Molecular Markers for New Promising Drought Tolerant Lines of Rice under Drought Stress *via* RAPD-PCR and ISSR Markers

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ABSTRACT

Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR) and inter simple sequence repeats (ISSRs) markers were performed to detect the genetic diversity among 6 new rice lines and 4 cultivars with different responses to drought tolerance and establish specific DNA markers associated with drought tolerance. Among 16 RAPD primers tested, only 5 produced bands polymorphic between lines with an average of 5.2 bands per primer (ranging from approximately 252 to 1232 bp) and 73.02 % were polymorphic. Among the tested ISSR primers, only five amplified polymorphic ISSR loci with an average number of 4.4 bands per primer (ranging from approximately 80 to 813 bp) and the mean percentage of ISSR polymorphism was 90.91. Based on band polymorphisms generated by RAPD-PCR and ISSR after using the primers, the highest similarity value (0.93) was found between P-5-3-b line and P-5-3-a line and the lowest value (0.44) was found between P-5-3-b line and Giza 172. The dendrogram separated all cultivars and new lines into two clusters and indicated that the cross of tolerant line (P-5-3-b) and susceptible cultivar (Giza 172) is suggested as the most suitable cross for drought tolerance analysis studies as they have the lowest similarity value (0.44) and also grouped in distinct cluster. Since two fragments of about (315 and 505 bp) were visualized using HP15 primer in the genomic DNA of the drought tolerant lines while were absent in the sensitive cultivars, they can be considered as positive drought tolerant markers. [Journal of American Science 2010; 6(12): 355-363]. (ISSN: 1545-1003).

Key words: RAPD-PCR, ISSRs, rice, drought stress, dendogram.

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1. INTRODUCTION:

Rice (*Oryza sativa*), one of the important food crops, is grown on 154 million hectares world- wide in a wide range of environments and about 45% of the world's rice is cultivated in rainfed ecosystems [Nazari and Pakniyat, 2008]. These areas often experience severe water deficits due to low and uneven rainfall distribution patterns and yields are largely reduced by drought. Drought stress is a serious limiting factor to rice production and yield stability in rainfed areas and 18 million tons of rice valued at US$ 3600 millions is lost annually to drought [Ribaut and Poland, 1999]. Development of drought resistant cultivars will considerably improve rainfed rice production. However, little progress has been made in improving the genetic potential of rice for drought resistance because lack of phenotyping facilities to precisely screen large germplasm for drought resistance, inherent variation in the field and only one experimentally droughted crop per year [Ribaut *et al.*,1997].

In Egypt, the cultivated varieties require large amount of water irrigation (16500 m3/ha).The available amount of irrigation water from River Nile is not only limited (55.5 million m3/year) but liable to decrease year after year due to competition between the agriculture, industry and human consumption in the fixed amount of water from River Nile, in addition to the competition between groove countries of River Nile. As well as about 15.20% from rice areas was suffering a decreasing of yield due to short of water [Mahasson *et. al*, 1999]. Accordingly, the future of rice cultivation in Egypt depends upon breeding for drought resistance because the cultivated varieties (lowland) require large amount of water and susceptibility of water deficits. The first trial for breeding drought tolerance in Egypt initiated at 1986 by ours obtaining on rice breeding for drought stress project (NARP No. 329). Investigations at this project included on genetical and physiological studies on the drought tolerance related characters as well as grain yield and its components [Soliman 1993a, Soliman 1993b].

The effect of drought stress on disease infections and quality characters susceptible and drought tolerance genotypes were studied also [Abou- zaid *et. al*, 1993, Wafaa *et. al*, 1998]. In the same time the breeding was done and we obtained on new promising drought tolerant lines of rice. These lines were evaluated for grain yield and its attributes as well as quality characters (Tables 1 and 2).cultivation of new drought tolerant lines in the large scale at sandy soil (light) by

using microjectsprinkler irrigation as well as cultivation of its lines under heavy soil on large scale [Ghazi and Soliman, 2008]. The findings of these experiments confirmed that the importance of these lines for solving of rice cultivation in Egypt and gave higher grain yield under drought stress than under normal irrigation (traditional method).

