# Genetic polymorphism among some Egyptian rice genotypes as revealed by RAPD, SSR and AFLP analyses

*(Received: 01. 09. 2010; Accepted: 09 .11 .2010)*

**Momtaz O. A. *, Hashem Mai M. *, Moghaieb R. E.A. ** and Hussein Mona H. ****
*Agriculture Genetic Engineering Research Institute (AG ERI); Agriculture Research Center (ARC); Giza, Egypt
**Department of Genetic, Faculty of Agriculture, Cairo University

## ABSTRACT

*In order to determine the genetic diversity among the five Egyptian rice cultivars, RAPD, SSR and AFLP analyses were performed. The data indicated that all of the three molecular markers gave different levels of polymorphism. A total of 59, 34 and 205 markers that show 52, 33 and 77% polymorphism percentages were resulted from the RAPD, SSR and AFLP analyses, respectively. Based on the data obtained, the three markers can be used to differentiate between the five rice genotypes. The genotype specific markers were determined; the cultivar Giza-178 scored 25 markers followed by Sakha-102 (18) then Giza-177(14), while the cultivar Sakha-101 scored only 9 markers. The AFLP analysis gave the highest number of genotype specific markers (43) followed by RAPD (28) then SSR analysis (8). The dendrogram based on combined RAPD, SSR, and AFLP data clustered the genotypes Giza 178 and Giza 182 in one cluster and Sakha 101,Sakha102 and Giza 177 in another cluster, where Sakha 101 and Sakha 102 are clustered in one sub cluster and Sakha 102 in another sub cluster. It can be concluded that the rice varieties Sakha 101 and Sakha 102 may originated from more closely related ancestors than those of the variety Giza 177, since they possess a higher degree of genetic similarity and they also may share this genetic similarity with the variety Giza 177. Similarly, the rice varieties Giza 178 and Giza 182 may have a closely related ancestor.*

*Keywords: Oryza sativa, fingerprinting, genetic polymorphism, molecular markers.*

## INTRODUCTION

Rice (*Oryza sativa*) (2n=24) family *Graminae* and subfamily *Oryzoidea* is the main source food for one third of the world population and occupies almost one-fifth of the total land area covered under cereals (Chakravarthi and Naravaneni, 2006). Egypt is one of the few countries which develop high yielding rice varieties. From the commercial point of view, 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). The major goal of evolutionary biology is the characterization and quantification of genetic diversity. Information on the genetic diversity and the analysis of genetic variation within and among elite breeding materials is of fundamental interest to plant breeders. It contributes to monitoring germplasm and can

also be used to predict potential genetic gains (Chakravarthi and Naravaneni, 2006).

Molecular markers have been proven to be powerful tools in the assessment of genetic variation and elucidation of genetic relationships within and among species on the contemporary of the morphological and biochemical markers which may be affected by environmental factors and growth practices (Xiao *et al.,* 1996, Ovesna *et al.,* 2002 and Higgins, 1984). A wide variety of DNA-based markers has been developed in the past few years and restriction fragment length polymorphism (RFLP) was the first molecular marker used for genome analysis and genome mapping (Botstein *et al.,* 1980). This was followed by advances in polymerase chain reaction (PCR) technology which led to a number of useful markers e.g. .random amplification of polymorphic DNA (RAPD) (Tingey and Deltufo, 1993) , simple sequence repeats (SSRs) (Levinson and Gutman, 1987) , amplified fragment length polymorphism (AFLP) (Mackill *et al.,*1996), which vary in their specificity and resolution . RAPD was the first PCR based marker technique in which a short random PCR primer is used to amplify a genomic DNA sequence. SSRs are present in the genome of all eukaryotes and consist of several repeats to over hundreds of nucleotide motif and flanked by sequence that can be used as primers so it is more specific than RAPD .Moreover AFLP is the latest form of molecular markers and it is highly sensitive as it is a method based on combined concept of RFLP and RAPD .

Molecular marker applications in rice studies starting from RAPD, SSRs and AFLP-based DNA fingerprinting are useful to determine phylogenetic trees, characterization and quantification of genetic diversity within and among elite breeding materials for monitoring germplasm, as well as, predicting potential genetic gains in addition to its usage as a tool for varietal protection to prove ownership or derivation of plant lines (Chakravarthi and Naravaneni., 2006 and Saker *et al.*, 2005).

