GENETIC DIVERSITY AMONG SOME RICE GENOTYPES WITH DIFFERENT DROUGHT TOLERANCE

GENETIC DIVERSITY AMONG SOME RICE GENOTYPES WITH DIFFERENT DROUGHT TOLERANCE BASED ON SSR MARKERS

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ABSTRACT. Rice is the most important food crop for the developing world. Hence, identifying rice genotypes to drought tolerance for using as donors in breeding is one of the most important challenges for rice research. Therefore, Molecular markers are useful tools to determine genetic diversity and identifying rice genotypes to drought tolerance. In the present study, A number of 41 rice genotypes with different drought tolerance from different geographic locations were evaluated for genetic diversity by using 15 SSR markers. A total of 68 alleles were detected of which 61(89.79%) were polymorphic. The number of alleles detected by a single marker varied from 2 to 8 alleles with an average of 4.71 alleles per locus. The polymorphic information content (PIC) values ranged from 0.07 (RM219) to 0.80 (RM263) with an average of 0.52. Genetic similarity coefficients of pair wise comparisons were estimated on the basis of the polymorphic microsatelite loci ranged from 0.23 to 0.91 indicating a wide range of genetic variation present among the studied genotypes. It was determined that the primers RM20A, RM302, RM212 and RM286 could be useful for selecting drought tolerant lines through MAS approach. The most significant application of these identified major QTLs for drought tolerance is to collect those favorable alleles into elite local line through marker assisted breeding. The results indicated the ability of SSR markers to identify the allelic diversity and genetic variation among the studied rice genotypes. These results recommended for using this material in future breeding programs to provide important source of genetic diversity for drought tolerance in rice.

Keywords: genetic variability; drought; molecular markers; *Oryza sativa*.

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the agronomically and nutritionally most important cereal crops, providing a staple food for nearly half of the global population (FAO, 2004). In Egypt, rice is one of the major water consuming crops and continuous flooding is the only method for irrigation. Rice occupies about 22% of the total cultivated area in Egypt during the summer season and it consumes about 20% of the total water resources. Due to the limited water resources in Egypt and increasing population, the total water requirements for rice crop caused a problem. Some rice cultivated areas especially that located at the end of the terminal canals in the northern part of the Nile Delta suffer from shortage of irrigation water during different growth stages, which are considered to be one of the most serious constraints to rice production in Egypt (Abd Allah *et al.*, 2010). One possible way to attain 43% increase in rice production without expansion of cultivation area in order to meet the demand of population growth is to breed drought-tolerant rice cultivars by using advanced stress-breeding methodology and molecular techniques (Salekdeh *et al.*, 2002). Genetic diversity is pre-requisite for any crop improvement program as it helps in the development of superior recombinants. It is a source of variation, which is raw material for any improvement work. Accurate assessment of the level and patternof genetic diversity is of great importance for crop breeding. Genetic diversity analysis is used for estimating and establishing of genetic relationship in germplasm collection, identifying diverse parental combinations to create segregating progenies with maximum genetic variability for further selection and introgression of desirable genes from diverse germplasm into the available genetic base (Islam *et al.*, 2012). Genetic diversity analysis guides breeders for rapid progress of breeding program.

Molecular markers are promising and effective tools for measuring genetic diversity in germplasm collection and elucidating their evolutionary relationships. Using molecular marker technology, it is now feasible to analyze the quantitative traits and identify the chromosomal regions associating with such characters known as quantitative trait loci (QTLs). Identifying such regions will help to increase the selection efficiency in the breeding program. Among molecular markers many systems had been used to identify and assess the genetic diversity and phylogenetic relationships in plant genetic resources. Simple sequence repeats (SSR) or microsatellites are simple, tandemly repeated, di- to tetra-nucleotide sequence motifs flanked by unique sequences (Hamada *et al.*, 1982). These markers become useful for genetic diversity analysis because they detect high level of allelic diversity, occur frequently throughout

plant genomes, and easily assayed by PCR. Microsatellite markers have been extensively used for various purposes, i.e. to identify genetic variation among rice species (Ren *et al.*, 2003), to analyze genetic structure within the cultivated rice (Garris *et al.*, 2005), to evaluate genetic diversity among strains of wild rice (Shishido *et al.*, 2006) and among cultivars of cultivated rice (Jayamani *et al.*, 2007).