Molecular tools facilitate the identification of genomic locations linked to traits of interest and help in indirect selection of such complex traits without the need for difficult phenotypic measurements. In the last few decades, new DNA molecular markers, based on the PCR technique, such as random amplified polymorphic DNA [RAPD; Williams *et al.* 1990] and inter simple sequence repeats [ISSRs; Zietkiewicz *et al.* 1994], among others, have become excellent tools for plant breeders [Lima-Brito *et al.* 2006]. When there is insufficient information about the genome sequence of a wild species, or there are economic constraints, one of the most adequate marker systems is RAPD amplification [Lima-Brito *et al.* 2006]. This technique gives fast results but also has limitations, such as dependence on the genetic background, low reproducibility, and level of polymorphism obtained [Zietkiewicz *et al.* 1994; Godwin *et al.* 1997 and Fern´andez *et al.* 2002]. In contrast to RAPD amplification, the ISSR markers are more feasible and reproducible [Godwin *et al.* 1997], and the distribution of ISSRs in the eukaryotic genome makes them highly informative [Tautz and Renz 1984].

They are also highly polymorphic and their use is cost effective, requiring no prior information of the sequence [Bornet *et al.* 2002]. In cereals, ISSR markers have been used to study genetic diversity and phylogenetic relationships [Kantety *et al.* 1995; Matos *et al.* 2001 and Fern´andez *et al.* 2002], for gene mapping [Kojima *et al.* 1998], for gene tagging in molecular assisted selection [Akagi *et al.* 1996 and Kaushik *et al.* 2003], and for DNA fingerprinting [Carvalho *et al.* 2005].

The objectives of this study were to use RAPD-PCR and ISSR markers to assess genetic diversity and identification of 6 new rice lines and 4 cultivars with different responses to drought tolerance by comparison of local cultivation and establish specific DNA markers associated with drought tolerance using RAPD-PCR and ISSR markers to assessed of breeders to selection drought tolerant genotypes on the molecular level at the laboratory only subsequently acceleration and facilitates of drought breeding programs.

2. MATERIALS AND METHODS:

This work was carried out in Molecular Genetics Lab. Genetic Dept., Fac. of Agric., Zagazig Univ.

Plant materials:

Six new drought tolerant lines and four sensitive cultivars were used in this study under drought stress (Table 3).

RAPD and ISSR amplification:

Total genomic DNA was extracted from young leaves by the CTAB (cethyltrimethylammonium bromide) method followed by an RNase-A treatment (Sigma, St. Louis, MO; R-4875) for 30 min at 37°C.

Primer:

A set of sixteen 10-mer oligonucleotides was analyzed for RAPD-PCR and a total of twenty primers were tested for ISSR. Based on the accurate amplified bands profiles and the produced polymorphic patterns of DNA fingerprinting selected five different primers were chosen for RAPD-PCR and another five primers for ISSR (Table 4).

RAPD- PCR reactions:

The RAPD amplification reactions were carried out in 50 µl containing 20 ng/µl of template DNA, 10× buffer (NH4)2SO4; Fermentas, St. Leon- Rot, Germany), 2.5mM $MgCl_2$ (Fermentas), 2.5mM dNTPs, 0.25 µM primer and 1 Unit *Taq* DNA polymerase (Fermentas). The RAPD amplifications occurred under the following conditions: an initial denaturation step at 94◦C for 7 min and 30 cycles at 94◦C for 1 min, 35◦C for 1 min and 72◦C for 2 min; the final elongation step was at 72◦C for 6 min.

ISSR- PCR reactions:

The ISSR amplification reactions were carried out in 25µl per tube, containing 2µl DNA (20 ng), 1 unit of *Taq* DNA polymerase enzyme, 2µl 10X buffer, 2 µl $MgCl_2$ (25 mM), 2µl $dNTP_S$ (2.5 mM of each), 2 µl primer (10 pmol) and 14.8µl H_2O. The following conditions were used for ISSR amplifications: an initial denaturation step of 94◦C for 5 min, followed by 45 cycles of denaturation at 94◦C for 30 s, a primer annealing step at 52◦C for 45 s, and an extension at 72◦C for 2 min; then a final extension was carried out at 72◦C for 5 min. The annealing temperature varied according to the melting temperature of each primer.