Molecular Markers also are useful in rice genome sequence mapping, physical mapping, gene identification, data base storage and visualization and identification of candidate genes for drought stress tolerance in rice by the integration of a genetic QTL maps with rice genome.

The objectives of this investigation were to determine the genetic variability and biodiversity among five Egyptian rice varieties at the molecular level using RAPD, SSRs and AFLP markers and to use the combined data to make a phylogenetic tree.

## MATERIALS AND METHODS

### Plant materials

Seeds of rice varieties provided by the Field Crop Research Institute, ARC (Sakha 101, Sakha102, Sakha104, Giza 177, Giza 178) were germinated at 28±2°C for 4 days in Petri dishes (Wang *et al* , 2007), then they were transferred into pots filled with sand till they became 10 day old seedlings.

### DNA isolation

DNA was isolated from leaves of 10 day old seedlings according to CTAB protocol with minor modification that is regularly being followed in GERT (Gene Expression and Regulation Technology) Laboratory. It was found that DNA isolated by modified CTAB method was of high purity and the yield was also substantial (Michiels *et al,* 2003). the DNA isolated for all the samples by this method was used for further analysis.

### RAPD analysis

PCR reactions were carried out in 25 µl volume containing 25ng of total genomic

DNA, 10 pmole primer,200 µM dNTPs,2 mM MgCl2,1X PCR buffer and 0.4 µl (2 units) Ampli taq polymerase (Fermentas UK). Ten-mer-oligonucleutides random primers were selected for analysis (Williams *et al.*, 1990). Primer names and their sequences are listed in Table (1). Amplification was performed in G-Storm thermal cycler with the following profile: 94°C for 3 min. (initial denaturation), 94°C for 50 sec, 36°C for 1 min, and 72 ° C for 90 sec for 40 cycles with a final extension at 72 ° C for 7 min.

***Table (1): RAPD and SSR primer names and sequences used to study the genetic diversity among five rice genotypes.***

| RAPD primers | | SSR primers | |
|---|---|---|---|
| **Name** | **sequence** | **Name** | **Sequence (5'--3')** |
| OP-B6 | 5'-TGCTCTGCCC-3' | RM10 | F –TTGTCAAGAGGAGGCATCG<br>R- CAGAATGGGAAATGGGTCC |
| OP-B7 | 5'-GGTGACGCAG-3' | RM519 | F- AGAGAGCCCCTAAATTTCCG<br>R- AGGTACGCTCACCTGTGGAC |
| OP-C11 | 5'-AAAGCTGCGG-3' | | |
| OP-C7 | 5'-GTCCCGACGA-3' | RM561 | F -GAGCTGTTTTGGACTACGGC<br>R GAGTAGCTTTCTCCCACCCC |
| OP-C5 | 5'-GATGACCGCC-3' | RM20 | F-ATCTTGTCCCTGCAGGTCAT<br>R –GAAACAGAGGCACATTTCATTG |
| OP-C10 | 5'-TGTCTGGGTG-3' | | |
| OP-B5 | 5'-TGCGCCCTTC-3' | RM320 | F- CAACGTGATCGAGGATAGATC<br>R- GGATTTGCTTACCACAGCTC |
| | | RM544 | F- CAACGTGATCGAGGATAGATC<br>R- GGATTTGCTTACCACAGCTC |
| | | RM346 | F- C G A G A GAGCCCATAACTACG<br>R- ACAAGACGACGAGGAGGGAC |
| | | RM536 | F- TCTCTCCTCTTGTTTGG CTC<br>R-ACACACCAACACGACCACAC |
| | | RM47 | F -ACTCCACTCCACTCCCCAC<br>R -GTCAGCAGGTCGGACGTC |

**SSR analysis**

The polymerase chain reaction was carried out in a Bio-Rad Thermal Cycler using RM primers (Chakravarthi and Naravaneni, 2006) (Table 1). PCR reaction mix included the following: DNA, 10 ng/µl; 10X buffer; 10 mM dNTPs; 50 mM MgCl2; 10 µM each of forward and reverse primers. The PCR profile start with 95°C for 5 min followed by 35cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1min extension at 72°C for 2 min. A final extension at 72°C for 7 min was included. The PCR products were electrophoresed in a 2% agarose gels (for SSRs) at 100 V for 2-3 h. The gel was then stained in ethidium bromide for 30 min, de-stained for

15-30 min and then observed on a UV transilluminator. The PCR products of SSR primers were run on 4% polyacrylamide gels to achieve better resolution of the bands. After the gel was dried completely, it was scanned using BioRad Model GS-700 Imaging Densitometer and the individual bands were scored for further analysis.

