines and their three parental lines. They found molecular differences among the used genotypes and concluded the possibility to develop elite lines resistant to blast using conventional breeding methods. Genetic diversity of rice (*Oryza sativa* L.) cultivars in Argentina was evaluated at the DNA level *(Giarrocco et al., 2007)*. They surveyed Sixty-nine accessions with 26 simple sequence repeat (SSRs) markers revealing the genomic relationship among cultivars. The 69 accessions used in this study were clustered in the same order using the UPGMA cluster analysis based Jaccard coefficient. The two major *O. sativa* groups, Indica and Japonica were resolved in the dendrogram and verified by the reference cultivars IR36, Bluebelle, Lemont, Katy, Cypress and Dawn. Herrera, et al. *(2008)* used a set of 48 simple sequence repeat (SSR) markers to assess the genetic diversity of 11 Venezuelan rice cultivars, released by the National Rice Breeding Program between 1978 and 2007. UPGMA-cluster-analysis based on genetic distance coefficients clearly separated all the genotypes and showed that the Venezuelan rice varieties are closely related. Molecular identification of seven Venezuelan cultivars could be done with nine primer pairs, which produced 10 genotype specific alleles. Although the genetic diversity was low, SSRs proved to be an efficient tool in assessing the genetic diversity of rice genotypes. Lapitan, et al. *(2007)* used 164 SSR primers to study the genetic relationships among 24 Philippine rice varieties. Cluster analysis of these cultivars was able to identify three groups at 40% level of similarity with additional sub-clusters within each group. Group 1 corresponded to the eight Japonica subspecies, whereas Groups 2 and 3 comprised the Indica. Cultivars under groups 1 and 2 are known for their aroma and good cooking and eating quality traits. Between the two rice subspecies, Indica gave more alleles than Japonica and likewise displayed a higher genetic diversity. Wan et al. *(2008)* used 13 ISSR (Inter simple Sequence Repeats) to access the genetic diversity among 34 populations of *Oryza meyeriana* distributing in Yunnan province, China.

A molecular profile for each of the commercial varieties was carried out using selected 28 Clear STMS markers with clear banding pattern. The profile will be used as a reference for the

breeder's rights and IPR and any other related issues. It will also help in assessment of seed purity for specific varieties. Molecular profiling also is an essential requirement for registering the new rice varieties. Profiling helps in detection of essentially derived varieties. (Figure 3) shows the molecular profile for Giza 177 using 28 SSR primers. The profile will be used as a reference for any of the above mentioned related issues.

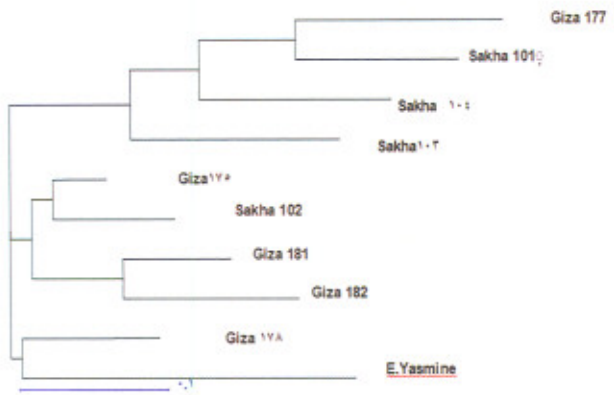

Figure 2: Dendrogram explaining the genetic relationship among the tested varieties.

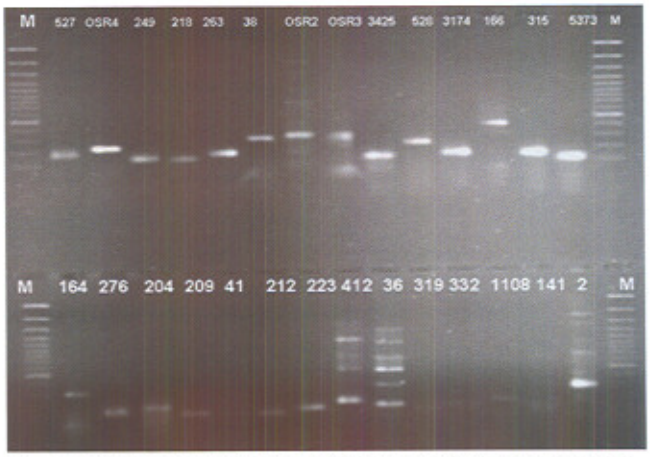

Figure 3: Molecular Profiling for Giza 177 with 28 SSR markers.

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