The objectives of this study are to investigate the genetic diversity among forty one rice genotypes differing in their tolerance to drought using SSR markers for developing unique fingerprint for each genotype, and to identify SSR markers linked with some drought tolerance traits or QTLs, and to identify the best genotype to be used as donors for water stress tolerance in breeding program in the future for development of new rice varieties that are equally beneficial for farmers and the scientific community.

MATERIALS AND METHODS
Plant materials
A number of 41 rice genotypes were used, including Egyptian commercial varieties, promising lines and introduced varieties from different geographic locations, which have been evaluated for drought tolerance (Freeg, 2014). The names, origin and type of these genotypes and their drought response are presented in *Table 1*.

DNA extraction
Genomic DNA was isolated from 21 days old seedlings using the CTAB (Cetyl Try methyl Ammonim Bromide) method described by (Murray and Thompson, 1988). Quantification of DNA was done by analyzing the DNA on 0.8% agarose gel with lambda (λ) *Hind* III DNA as standard. Based on the intensity and thickness of genomic DNA bands, as compared to lambda (λ) *Hind* III DNA, the concentration and quality of DNA in individual samples were determined. The concentration of DNA was adjusted to approximately 15 ng/µl for PCR reaction.

SSR analysis
A number of 15 SSR markers covering all the 12 chromosomes on rice genome were selected for the genetic diversity analysis based on the Gramene Markers Database (http://www. gramene. org/markers). These markers were chosen based on ability to be high polymorphic among rice genotypes in the preliminary screening performed in previous studies and most of them were reported to be related to drought tolerance traits /QTLs. The PCR amplification reactions were done in 10 µl reaction mixtures, containing 1 µl of template DNA, 0.5 µl of each forward and reverse primer, 5 µl of PCR master mix (Ferments) and 3 µl ddH$_2$O. Thermal cycler was used with the following PCR profile: an initial denaturation step at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 30 seconds and primer elongation at 72°C for 1 min and then a final extension at 2°C for 5 min. Amplified products were stored at -20°C until further use.

Data analysis
The amplified bands were scored for each SSR marker based on the presence or absence of bands, generating a binary data matrix of 1 and 0 for each marker system. Polymorphic information content

(PIC) values were calculated for each SSR marker by using the formula described by Anderson *et al.* (1993) in the following formula: PIC $= 1 - \Sigma Pij^2$, where pij is the frequency of jth allele at the locus I and summation extends over n alleles. Matrix was then analyzed using the PAST, ver. 1.90 (Hammer *et al.*, 2001).

The data matrix were used to calculate genetic similarity based on Jaccard's similarity coefficients, and dendrogram displaying relationships among 41 rice genotypes was constructed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) method.

Table 1 - Origin and type of the 41 rice genotypes under study

No.	Genotypes	Origin	Types	DT	No.	Genotypes	Origin	Types	DT
1	Giza177	Egypt	Japonica	s	22	Giza14	Egypt	Japonica	s
2	Giza178	Egypt	Indica/Japonica	MT	23	Giza175	Egypt	Japonica	s
3	Giza182	Egypt	Indica	s	24	GZ5121-5-2	Egypt	Indica	MT
4	Sakha101	Egypt	Japonica	s	25	GZ8450-19-6-5-3	Egypt	Japonica	MT
5	Sakha102	Egypt	Japonica	s	26	GZ8452- 7-6 -5-3	Egypt	Japonica	MT
6	Sakha103	Egypt	Japonica	s	27	Augusto	Italy	Indica	MT
7	Sakha104	Egypt	Japonica	s	28	Eurosis	Italy	Indica	MT
8	Sakha105	Egypt	Japonica	s	29	Sisr215	Italy	Japonica	MT
9	E. yasmine	Egypt	Indica	s	30	Douradao	China	Japonica	T
10	GZ1368	Egypt	Indica	MT	31	Handao11	China	Indica	S
11	Hybrid1	Egypt	Indica	s	32	Handao44	China	Japonica	MT
12	IRAT170	Ivory Coast	Indica	T	33	Handao279	China	Indica	MT
13	Moroberekan	Guinean	Tropical Japonica	T	34	IAPAR9	China	Indica	MT
14	IET1444	India	Indica	T	35	Nong Xan2	China	Indica	S
15	Gaori	Korea & IRRI	Japonica	T	36	Qinai	China	Japonica	MT
16	A22	Srilanka	Indica	T	37	Tp21	China	Japonica	T
17	Vandana	India	Indica	T	38	Zheng Zhou	China	Japonica	S
18	IR600080-46A	IRRI	Indica	T	39	Luxor	Italy	Japonica	S
19	IR78875-176-B-2-B	IRRI	Indica	T	40	L469PB08	Italy	Indica	S
20	IR80508-B-194-1-B	IRRI	Indica	T	41	L696	Italy	Indica	S
21	IR81025-B-347-3	IRRI	Indica	T					