**AFLP analysis**

AFLP was carried out as described by Vos *et al.* (1995) using the GIBCO BRL system I (Cat. No.10544) according to the manufacturer's protocol .

**Electrophoresis**

The RAPD-PCR products were analyzed directly on 1.5% agarose gels in TAE buffer, visualized by staining with ethidium bromide and transillumination under short- wave UV light. Amplified DNA products using SSR primers were examined by horizontal electrophoresis 3.5% metaphor (FMC Bioproducts, Rockland, ME) agarose gels. AFLP amplification products were separated in a vertical denaturating 6% polyacrylamid gel in Sequi-Gen cell (BioRad Laboratories Inc.) as described by Bassam *et al.* (1991). The DNA fragments were visualized by autoradiography.

**Data analysis**

All the genotypes were scored for the presence and absence of the RAPD, SSR and AFLP marker bands. The data were entered into a binary matrix as discrete variables, 1 for presence and 0 for absence of the character and this data matrix was subjected to further analysis. The Excel file containing the binary data was imported into NT Edit of NTSYS-pc 2.02J. The 0/1 matrix was used to calculate similarity as DICE coefficient using SIMQUAL subroutine in SIMILARITY routine. The resultant similarity matrix was employed to construct dendrograms using sequential agglomerative hierarchical nesting(SAHN) based unweighted pair group method with arithmetic means (UPGMA) to infer genetic relationships and phylogeny.

## RESULTS AND DISCUSSION

The feasibility of using RAPD analysis for DNA fingerprinting of U.S. rice cultivars was reported by Mackill *et al.*(1996). In the present study the genetic polymorphism between five cultivated Egyptian rice genotypes was determined using RAPD, SSR and AFLP analyses. Seven RAPD primers generating reproducible, informative and easily scorable RAPD profiles were pre-selected. The seven primers produced multiple band profiles with a number of amplified DNA fragments varying from 6 to 10, with a mean number of 8.42 markers per primer. The primer OPC-5 gave the highest number of fragments (10), while the minimum number of fragments (6) was amplified with the primer OPC-10. The total number of generated bands was 59, which show 88.13% polymorphism between them. A total of fifty two (52) polymorphic bands was observed with the seven selected primers ranging from 5 to 10 per primer. The highest number of polymorphic bands (10) was obtained with primer OPC-5, with 100% polymorphism, while the primer OPC-11 gave the lowest number of polymorphic bands(5) with 55.5% polymorphism. The average number of polymorphic fragments per primer among the 5 rice varieties was 7.4 (Table 2).

***Table (2): The RAPD primers which generate the different unique markers.***

| Primers | Total #of band | Monomorphic bands | Polymorphic bands | Band size range(bp) | % of polymorphism |
|---|---|---|---|---|---|
| **OP-B6** | 9 | 3 | 6 | 300-900 | 66.6 |
| **OP-B7** | 8 | 0 | 8 | 700-1400 | 100 |
| **OP-C11** | 9 | 4 | 5 | 60-255 | 55.5 |
| **OP-C7** | 9 | 0 | 9 | 80-1350 | 100 |
| **OP-C5** | 10 | 0 | 10 | 300-1800 | 100 |
| **OP-C10** | 6 | 0 | 6 | 400-2200 | 100 |
| **OP-B5** | 8 | 0 | 8 | 100-1100 | 100 |
| ***Total*** | 59 | ------ | 52 | ------- | 88.13 |

Nine SSR primers were utilized to provide genetic diversity among the five Egyptian rice varieties belonging to Indica and Japonica types. The data revealed that the nine SSR primer sets which are distributed through the 6 different rice chromosomes gave 34 alleles, eight alleles with RM-10, six with RM-47, four for each of RM-20 and RM-320, three for each RM-346 and RM-536 and two alleles for each of RM- 519, RM- 561and RM- 544. Similarly, Chakravarthi and Naravaneni, (2006), utilized 30 RM primers to provide genetic diversity among 15 elite rice lines belonging to Indica and Japonica types that showed polymorphism between them and resulted in scoring 462 bands. Also Wu and Tanksley (1993) detected comparable number of alleles per locus, 2 to 5 at different loci within 6 cultivars of the Japonica subspecies and 2 to 6 alleles within 8 cultivars of Indica subspecies. All primers showed different levels of polymorphism among the five rice varieties (Figure 1 and Table 3). Most of the alleles were polymorphic, thus revealing 98.6 % polymorphism which is higher than those given by RAPD. Also many studies have reported significantly greater allelic diversity of microsatellite markers than other molecular markers (McCouch *et al.*,2001).