Both RAPD and ISSR amplification reactions were carried out on a Perkin-Elmer Gene Amp PCR system (model 2400), and each reaction was repeated twice.

Band analysis:

The reaction products were analyzed by electrophoresis on 1.4% agarose gels, stained with ethidium bromide, and photographed under UV transilluminator by digital camera with UV filter adaptor.

The synthetic DNA, ladder 100 bp (Pharmacia) was employed as molecular markers for bands molecular weight. Each amplified band profile was defined by the presence or absence of bands at particular positions on the gel. Profiles were considered different when at least one polymorphic band was identified. Fragments were scored as 1 if present or 0 if absent based on standard marker using GelAnalyzer 3 (Egygene) software. Pairwise combinations, genetic similarity and genetic distances were estimated following Lynch (1990 and 1991). The computer package SPSS was used to construct a dendrogram based on the matrix of distance using Unweighted Pair Group Method with Arithmetic averages (UPGMA) (Sneath and Sokal 1973).

3. RESULTS:

RAPD and ISSR analysis:

The total number of amplified fragments, number of monomorphic fragments, number of polymorphic fragment and percentage of polymorphism obtained per each RAPD and ISSR primer are shown in table (5). Among the 16 RAPD primers tested, only 5 produced bands polymorphic between lines. An average of 5.2 bands per primer was amplified (ranging from approximately 252 to 1232 bp) and 73.02% were polymorphic. The oligonucleotide OPA-05 and OPA-11 presented the highest percentage of RAPD polymorphism (100 %; table 5). OPB-10 oligonucleotides, presented one unique band (665 bp) to Giza177 cultivar and OPD-07 oligonucleotides, presented one unique band (589 bp) to Giza 159 cultivar while, OPA-11 oligonucleotides, presented two unique bands (252 and 292 bp) to P-2-1-2-1 line and P-58-1-2 line respectively (Table 6).

Among the tested ISSR primers, only five amplified polymorphic ISSR loci. An average number of 4.4 bands per primer were amplified (ranging from approximately 80 to 813 bp) and the mean percentage of ISSR polymorphism was 90.91 (table 5). The oligonucleotide HP12 amplified the highest number of ISSR loci (6 bands) but primers HP9, HP12 and HP14 gave the highest percentage of polymorphism (100 %; table 5). HP12 oligonucleotides, presented two unique bands (519 and 773 bp) to P-5-3-b line while, HP14 and HP15 oligonucleotides, presented one unique band (783 and 453 bp) respectively to P-72-

11-1-1 line (Table 7).

The patterns obtained with HP15 oligonucleotide for lines suggested that this primer has the ability to produce drought tolerant markers. Since two fragments of about (315 and 505 bp) were visualized using HP15 oligonucleotide in the genomic DNA of the drought tolerant lines while were absent in the sensitive lines, they can be considered as positive drought tolerant markers (Table 7 and Figure 1).

Phylogenetic relationship among new drought tolerant lines and local cultivars (Susceptible) based on amplified RAPD-PCR and ISSR fragments (bands): The similarity coefficient values among all cultivars and new lines based on band polymorphisms generated by RAPD-PCR and ISSR after using the primers are presented in Table (8). The highest similarity value (0.93) was found between P-5-3-b line and P-5-3-a line and the lowest value (0.44) was found between P-5-3-b line and Giza 172.

Table (1): The average mean of quality characters and days to heading for new promising drought tolerant lines and local varieties of rice under drought stress.

	Day heading	Protein content	Milling characters			
			Brown rice	Brain and germ	Hask	White rice
P-58-1-2	96.0	9.81	68.0	9.9	32.0	58.1
P-5-3-b	83.0	10.43	66.5	10.3	33.5	56.3
P-5-3-a	78.0	10.22	65.3	10.1	34.7	55.2
58-1-2-1	95.0	9.60	67.5	10.2	32.5	57.3
P-2-1-2-1	82.0	8.34	79.10	8.0	21.0	71.0
Sakha 104	93.0	6.50	64.5	9.0	35.5	55.5
G-177	85.0	6.20	64.0	8.7	36.0	55.3
G-172	102.0	6.60	67.0	8.6	33.0	58.4
G-159	105	6.10	65.0	8.8	35.0	56.2
P-72-11-1-1	38.0	7.93	64.4	8.2	35.6	56.2
L.S.D 0.05	6.5	1.5	4.5	1.5	3.5	5.5