DT= drought tolerance; T= drought tolerant; MT= moderately drought tolerant; s= drought susceptible

RESULTS AND DISCUSSION

Allelic diversity of microsatellite markers

A total of 68 alleles were detected of which 61 (89.7%) were polymorphic (*Table 2*). The number of alleles detected by a single marker ranged from 2 (RM20, RM302 and RM212) to 8 (RM259 and RM260) with an average of 4.71 alleles per locus. The number of alleles per SSR locus detected in this study corresponded well with Sohrarbi *et al.* (2013) and Ram *et al.* (2007). However, Giarrocco *et al.* (2007), who used 26 SSR loci to estimate genetic relationship among 69 Argentine rice accessions reported higher allelic diversity (8.42 alleles per locus; range of 3-21). Jayamani *et al.* (2007) also reported similar values (7.7 alleles per locus; range of 3-16) from a fingerprinting study of 178 Portuguese rice accessions at 24 SSR loci. Zeng *et al.* (2004) reported much lower values of 4.3 alleles per locus (range of 2-9). In contrast, Brondani *et al.* (2006) determined 6-22 alleles per locus (average 14.6) from 192 accessions of Brazilian landrace rice. The reason for the wide variation in the number of alleles detected was due to the different sets of germplasm, number of genotypes, number and distribution of SSR loci and method of gel electrophoretic detection in different studies. The low number of alleles was usually obtained from a collection of breeding lines and closely related cultivars such as those used in Zeng *et al.* (2004).

High number of alleles was expected to be found when a large number of landraces from a wide range of geographical origins is included in the study (Brondani *et al.*, 2006). *Fig. 1* shows a gel image of amplified fragments produced by primer RM 219, which gave four polymorphic bands ranged from 330 to 450 bp.

There was a positive correlation between the number of alleles detected at a locus and the number of repeats within the targeted microsatellite DNA (r=0.224). Ni *et al.* (2002) and Yu *et al.* (2003) found positive correlation between number of alleles amplified and number of repeats within a microsatellite marker. However, no correlations between the number of alleles detected and the number of SSR repeats were found by Behera *et al. (*2012), Nguyen *et al.* (2012) and Sajib *et al.* (2012). Cho *et al.* (2000) and Jain *et al.* (2004) observed that SSR loci with di-nucleotide repeats detected greater number of alleles than those with tri-nucleotide repeats. The overall size of amplified products ranged from 70 bp (RM20A) to 710bp (RM260). The effective number of alleles per locus ranged from 1.08 (RM219) to 5.01 (RM263) with an average of 2.62. Unique allele, an allele that was observed in only one of the 41 rice genotypes, were identified at six loci in total of 15 loci, with the maximum of three unique alleles in RM219 locus. Nguyen *et al.* (2012) observed unique alleles at 16 loci in total of 30 loci with the maximum of three unique alleles as they studied the

genetic diversity among 41 rice accessions using 30 SSR markers. The names of genotypes which had unique alleles are presented in *Table 3.*

Table 2 - **List of the used 15 SSR markers including name, chromosome position, repeat motif, allele size range, number of amplified alleles, polymorphic bands (PM), effective number of alleles (ENA), polymorphic information content (PIC) and unique alleles**