***Table (3): Primer code, the total number of alleles, monomorphic alleles, polymorphic alleles and percentages of polymorphism as revealed by SSR analysis.***

| Primers | Total number of allels | Polymorphic allels | Band size range(bp) | % of polymorphism |
|---|---|---|---|---|
| RM10 | 8 | 7 | 50-300bp | 87.5 |
| RM519 | 2 | 2 | 250-290bp | 100 |
| RM561 | 2 | 2 | 390-400bp | 100 |
| RM20 | 4 | 4 | 400-500bp | 100 |
| RM320 | 4 | 4 | 140-230bp | 100 |
| RM544 | 2 | 2 | 250-255bp | 100 |
| RM346 | 3 | 3 | 150-175bp | 100 |
| RM536 | 3 | 3 | 620-650bp | 100 |
| RM47 | 6 | 6 | 140-265bp | 100 |
| **Total** | 34 | 33 | | 98.6 |

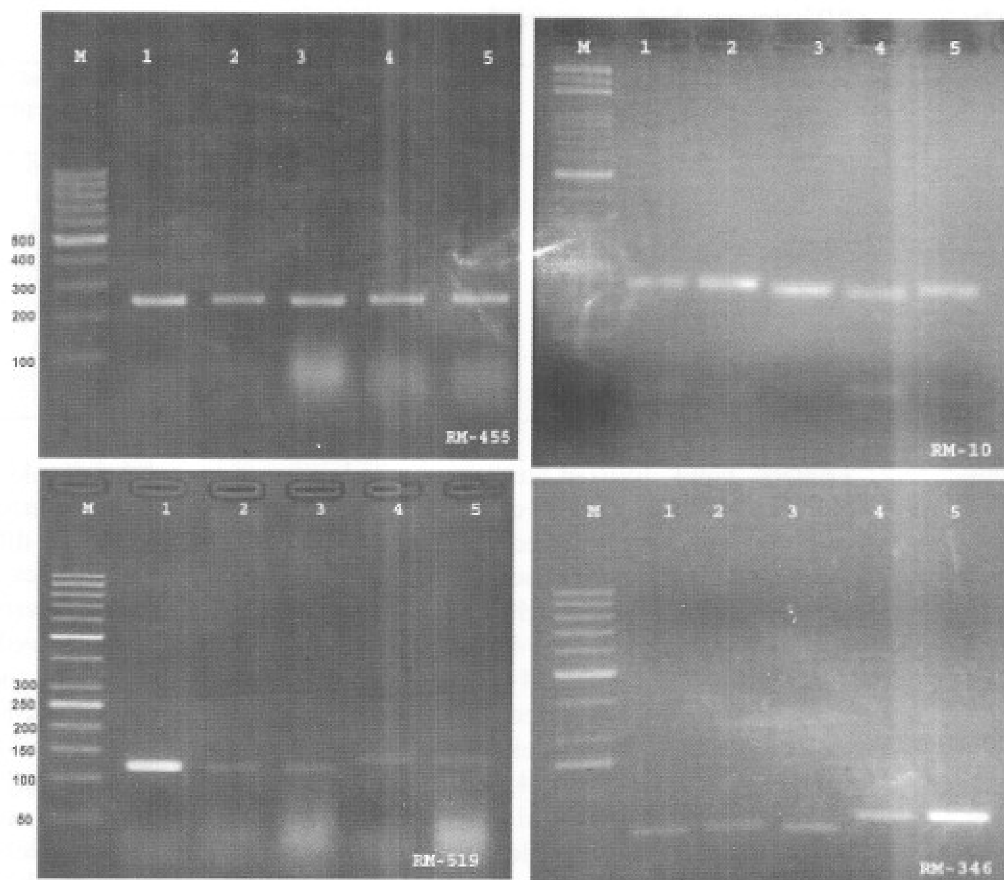


***Fig. (1): SSR profiles of four RM primers characterizing the studied five rice genotypes. M: molecular marker, Lanes (1-5) are the rice genotypes Sakha101, Sakha102, Giza 177, Giza 178, and Giza 182, respectively.***