The dendrogram of genetic distances among all tested genotypes based on band polymorphisms generated by RAPD-PCR and ISSR after using the primers is shown in (Fig. 2). The dendrogram separated all cultivars and new lines into two clusters. First cluster divided into two subclusters, first subcluster included P-58-1-2, P-5-3-b, P-5-3-a and second subcluster formed a separate subcluster with P-58-1-2-1. Second cluster was further divided into two subclusters.

Among the two subclusters, first subcluster formed a separate subcluster

with P-2-1-2-1, Sakha 104 and Giza177and second subcluster included Giza172, Giza159 and P-72-11-1-1

Table (2): The average mean of grain yield per plant and its attributes for new promising drought tolerant lines and local varieties of rice under drought stress.

	Culm length	Panacle length	No. of tillers/plant	100 Grain weight	Grain yield/plant
P-58-1-2	124.0	27.0	11.0	4.2	33.5
P-5-3-b	90.2	24.0	22.0	1.95	40.4
P-5-3-a	78.0	23.3	13.0	2.1	25.8
58-1-2-1	120.0	27.2	10.5	3.95	32.6
P-2-1-2-1	80.5	24.8	20.5	2.15	42.2
Sakha 104	110.5	22.0	8.5	1.92	26.2
G-177	115.5	22.5	7.6	1.98	24.5
G-172	120.0	21.0	6.8	1.85	20.6
G-159	120.0	20.0	7.0	1.78	19.5
P-72-11-1-1	79.2	24.4	19.4	1.8	40.6
L.S.D 0.05	16.366	3.793	7.714	0.533	11.57

Table (3): The Six new drought tolerant lines and the four sensitive cultivars which used in this study and its pedigree.

1	Cultivar Code	Cultivar Name	Code of tolerance	Pedigree
1	P-58-1-2	New line	Drought Tolerant	Selected line from (Gisa 159 x IET 1444)
2	P-5-3-b	New line	Drought Tolerant	Selected line from IR 4786-13-2-1 after treated by EMS 0.5%
3	P-5-3-a	New line	Drought Tolerant	Selected line from IR 4786-13-2-1 after treated by EMS 0.5%
4	P-58-1-2-1	New line	Drought Tolerant	Selected line from (Gisa 159 x IET 1444)
5	P-2-1-2-1	New line	Drought Tolerant	Selected line from (arbida x bluebell)
6	Sakha 104	Cultivar	Sensitive	Local modern Egyptian cultivar, salt tolerance
7	Giza177	Cultivar	Sensitive	Local modern Egyptian cultivar, salt tolerance
8	Giza 172	Cultivar	Sensitive	Local Egyptian variety
9	Giza 159	Cultivar	Sensitive	Local Egyptian variety
10	P-72-11-1-1	New line	Drought Tolerant	Selected line from Moroerkan after treated by 25 Rad

Table (4): Sequence and operon codes of the RAPD and ISSR primers used to detection of variation in different new drought tolerant lines and local varieties

Primer codes	Sequence (5- to 3-)
RAPD	
OPA-05	AGG GGT CTT G
OPA-11	CAA TCG CCG T
OPB-10	CTG CTG GGA C
OPC-02	GTG AGG CGT C
OPD-07	TTG GCA CGG G
ISSR	
HB9	(GT)6GG
HB12	(CAC)3GC
HB13	(GAG)3GC
HB14	(CTC)3GC
HB15	(GTG)3GC

Table (5): Number of monomorphic fragments, polymorphic fragments and percentage of polymorphism obtained per each RAPD and ISSRprimer for all cultivars and new lines

Primers	Range of fragment sizes (bp)	Total No. of fragments	Monomorphic fragments	Polymorphic fragments	Polymorphism %
RAPD					
OPA-05	276-788	5	0	5	100
OPA-11	252-339	5	0	5	100
OPB-10	524-1232	4	1	3	75
OPC-02	414-896	8	3	5	62.5
OPD-07	474-1004	4	3	1	25
Total	252-1232	26	7	19	73.02
Average		5.2	1.4	3.8	
ISSR					
HP9	79-181	3	0	3	100
HP12	343-813	6	0	6	100
HP13	506-748	3	1	2	66.67
HP14	287-783	5	0	5	100
HP15	251-505	5	1	4	80
Total	79-813	22	2	20	90.91
Average		4.4	0.4	4	

Table (6): RAPD Primers, molecular weight (bp), monomorphic, polymorphic and unique bands for 6 new promising drought tolerant lines and 4 local cultivars of rice.