Primers	Ch. position.	Repeat motif	No. of alleles	Bands size (bp)	PM	ENA	PIC	Unique alleles
RM20A	12	(ATT)14	2	70- 95	1	1.33	0.25	-
RM70	7	(ATT)33	6	350-580	6	2.88	0.65	2
RM159	5	(GA)19	5	280-505	5	3.62	0.72	1
RM160	9	(GAA)23	5	245- 530	5	2.35	0.58	2
RM219	9	(CT)17	4	330-450	3	1.08	0.07	3
RM222	10	(CT)18	7	240-555	7	4.94	0.8	-
RM243	1	(CT)18	3	230-635	2	1.72	0.42	-
RM280	4	(GA)16	4	270-625	4	2.41	0.58	1
RM286	11	(GA)16	3	355-520	2	1.88	0.47	-
RM263	2	(CT)34	7	510-680	7	5.01	0.8	1
RM212	1	(CT)24	2	145-355	1	1.27	0.21	-
RM302	1	(GT)30(AT)8	2	97-159	2	1.52	0.34	-
RM259	1	(CT)17	8	170-730	8	3.14	0.68	-
RM260	12	(CT)34	8	280-710	8	3.68	0.73	-
RM152	8	(GGC)10	2	625-640	-	-	-	-

Table 3 - **Unique allele identified in 41 genotypes under study**

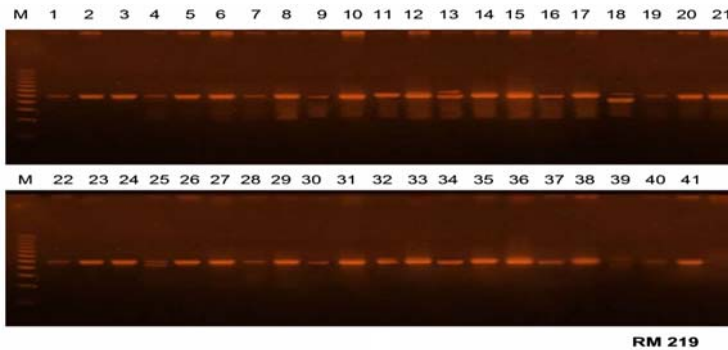| Genotypes	No. of unique allele	SSR marker	Size (bp)
Eurosis	1	RM70	530
Sisr 215	1	RM70	350
Egyptian yasmine	1	RM159	338
Giza 178	1	RM160	450
Giza 175	1	RM160	245
Morobrekan	1	RM219	450
Gz8450-19-6-5-2	1	RM219	380
IR60080-46A	1	RM219	330
IRAT170	1	RM280	625
ZhengZho	1	RM263	680

Figure 1 - Banding pattern for all the 41 genotypes amplified by the RM 219.M:100bp ladder.1:41: the used genotypes.

Polymorphism information content (PIC)

The PIC value provides an estimate of discriminating power of the marker. As shown in *Table 2*, the PIC values for the 14 polymorphic SSR markers used in this study varied from 0.07 (RM219) to 0.80 (RM222 and RM263) with an average of 0.52. The estimated PIC values were found to be relatively high and thus might be due to higher genetic diversity in the selected genotypes. This result is consistent with Sajib *et al.* (2012), who observed tremendous variations in PIC values for all tested SSR loci (from 0.14 to 0.71 with an average of 0.48). Higher averages of PIC values (0.57 and (0.71) were reported by Zeng *et al.* (2004) and Ram *et al.* (2007), respectively. According to Botstein *et al.* (1980), there were eight highly informative markers (PIC>0.50), four informative markers (50<PIC<0.25) and two slightly informative markers (PIC<0.25). Highly significant correlation coefficient was found between PIC values and the number of alleles detected per locus (r=0.81**). The higher the number of alleles detected per locus, the higher the PIC value (Behera *et al.*, 2012). A positive correlation between PIC value and the number of repeats per microsatellite marker was also detected (*r*=0.243). Similar results were obtained by Ni *et al.* (2002).