In the present investigation the genomes of the studied five rice genotypes were further analyzed by AFLP using five selective primer combinations which generated a total of 205 bands. Figure (2) illustrates the AFLP profile of the five rice varieties as revealed by the five primer combinations. The number of markers observed per primer combination ranged from 26 to 69 with an average of 41 markers per primer. The total accounting marker number was 205 amplified bands, representing 77 % polymorphism (Table 4) and an average number of polymorphic bands of 31.6 per AFLP primer combination. The highest percentage of polymorphism was obtained with 1/3(M-CAA/ E-ACA) (84.05%) which produced the highest polymorphic bands (58) and the lowest number was obtained with 7/3(M-CTG/ E- ACA) (32) with the lowest polymorphism which is 55%. In another study, Saker *et al.*( 2005) found that AFLP amplified 50-90 fragments per primer combination in Egyptian rice cultivars and the percentage of polymorphism per primer assay was 45.5%. While Mackill *et al.* (1996) used seven EcoRI and seven MseI primers in 18 combinations in AFLP analysis and generated 147 polymorphic bands among rice varieties (12 Japonica and 2

Indica varieties) with 24% polymorphism. Also, Zhu *et al.* (1998) detected 179 polymorphic bands (44% polymorphism), when using four combinations of AFLP primers which generated a total of 410 AFLP bands.

The genotype specific markers across the different analyses used were determined. The data indicated that, RAPD analysis was able to distinguish between all of the rice genotypes. The cultivar Sakha 101 was characterized by three RAPD markers, while Sakha 102 was characterized by two. Twelve markers distinguished the cultivar Giza 177 from the other cultivars and five markers were found to be specific for the cultivar Giza178 while Giza 182 was characterized by seven markers (Table 5). Similar results were reported by Saker *et al.* (2005) and Choudhury *et al.* (2001), where RAPD analysis was able to differentiate between different rice genotypes.

A total of 6 out of 9 primers used revealed 8 genotype specific SSR alleles characterizing four rice varieties (Table 5), while in another study, a total of 2 primers out of 6 revealed 4 unique SSR alleles (2 positive and 2 negative) for characterizing two rice varieties (Saker *et al.,* 2005) .The primers RM-20 and RM-346 characterized Sakha 101 by two specific SSR markers at 400 and 150 bp respectively. Sakha 102 was characterized by only one RM-536 marker with the molecular size of 630 bp. On the other hand, the primerRM-320 characterized Giza 178 by two SSR markers at the molecular size of 200 and 230bp, while Giza 182 was characterized by three SSR allels.

In the present study, all of the five AFLP primer combinations used were able to characterize the rice cultivars used. The highest number of genotype specific AFLP markers produced by Giza 178 (19 markers), followed by Sakha 102 which was characterized by 15 unique AFLP markers, Giza 182 that was characterized by 3 unique AFLP markers , while Giza 177 was characterized by 2 unique AFLP marker, Sakha 101 producing only four unique AFLP markers (Table 5).

***Table (4): AFLPs primer combination sequence and their codes, total number of generated and polymorphic bands and polymorphism percentages.***

| Primer combination sequence | Combination Code | Total # of bands | Polymorphic bands | Band size range(bp) | Polymorphism % |
|---|---|---|---|---|---|
| M-CAA / E-ACA | 1:3 | 69 | 58 | 52- 1500 | 84.05 |
| M-CAA / E-AAC | 1:1 | 40 | 34 | 85- 500 | 85.00 |
| M-CAA / E-ACG | 1:5 | 32 | 23 | 40- 420 | 71.87 |
| M-CTG / E-ACA | 7:3 | 38 | 30 | 146- 980 | 78.94 |
| M-CTG / E-ACA | 7:5 | 26 | 13 | 65-398 | 50.00 |
| Total | | 205 | 158 | | 77.00 |