Primer	M.W (pb)	P-58-1-2	P-5-3-b	P-5-3-a	P-58-1-2-1	P-2-1-2-1	Sakha 104	Giza 177	Giza 172	Giza 159	P-72-11-1-1	Polymorphism
OPA-05	788	0	0	0	0	1	1	1	1	1	1	Polymorphic
	676	1	1	1	0	0	0	0	0	0	0	Polymorphic
	633	0	0	0	0	0	0	0	1	1	1	Polymorphic
	309	0	0	0	0	1	1	1	1	1	1	Polymorphic
	276	1	1	1	0	0	0	0	0	0	0	Polymorphic
OPA-11	339	0	0	0	0	1	1	1	1	0	0	Polymorphic
	317	0	0	0	0	0	0	0	0	1	1	Polymorphic
	313	0	1	1	1	0	0	0	0	0	0	Polymorphic
	292	1	0	0	0	0	0	0	0	0	0	Unique
	252	0	0	0	0	1	0	0	0	0	0	Unique
OPB-10	1232	0	0	0	1	0	0	0	0	1	0	Polymorphic
	881	0	1	1	1	1	0	1	0	1	1	Polymorphic
	665	0	0	0	0	0	0	1	0	0	0	Unique
	524	1	1	1	1	1	1	1	1	1	1	Monomorphic
OPC-02	896	1	1	1	1	1	1	1	1	1	1	Monomorphic
	786	0	0	0	0	0	0	0	1	1	1	Polymorphic
	744	1	1	1	1	1	1	1	0	0	0	Polymorphic
	706	0	0	0	0	0	0	0	1	1	0	Polymorphic
	682	0	1	0	1	0	0	0	0	0	0	Polymorphic
	629	0	1	0	1	0	0	0	1	1	0	Polymorphic
	560	1	1	1	1	1	1	1	1	1	1	Monomorphic
	414	1	1	1	1	1	1	1	1	1	1	Monomorphic
OPD-07	1004	1	1	1	1	1	1	1	1	1	1	Monomorphic
	741	1	1	1	1	1	1	1	1	1	1	Monomorphic
	589	0	0	0	0	0	0	0	0	1	0	Unique
	474	1	1	1	1	1	1	1	1	1	1	Monomorphic
Total		11	14	12	13	13	11	13	14	17	13	

Table (7): ISSR Primers, molecular weight (bp), monomorphic, polymorphic and unique bands for 6 new promising drought tolerant lines and 4 local cultivars of rice.

Primer	M.W (pb)	P-58-1-2	P-5-3-b	P-5-3-a	P-58-1-2-1	P-2-1-2-1	Sakha 104	Giza177	Giza 172	Giza 159	P-72-11-1-1	Polymorphism
HP9	181	0	1	1	0	0	0	0	0	0	0	Polymorphic
	160	0	0	0	0	0	0	1	1	0	0	Polymorphic
	79	1	0	0	0	0	0	0	1	0	0	Polymorphic
HP12	813	0	0	0	1	1	1	1	1	1	1	Polymorphic
	773	0	1	0	0	0	0	0	0	0	0	Unique
	621	0	0	0	0	0	1	1	1	0	0	Polymorphic
	519	0	1	0	0	0	0	0	0	0	0	Unique
	512	0	0	0	1	1	1	1	1	1	1	Polymorphic
	343	0	0	0	1	1	1	1	1	1	1	Polymorphic
HP13	748	1	1	1	1	1	1	1	1	1	1	Monomorphic
	566	0	0	0	1	1	1	0	1	1	1	Polymorphic
	506	0	0	0	1	1	1	0	1	1	1	Polymorphic