Similarity and cluster analysis

Genetic similarity coefficients of pair-wise comparisons estimated on the basis of the polymorphic microsatelite loci ranged from 0.225 to 0.913, with an average of 0.499, indicating a wide range of genetic variation among the genotypes. The highest similarity coefficient (0.913) was found between the genotypes L469pB08 and L696, which are both *Indica* type and drought sensitive genotypes. While, the lowest similarity coefficient (0.225) was found between IR78875-176-B-2B (*Indica* type) and Luxor (*Japonica* type). These results were in agreement with those of Chakravarthi and Naravaneni (2006), who reported low

similarity coefficient between *Japonica* type and *Indica* type varieties, and with those of Kanawapee *et al.* (2011), who reported relatively high level of similarity between closely related genotypes. The genetic relationship among rice genotypes are presented in a dendrogram, based on informative microsatellite alleles (*Fig. 2*).

The 41 genotypes were divided into two groups at 0.44 similarity level. The group A represented most of the drought-sensitive genotypes; it divided into three subgroups at 0.56 similarity level: subgroup A1 contained two genotypes (Japonica background), subgroup A2 contained eight genotypes all of them are *Indica* type and the third subgroup A3, contained nine genotypes all of them are Japonica. The group B included 22 genotypes, it divided into two subgroups, as B1 and B2, the subgroup B1 contained one genotype, representing the only aromatic genotype among the 41 studied genotypes. The subgroup B2 further divided into three sub-subgroups at 0.56 similarity level: sub-subgroup B2a contained five genotypes. The sub-subgroup B2b included nine genotypes, representing most of the drought tolerant genotypes and the third sub-subgroup B2c contained seven genotypes. The clustering was largely depending on drought tolerance according to absence or presence of the banding by SSR markers rather than genetic background. The obtained results reflected the existence of considerable

amount of molecular diversity among the tested genotypes and hence demonstrated of the feasibility of genetic improvement of drought tolerance using those genotypes in breeding program. The results also demonstrated the powerfulness of molecular analysis in assessing the genetic diversity. The molecular results were collinear with the field results regarding the existence of genetic diversity. Similar trends were reported as SSR markers have been widely used to characterize rice germplasm and evaluate genetic relationships among cultivars. Chandra *et al.* (2004) studied the extent of genetic diversity among 27 rice accessions from diverse hydrological habitats using 26 SSR markers, a dendrogram was constructed based on the similarity index. Clustering represented the genetic similarity among the accessions as well as their hydrological habitat adaptation. Allelic variations among 169 SSR loci have been used to evaluate genetic diversity among 234 accessions of rice and clearly detected five distinct groups, corresponding to *Indica, aus, aromatic, temperate Japonica* and *tropical Japonica* (Garris *et al.*, 2005). Siwach *et al.* (2004) was able to differentiate 24 rice genotypes into two major groups, corresponding to Basmati and non-Basmati types, based on SSR analysis at 50 loci. Cluster analysis based on 32 SSR loci clearly placed 35 Asian rice cultivars into two major groups, i.e. aromatic and non-aromatic coarse grain rice

(Pervaiz *et al.*, 2009). Analysis based on SSR markers was able to divide Indian elite rice cultivars into clusters according to complex physiological characters namely early duration maturity, medium duration maturity and semi deepwater and deepwater rice (Davierwala *et al.*, 2000). Sohrabi *et al.* (2013) used 15 SSR markers to study the genetic diversity among 50 upland rice accessions, a dendrogram was constructed and accessions were clustered into seven groups, the most of the accessions were clustered according to their geographical origin.

Identified expected (MAS) markers

The primer RM20A showed its ability to amplify two DNA fragments with different sizes (95 and 70bp), the first fragment was found in 13 genotypes only. Lin *et al.* (2007) reported that RM20A (on chromosome 12) was associated with leaf rolling trait as indicator of drought tolerance. The results were in agreement with those obtained from the phenotypic studies where this fragment (95bp) was appeared only in tolerant and moderately tolerant genotypes. The primer RM302 recognized also two DNA regions (97bp and 159bp), the smaller one (97bp) was found in 14 genotypes; according to the phenotypic data this band may be associated with drought tolerance. The primer RM212 amplified two DNA fragments (145bp and 355bp), the 355bp fragment was found in the genome of 10 genotypes