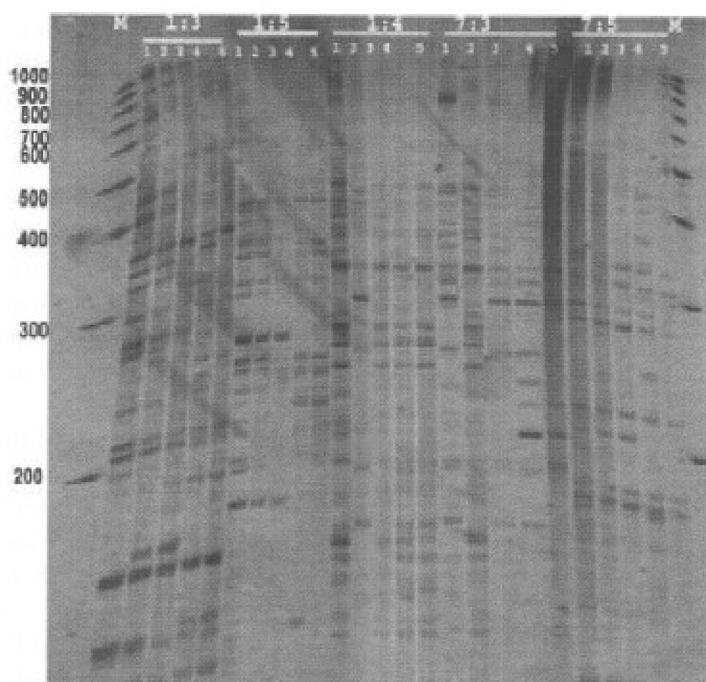


***Fig. (2): AFLP profile of the five rice varieties as revealed by the five primer combinations. Lanes (1-5) are the rice genotypes Sakha101, Sakha102, Giza 177, Giza 178, and Giza 182, respectively.***

The combined data resulted from the similarity matrices generated correct relation-nships based on large and different genome regions where it characterized all the investigated rice varieties by a large number of unique markers. The three DNA analyses were able to differentiate between the five rice varieties. Obviously AFLP technique was able to differentiate rice varieties under study by a higher number of unique markers compared to RAPD and SSR techniques; these could be due to the different marker systems. The AFLP technique is a novel polymerase chain reaction (PCR) - based molecular marker assay (Vos *et al.* 1995) that has the capacity to detect a higher number of loci and thus a higher rate of polymorphism in a single assay than RFLPs or RAPDs (Powell *et al.* 1996). The highest percentage of similarity was detected between Sakha 101 and Sakha 102 (78.8%) followed by 77.2% between Giza 178 and Giza 182 and 71.1% between Sakha 101 and Giza 177. On the other hand the lowest similarity matrix (47.4%) was obtained between Giza 178 and Sakha 102. The dendrogram built on the basis of the combined data from RAPD, SSR and AFLP analyses represents the genetic distances among the five rice varieties (Figure 3). The dendrogram includes two main clusters, the first containing Giza 178 and Giza 182, while the second cluster contains two sub clusters. The first sub cluster contains Giza 177 and the second contains Sakha101 and Sakha 102.

In conclusion the combined RAPD, SSR, and AFLP dendrogram clustered the genotypes Giza 178( Giza 175/ Milyang49) and Giza 182 ( Giza181/IR39422-163-1-3//Giza/81) in one

cluster and Sakha 101 (Giza176/ Milyang79), Sakha102(Gz4096/ Giza 177) and Giza 177(Giza 171/Yomji No.1//Pi No. 4) are clustered in the other cluster ,where Sakha 101 and Sakha 102 are clustered in one sub cluster and Giza 177 in another sub cluster. Collectively, the rice varieties Sakha 101 and Sakha 102 might be originated from a more closely related ancestors than the variety Giza 177 and possess high degree of genetic similarity and they also may share this genetic similarity with the variety Giza 177. Similarly, the rice varieties Giza 178 and Giza 182 may possess a high degree of genetic similarity; also their ancestors might be closely related.

***Table (5): Genotype –specific markers as revealed by RAPD, SSR and AFLP analyses.***

| Genotype | RAPD markers | SSR markers | AFLP markers | Total no. of bands/ genotype |
|---|---|---|---|---|
| **Sakha101** | C-11 (100,400,500) **Total:3** | RM20 (400), RM 346(150) **Total:2** | 1/1 (500, 395, 180). 7/3 (580) **Total:4** | 9 |
| **Sakha 102** | C-07(500), C-05(900) **Total:2** | RM 536(630) **Total:1** | 1/3 (730); 1/1(330, 260,185,182); 7/3(980, 900, 398,392,280,278, 270, 268, 260, 254) **Total:15** | **18** |
| **Giza177** | B-06(300,600,850), B-07 (740,1400), C-11(500), c-07(80,300), c-05 (300,600,1600 ,1800) **Total:12** | | 1/5 (155, 133) **Total:2** | **14** |
| **Giza178** | B-06( 930), B-05 (700), B-07 (760,1200) **Total:4** | RM320 (200,230) **Total:2** | 1/3(950, 930, 460, 378,377, 290, 274, 246, 239) 1/5(154, 152); 7/3(410, 255, 245, 23); 7/5 (275, 188, 150, 148). **Total:19** | **25** |
| **Giza 182** | B-05 (500,770), B-06 (475,530,1000), B-07 (800), C-05 (635) **Total:7** | RM10 (250), RM47(160,265) **Total:3** | 1/3 (850, 465); 7/3 (750) **Total:3** | **13** |
| **Total no. of bands/ marker** | **28 bands** | **8 alleles** | **43 marker** | |