%

Table (7):

Primer	M.W (pb)	P-58-1-2	P-5-3-b	P-5-3-a	P-58-1-2-1	P-2-1-2-1	Sakha 104	Giza177	Giza 172	Giza 159	P-72-11-1-1	Polymorphism
HP14	783	0	0	0	0	0	0	0	0	0	1	Unique
	665	0	0	0	0	1	0	0	0	0	1	Polymorphic
	432	1	1	1	1	1	0	0	1	1	1	Polymorphic
	355	0	1	1	0	0	0	0	0	0	0	Polymorphic
	287	0	1	1	1	1	0	0	1	1	1	Polymorphic
HP15	505	1	1	1	1	0	0	0	0	0	1	+ mol. Marker
	454	0	0	0	0	0	0	0	0	0	1	Unique
	401	1	1	1	1	0	1	0	1	1	0	Polymorphic
	313	1	1	1	1	0	0	0	0	0	1	+ mol. marker
	251	1	1	1	1	1	1	1	1	1	1	Monomorphic
Total		7	11	9	12	10	9	7	13	10	14	

Table (8): The similarity coefficient values among all cultivars and new lines based on band polymorphisms generated by RAPD-PCR and ISSR after using the primers

Cultivar	P-5-3-b	P-5-3-a	P-58-1-2-1	P-2-1-2-1	Sakha 104	Giza177	Giza 172	Giza 159	P-72-11-1-1
P-58-1-2	0.79	0.86	0.67	0.58	0.65	0.58	0.54	0.47	0.53
P-5-3-b		**0.93**	0.74	0.55	0.51	0.51	**0.44**	0.47	0.49
P-5-3-a			0.74	0.61	0.58	0.58	0.47	0.51	0.56
P-58-1-2-1				0.74	0.71	0.63	0.63	0.74	0.69
P-2-1-2-1					0.86	0.82	0.75	0.75	0.81
Sakha 104						0.86	0.82	0.72	0.70
Giza177							0.72	0.65	0.67
Giza 172								0.82	0.74
Giza 159									0.81

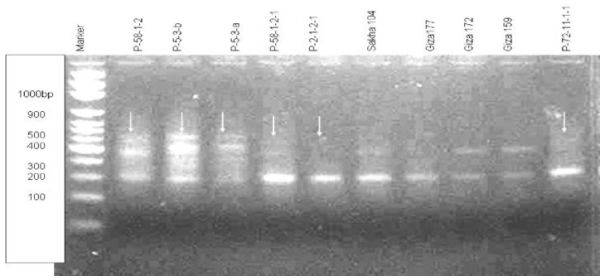

Fig. (1): Results of ISSR amplification based on the use of HP15 primer in the six new drought tolerant lines and the four sensitive cultivars

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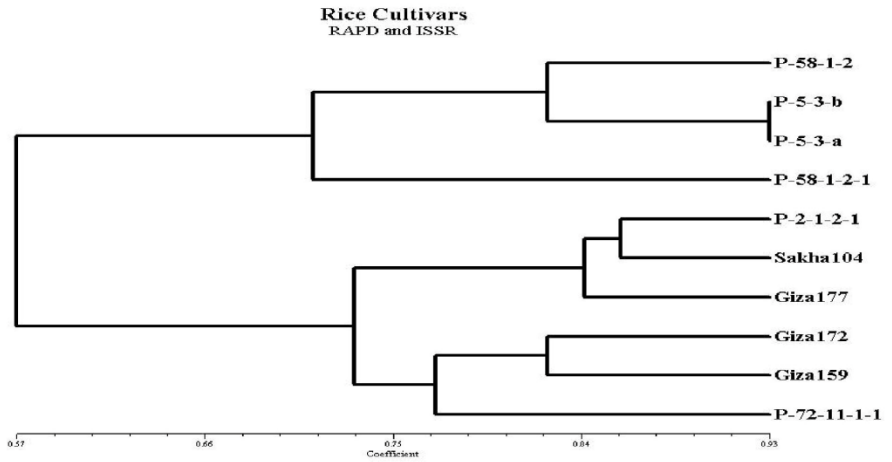