only, from the phenotypic data this band may be associated with drought tolerance and maximum root length in rice, as it appeared only in tolerant genotypes. Similar results were reported by many authors like McCouch *et al.* (2002), who reported that these two primers RM212 and RM302 are located on chromosome 1of rice genome between 135.8 and 143.7 cM. This region has been found to be linked with several drought resistance traits, such as plant height, biomass, deep root mass, leaf drying, relative water content, osmotic adjustment, basal root thickness, tiller number and deep root to shoot ratio, grain yield and panicle length, canopy temperature in IR20/Nootripathu RI lines under drought stress (Boopathi, 2004; Kanagaraj *et al.*, 2010). RM302 was associated with relative water content (RWC) under water stress in CT9993/IR62266 doubled haploid (DH) lines (Babu, 2003). RM212 was linked to root depth, penetrated root thickness, deep root to shoot ratio, deep root dry weight, deep root per tiller in CT9993/IR62266 DH lines (Kamoshita *et al.*, 2002), and root length, root thickness and root weight in Bala/Azucena RI lines of rice (Price *et al.*, 2000). RM286 amplified three DNA fragments (355, 415 and 520 bp), the smallest one was found in only five genotypes, from the phenotypic data this fragment exist only in tolerant genotypes and it may be associated with drought tolerance in rice.
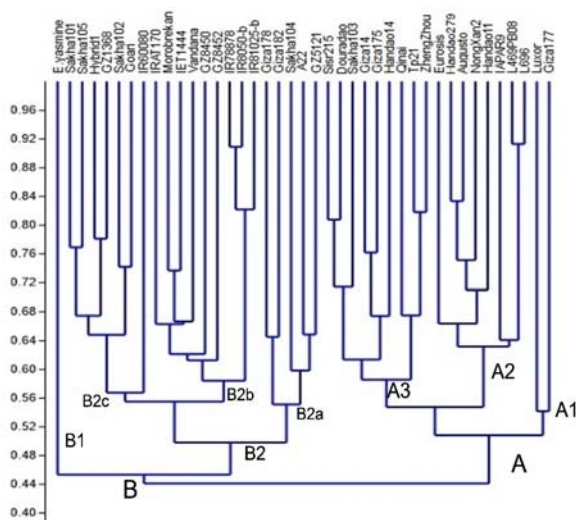
Figure 2 - UPGMA cluster analysis for all the 41 rice genotypes based on Jaccard's similarity coefficient, using SSR markers.

CONCLUSION

From the study, it could be concluded that the markers RM20A, RM302, RM212 and RM286 would be useful for more efficient way for selecting drought tolerant lines through MAS approach. The most significant application of these identified major QTLs for drought tolerance is to collect those favorable alleles into elite local line through marker assisted breeding. In addition, the informative and highly informative markers identified in this study could be utilized in further studies for association mapping and marker assisted selection for drought tolerance in Egyptian rice genotypes.

REFERENCES

Abd Allah A.A., Badawy A.T., Zayed B.A., El-Gohary A.A., 2010 - The role of root system traits in the drought tolerance of rice (*Oryza sativa* L.).* Int. J. Agric. Bio. Sci., 1(2): 83-87.

Anderson J.A., Churchill G.A., Autrique J.E., Tanksley S.D., Sorrells, M.E., 1993 - Optimizing parental selection for genetic linkage maps. Genome, 36(1): 181-186.

Babu R.C. 2003 - Genetic analysis of drought resistance in rice by molecular markers: association between secondary traits and field performance. Crop Sci., 43: 1457-1469.

Behera L., Sahu R., Datta S., Nanda A., Sahu S., Pradhan S., Rao G., Singh O., 2012 - DNA fingerprinting and assessment of genetic diversity of high yielding rice varieties using microsatellite markers. Int. J. Agric. Env. Biotech., 5(2): 89-99.

Boopathi N.M., 2004 - Quantitative trait loci mapping of drought resistance traits in rice (*Oryza sativa* L.) lines adapted to target population of environment. Ph. D. (Biotechnology) Thesis, Tamil Nadu Agricultural University, Coimbatore, India.

Botstein D., White R.L., Skolnick M., Davis R.W., 1980 - Construction of a genetic linkage map in man using

restriction fragment length polymorphism. Am. J. Hum. Genet., 32: 314-331.