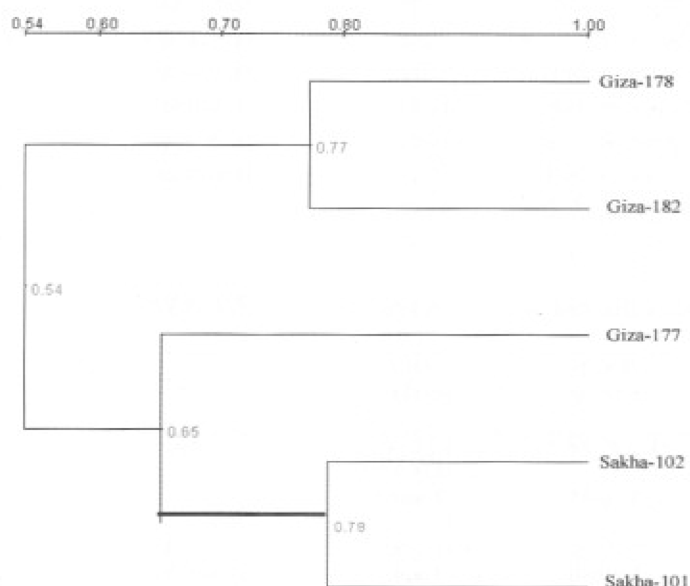


***Fig. (3): Cluster analysis of the five rice cultivars used as revealed by combined RAPD, SSR and AFLP data.***

## REFERENCES


**Bassam, J.B.; Caetano-Anolles, G. and Gresshoff, P.M. (1991).** Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal.Biochem. 196: 80-83.

**Botstein, D.,White, R.; Skolnick, M. and Davis R. (1980).** Construction of a genetic linkage map in man using restriction fragment length polymorphism. Am. J. Genet. (32): 314 – 331.

**Chakravarthi, B. K. and Naravaneni, R. (2006).** SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa*. L). African Journal of Biotechnology Vol. 5 (9): 684-688.

**Choudhury, R.; Kohli, S.; Srinivasan, K.; Mohapatra, T. and Sharma, R. (2001).**Identification and classification of aromatic rice based on DNA finger printing. Euphytica (118): 243-251.

**Higgins, M. (1984).** Synthesis regulation of major protein seeds. Ann. Rev. Plant Physiol. (35): 191-221.

**Levinson, G. and Gutman, G.A. (1987)** .Slipped-strand mis-pairing: a major mechanism of DNA sequence evolution. Molecular Biology vol (4):203-221.

**Mackill, D.J.; Zhang, Z.; Redona, E.D.and Colowit, P.M. (1996).** Level of polymorphism and genetic mapping of AFLP markers in rice.Genome 39: 969-977.

**McCouch, S.R.; Temnykh, S.; Lukashova, A.; Coburn, J.; Declerck, G.; Cartinhour, S.;Harrington, S.; Thomson, M.; Septiningsi, E.; Semon, M.; Moncada, P. and Jiming, L. (2001).** Microsatellite markers in rice: Abundance, diversity and

applications. In: Rice Genetics IV. IRRI. Manila, Philippines. 117-135.

**Michiels, A.; Vanden, E.W.; Tucker, M.; Van, R.L. and Van, L.A. (2003).** Extraction of high quality genomic DNA from latex containing plants. Anal. Biochem. 315: 85-89.

**Ovesna, J.; Polakova, K. and Lisova, L. (2002).** DNA analyses and their applications in plant breeding. Czech. J. Genet. Plant Breed. (38): 29-40.

**Powell w.; Morgante, M.; Andre, C.; Hanafy, M.; Vogel, J.; Tingey, S. and Rafalski, A. (1996).** The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Molecular breeding (2): 225-238.