Fig. (2): The dendrogram of genetic distances among all tested genotypes based on band polymorphisms generated by RAPD-PCR and ISSR after using the primers

4. DISCUSSION:

Molecular characterization revealed 73.02% polymorphism of RAPD markers and 90.91% polymorphism of ISSR markers between lines (table5). The difference is perhaps explained by the difference in the DNA segments targeted by the two methods, and is consistent with some previous studies which reported that ISSR markers are more polymorphic than RAPD markers (Zietkiewicz et al. 1994; Godwin *et al*. 1997 and Nagaoka and Ogihara 1997). Fern´andez et al. (2002) used RAPD and ISSR markers for DNA fingerprinting, because they provide a quick, reliable and highly informative system and can also be used to establish genetic relationships. Although in our work the ISSR markers showed higher percentage of polymorphism than RAPD markers, we believe that both could be useful for DNA and genomic fingerprinting.

Regarding to fingerprinting of new drought tolerant lines with comparison of local cultivars (drought susceptible), four unique bands were detected between genotypes at RAPD-PCR. The first unique band posses 292 bp length with OPA-11 primer and it was found in P-58-1-2 line (drought tolerant), the second unique band (252 bp) with OPA-11 distinguish P-2-1-2-1 line (drought tolerant). The third and fourth unique bands, i.e., 665 bp with OPB- 10 and 589 bp at OPD-O7, they identified of Gisa 174 and Giza 159, respectively. While, ISSR results gave four unique bands also but for drought tolerant lines. The first and second unique bands identified P-5-3-b line; these bands were

773 bp and 519 bp with HP-12 primer. As well as, P-72-11-1-1 line posse two unique bands (783 bp with HP-13 primer and 454 bp with HP-15 primer). These results determine the fingerprinting of very important drought tolerant lines because they interred into confirmed experiments in season 2010 under seed production and possess height yield under drought stress.

There was close relationship between some of the cultivars and new lines used in this study, presumably they might have been collected from similar locations or these cultivars and new lines may have been derived from the same pedigree. The high similarity between P-5-3-b line and P-5-3-a line indicating, that these lines are closely related because they were developed from the same genotype (IR4786-13-2-1). SIf there is possibility of several crosses, two patents should be crossed in order they have the QTLs involved in drought tolerance. On this basis, the cross of tolerant line (P-5-3-b) and susceptible cultivar (Giza 172) is suggested as the most suitable cross for drought tolerance analysis studies as they have the lowest similarity value (0.44) and also grouped in distinct cluster.

Nagaoka and Ogihara (1997) have reported that the ISSR primers produced several times more information than RAPD markers in wheat. Fern´andez *et al.* (2002) have studied 16 barley cultivars form different countries and they have found high similarity index by ISSRs than by RAPDs. It may be due to highly polymorphic, abundant nature of the microsattelites due to slippage in DNA eplication. Galvan *et al.* (2003) concluded that ISSR would be a better tool than RAPD for phylogenetic studies.

Through RAPD and ISSR techniques, which are relatively cheap and require small quantities of DNA, it was possible to identify one primer (HP15) from ISSR that generated polymorphic bands in tolerant and non-tolerant lines (315 and 505 bp).These bands can be considered as potential markers to identify drought tolerant lines or may even be more useful when converted into a simple-sequence PCR- based marker that can be used for large-scale drought tolerance screening of cultivars. On the other hand, RAPD-PCR did not detect positive drought marker. Present results suggest that one characteristic is not good predictor of genetic marker.

Polymorphic bands were determined drought tolerance in rice at present study confidence as a very important results because it is the first molecular markers for drought tolerance in Egyptian rice genotypes. These molecular markers could be assessing to acceleration

of detection to drought tolerant genotypes on the bases of molecular markers at laboratory conditions only with comparison to field screening, which is very difficult and less accuracy.

Pakniyat *et al*. (2004) introduced markers linked to salt tolerance in cultivated and wild barley using RAPD-PCR. Pakniyat and Tavakol (2007) found markers related to drought tolerance in bread wheat genotypes using these markers. Also Nazari and Pakniyat (2008) found markers associated with drought tolerance in wild and cultivated barley genotypes using RAPD markers.

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