Brondani C., Borba T.C.O., Rangel P.H.N., Brondani R.P.V., 2006 - Determination of genetic variability of traditional varieties of Brazilian rice using microsatellite markers. Gen. Mol. Biol., 29(4): 676-684.

Chakravarthi B.K., Naravaneni R., 2006 - SSR marker based DNA fingerprinting and diversity study in rice (*Oryza sativa.* L). Afr. J. Biotechnol., 5(9): 684-688.

Chandra S., Babu R.C., Boopathi N.M., Gomez S.M., Yogameenakshi P., Kumar S.S., Chezian P., Vivek K.T., Shanmugasundaram P., **2004** - Genetic analysis of rice (*Oryza sativa* L.) accessions from diverse hydrological habitats using microsatellite markers. Plant Archives, 4(2): 267-274.

Cho Y.G., Ishii T., Temnykh S., Chen X., Lipovich L., McCouch S.R., 2000 - Diversity of microsatellites derived from genomic libaries and Gene bank sequences in rice (*Oryza sativa* L.).Theor. Appl. Genet., 100: 713-722.

Davierwala A.P., Chowdari K.V., Kumar S., Reddy A.P.K., Ranjekar P.K., Gupta V.S., 2000 - Use of three different marker systems to estimate genetic diversity of Indian elite rice varieties. Genetica, 108(3): 269-284.

FAO (Food and Agriculture Organization), 2004 - The State of Food and Agriculture 2003-2004. Agricultural Biotechnology: Meeting the Needs of the Poor, Rome.

Freeg H.A., 2014 - Phenotypic and genotypic analysis for some Rice (*Oryza sativa* L.) under water stress conditions. M.Sc. thesis. Kaferelshiekh University.

Garris A.J., Tai T.H., Coburn J., Kresovich S., McCouch S., 2005 - Genetic structure and diversity in (*Oryza sativa* L.) Genetics, 169(3): 1631-1638.

Giarrocco L.E., Marassi M.A., Salerno G.L., 2007 - Assessment of the genetic diversity in Argentine rice cultivars with SSR markers. Crop Sci., 47(2): 853-858**.**

Hamada H., Petrino M.G., Kakunaga T., 1982 - A novel repeated element with Z-DNA-forming potential is widely found in evolutionarily diverse eukaryotic genomes. Proceedings of the National Academy of Sciences of the United States of America, 79 (21): 6465-6469.

Hammer Q., Harper D.A.T., Ryan P.D., 2001 - PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica. 4(1): 9. http://palaeo-electronica.org/2001_1 /past/issue1_01.htm.

Islam A.S.F., Ali M.R., Gregorio G.B., Islam M.R., 2012 - Genetic diversity analysis of stress tolerant rice (*Oryza sativa* L.). African J. Biotech., 11(85): 15123-15129.

Jain S., Jain R.K., McCouch S.R., 2004 - Genetic analysis of Indian aromatic and quality rice (*Oryza sativa* L.) germplasm using panels of fluorescently-labeled microsatellite markers. Theor. Appl. Genet., 109: 965-977.

Jayamani P., Negrão S., Martins M., Maçãs B., Oliveira M.M., 2007 - Genetic relatedness of Portuguese rice accessions from diverse origins as assessed by microsatellite markers. Crop Sci., 47(2): 879-884.

Kamoshita A., Zhang J., Siopongco J., Sarkarang S., Nguyen H.T., Wade L.J., 2002 - Effects of phenotyping environment on identification of quantitative trait loci for rice root morphology under aerobic conditions. Crop Sci., 42: 255-265.

Kanagaraj P., Prince K.S.J., Sheeba J.A., Biji K.R., Paul S.B., Senthil A., Babu R.C., 2010 - Microsatellite markers linked to drought resistance in rice (*Oryza sativa* L.). Current Sci., 98(6): 836-839.

Kanawapee N., Sanitchon J., Srihaban P., Theerakulpisut P., 2011 - Genetic diversity analysis of rice cultivars (*Oryza sativa* L.) differing in salinity tolerance based on RAPD

and SSR markers. Elec. J. Biotech., 14(6): 1-17.