**Saker, M.M.; Youssef, S.S.; Abdallah, N.A.; Bashandy, H.S. and El Sharkawy, A.M. (2005).** Genetic analysis of some Egyptian rice genotypes using RAPD, SSR and AFLP. African Journal of Biotechnology Vol. 4 (9):882-890.

**Tingey, S.V. and Deltufo, J.P. (1993).** Genetic analysis with Random Amplified Polymorphic DNA. Plant Physiol. 101: 349-352.

**Vos, P.; Hogers, R.; Bleeker, M.; Reijans, M.; Van de Lee, T.; Hornes, M.; Frijters, A.; Pot, J.; Peleman, J.; Kuiper, M. and Zabeau, M. (1995).** AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23: 4407-4414.

**Wang, C.; Wen, G.; Lin, X. and Zhang, D. (2007).** Identification and fine mapping of a new bacterial blight resistance gene, Xa 31 (t) in rice. Plant Genomics in China VIII, Poster presentation on August 18-20.

**Weising, K.; Atkinson, G. and Gardner, C. (1995).** Genomic fingerprinting by microsatellite-primed PCR: a critical evaluation. PCR Methods and Applications 4: 249-255.

**Williams, K.; Kubelik, A.; Livak, K.; Rafalski, J. and Tingey, V. (1990).** DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acid Res. 18: 6531–6535.

**Wu, K. and Tanksley, S. (1993).** Abundance, polymorphism and genetic mapping of microsatellites in rice. Mol. Gen. Genet. (241): 225-235.

**Xiao, J.; Li, J.; Yuan, L.; Mccouch, S. and Tanksley, S.D. (1996).** Genetic diversity and its relationship to hybrid performance and heterosis in rice as revealed by PCR- based markers. Theor. Appl. Genet. (92):637-64.

**Zhu, J.; Gale, M.; Qurrie, S.; Jackson, M. and Bryan, B. (1998).** AFLP markers for the study of rice biodiversity. Theor. Appl. Genet. (96): 601-611.

## الملخص العربي

### درجة التباين الوراثي بين بعض التراكيب الوراثية للأرز المصري طبقا لتحليلات ال RAPD,SSR , AFLP

أسامة أحمد ممتاز*مي محمد هاشم*رضا علواني مغيب**منى هاشم حسين**
*معهد بحوث الهندسة الوراثية الزراعية - مركز البحوث الزراعية - الجيزة-مصر
** قسم الوراثة- كلية الزراعة- جامعة القاهرة- مصر

لدراسة و تحديد الأختلافات الوراثية بين خمسة من الأصناف المنزرعة من الأرز المصرى, تم إجراء تحليلات ال RAPD,SSR, and AFLP . اوضحت النتائج ان كل من الثلاث واسمات الجزيية المستخدمة اظهرت درجات مختلفة من التباين الوراثي . كان عدد الواسمات الكلية المتحصل عليها 59 and 34 ,205 واسم و قد اظهرت فيما بينها 52 ,77,33% تباينا لكل من الإ ختبارات التالية على التوالى AFLP, SSR, RAPD و طبقا للنتائج المتحصل عليها فإنه يمكن إستخدام هذه الواسمات الثلاث فى التمييز بين الخمسة اصناف من الأرز المستخدمة. تم تحديد الواسمات الجزيئية المحددة لكل صنف ووجد ان هناك 25 واسماً مميزا للصنف جيزة 178 بينما سجل الصنف سخا 102 (18واسما) و الصنف جيزة177(14 واسماً) فى حين ان الصنف سخا 101 سجل 9 واسمات وراثية. اتضح من النتائج ان تحليلات ال AFLP اعطت اعلى نسبة من الواسمات المحددة للتراكيب الوراثية(43واسم) يليها الRAPD(28) ثم ال SSR (8). اوضحت شجرة القرابة الوراثية المبنية على النتائج الكلية المتحصل عليها من النتائج المجمعة من ال RAPD , SSR, AFLP ان جيزة 178وجيزة 182 كانا شديدى القرابة وسخا 101,سخا 102, و جيزة 177 ايضا على درجة عالية من القرابة و يمكن القول بأن الصنف سخا 101 و سخا 102 ربما قد نشآ من سلف اكثر قرابة من من جيزة 177 حيث انهما اظهرا درجة عالية من التشابه الوراثى و قد يشاركون الصنف جيزة 177 هذا التشابه.