Lin M.H., Lin C.W., Chen J.C., Len Y.C., Cheng S.Y., Liu T.H., Jan F.J., Wu S.T., Ku H.M., 2007 - Tagging rice drought-related QTL with SSR DNA markers. Crop Environ. Bioinf., 4: 65-76.

McCouch S.R., Teytelman L., Xu Y., Lobos K.B., Clare K., Walton M., Fu B., Maghirang R., Li Z., Xing Y., Zhang Q., Kono I., Yano M., Fjellstrom R., DeClerck G., Schneider D., Cartinhour S., Ware D., Stien L., 2002 - Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). DNA Res., 9(6): 199-207.

Nguyen T.T.T., Nguyen N.M.T., Hoang L.H., Furuya N., Tsuchiya K., 2012 - Genetic diversity in Vietnamese upland rice germplasm revealed by SSR markers. J. Fac. Agr., Kyushu Univ., 57 (2): 383-391.

Ni J., Colowit P.M., Mackill D.J., 2002 - Evaluation of genetic diversity in rice subspecies using microsatellite markers. Crop Sci., 42: 601-607.

Pervaiz Z.H., Rabbani M.A., Pearce S.R., Malik S.A., 2009 - Determination of genetic variability of Asian rice (*Oryza sativa* L.) varieties using microsatellite markers. Afric. J. Biotech. 8(21): 5641-5651.

Price A.H., Steele K.A., Moore B.J.,. Barraclough P.B., Clark L.J., 2000 - A combined RFLP and AFLP map of upland rice (*Oryza sativa* L.) used to identify QTLs for root-penetration ability. Theor. Appl. Genet., 100: 49-56.

Ram S.G., Thiruvengadam V., Vinod K.K., 2007 - Genetic diversity among cultivars, landraces and wild relatives of rice as revealed by microsatellite markers. J. Appl. Genet., 48(4): 337-345.

Ren F., Lu B.R., Li S., Huang J., Zhu Y., 2003 - A comparative study of genetic relationships among the AA-genome *Oryza* species using RAPD

and SSR markers. Theor. Appl. Genet., 108(1): 113-120.

Sajib A.M., Hossain M.M., Mosnaz A.T.M.J., Hossain H., Islam M.M., Ali M.S., Prodhan S.H., 2012 - SSR marker-based molecular characterization and genetic diversity analysis of aromatic landraces of rice (*Oryza sativa* L.). J. Bio Sci. Biotech., 1(2): 107-116.

Salekdeh G.H., Siopongco J., Wade L.J., Ghareyazie B., Bennett J., 2002 - Proteomic analysis of rice leaves during drought stress and recovery. Proteomics, 2(9): 1131-1145.

Shishido R., Kikuchi M., Nomura K., Ikehashi H., 2006 - Evaluation of genetic diversity of wild rice (*Oryza rufipogon* Griff.) in Myanmar using simple sequence repeats (SSRs). Gen. Res. Crop Evol., 53(1): 179-186.

Siwach P., Jain S., Saini N., Vijay K., Chowdhury V.K., Jain R.K., 2004 - Allelic diversity among basmati and non-basmati long-grain *Indica* rice varieties using microsatellite markers. J. Plant Biochem. Biotechnol., 13(1): 25-32.

Sohrabi M., Rafii M.Y., Hanafi M.M., Latif M.A., 2013 - Genetic divergence of Malaysian upland rice revealed by microsatellite markers. POJ, 6(3): 175-182.

Yu B., Xu W.J., Vijaykumar C.H., Ali J., Fu B.Y., Xu J.L., Jian Y.Z., Maghirang R., Domingo J., Acquino C., Virmani S.S., Li Z.K., 2003 - Molecular diversity and multilocus organization of the parental lines used in the international rice molecular breeding program. Theor. Appl. Genet., 108: 131-140.

Zeng L., Kwon T.R., Liu X., Wilson C., Grieve C.M., Gregorio G.B., 2004 - Genetic diversity analyzed by microsatellite markers among rice (*Oryza sativa* L.) genotypes with different adaptations to saline soils. Plant Sci., 166(5): 1275-1285.