International Journal of Biotechnology Research Vol. 3(3), pp. 045-054, April 2015
Available online at http://academeresearchjournals.org/journal/ijbr
ISSN 2328-3505 ©2015 Academe Research Journals

Full Length Research Paper

Marker assisted selection for genetic improvement of drought tolerance in hybrid rice (*Oryza sativa* L.)

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Accepted 13 April, 2015

Ten rice cultivars (six CMS lines and four restorers) were used as parents to investigate the effect of mannitol induced drought stress on seedling traits, protein profile and molecular characteristics. Analyses of variance revealed significant differences for all studied traits and genotypes for mannitol levels. It was evident from the data that mannitol levels increased fresh weight linearly and decreased the other traits for most parents. Results of SDS-PAGE showed polymorphic variations among genotypes as well as among mannitol levels. Ten SSR markers associated with drought tolerance QTLs were evaluated for their use in marker assisted selection (MAS). The obtained results reflect the existence of considerable amount of molecular diversities among the tested genotypes. A total of 15 alleles for 10 SSR loci were detected among 34 rice genotypes (10 parents and 24 hybrids). Two specific DNA bands, the first with 100 bp molecular size appearing by RM201 marker and the second with molecular size of 80 bp appearing by RM451 marker, may play an important role in drought response in the used rice genotypes. Results proved also that the CMS parent (G46A) and the restorer parent (IR25571R) were not able to tolerate drought, but the other eight parents appeared as drought tolerant rice genotypes.

Key words: Marker assisted selection, SSR markers, drought tolerance, rice.

INTRODUCTION

Rice, one of the most important food crops for over half of the world's population, accounts for around 23% of the global calorie intake (Bernier et al., 2008; Li et al., 2011). Increasing population pressure, global warming and unpredictable rainfall patterns have induced severe drought spells in the major rice growing areas of the world in the past few years. To meet the growing demands of global population, rice productivity needs to be significantly increased (Awasthi and La, 2014).

Drought is a more complex phenomenon than most other stresses, such as salinity, submergence, pests, and diseases. It can occur at any point during crop production and for any length of time, affecting a large array of physiological, biochemical, and molecular processes. The genetic mechanisms that condition the expression of drought tolerance in rice plants are poorly understood. Since drought tolerance is a complex trait controlled by polygenes, and is dependent on the phenotype evaluated, it is one of the most difficult traits to study and characterize. Understanding the genetic basis of drought tolerance in rice is fundamental to enable breeders and molecular biologists to develop new varieties with more drought tolerance characters (Nguyen and Buu, 2008).

Plant response to stress conditions occurs through a number of changes at physiological and developmental levels, brought about by altering the expression of stress inducible genes (Philippe et al., 2010). In general, genes associated with drought resistance are numerous and have been shown to interact with the environment, and thus the networks involved in drought tolerance are quite complex in nature. Therefore, progress in improving the drought tolerance of rice is slow (Lin et al., 2007).

Mannitol, a member of sugar alcohols, is an osmotic adjustment chemical to control osmotic potential in the culture media or nutrient solutions in order to induce water deficit conditions for protein expression or proteomic studies (Zang and Komatsu, 2007).

Molecular markers have been used to identify many

drought tolerant associated QTLs in the past decade. Loci affecting root systems, osmotic adjustment, leaf rolling, leaf drying, and relative water content have also been reported. Root-related traits under drought stress were mapped in several studies including root penetration ability (or index), root thickness, root dry weight, pulling force, and root length (Price et al., 2000; Zheng et al., 2000; Zhang et al., 2001; Robin et al., 2003; Nguyen et al., 2004).

The present study aimed at: (1) investigating the effect of mannitol induced drought stress on seedling traits and protein profile for ten rice cultivars; and (2) validating ten major drought QTL markers associated with drought tolerance in different populations (ten parents and 24 commercial rice hybrids).

So, this study "Marker assisted selection for drought tolerance rice in Egypt" was performed with the following purposes: (1) to introduce and apply new approaches through molecular techniques; and (2) to develop new rice varieties for drought tolerance.

MATERIALS AND METHODS

The present study was conducted at the laboratories of the Department of Genetics, Faculty of Agriculture, Kafrelsheikh University, Egypt during years 2013 and 2014.

Plant material and drought treatments

Six CMS lines and four restorers obtained from Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt were used as parents in this study (Table 1). Seeds of the ten parents were dehusked by hand, sterilized in 70% ethanol for 2-3 min, followed by treatments in 5% commercial bleach (Clorox; 6.0% sodium hypochlorite) for 40 min, 30% commercial bleach for 30 min and then rinsed 4-5 times with sterile distilled-water. Surface sterilized seeds were germinated on MS medium containing 3% sucrose and 0.8% agar (w/v) in 250 ml glass vessels. The media were adjusted to pH 5.7 before autoclaving. Seedlings were cultured *in vitro* under the conditions of 25±2°C, 85-90% relative humidity and 60±5 µmol $m^{-2}s^{-1}$ photosynthetic photon flux (PPF) with 16 hd^{-1} photoperiod. After seven days, rice seedlings were placed on MS-liquid media under the photoautotrophic condition. Fourteen days old rice seedlings were treated with 0 (control) and 100 mM mannitol as drought stress for seven days (Sutee et al., 2008). Rice seedlings were harvested and stored at -20°C prior to the analysis.

Evaluation of parents for drought stress

Fresh weight, shoot height, root length and number of roots/seedling were measured as described by Cha-um et al. (2006); these traits putatively contribute to drought tolerance in rice.

Protein extraction and separation through SDS-PAGE

Protein was extracted by grinding 100 mg lyophilized plant material under liquid nitrogen to fine powder in a mortar and pestle. The powder was homogenized with buffer containing 100 mM TrisHCl (pH 7.5), 1% SDS and 0.1% ß-mercapto ethanol, centrifuged at 12,000 rpm for 10 min at 4°C and supernatant was collected. Discontinuous sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to Laemmli (1970) for protein bands separation with 12% acrylamide gels. About 25 µl of protein from each extract sample were loaded. For detection of proteins, gels were stained with 0.03% Coomassie Brilliant Blue G250.

DNA isolation

For DNA studies, the six CMS lines and four restorers were used to produce 24 commercial hybrids using line x tester mating design (Table 2). For DNA isolation, all hybrids beside their parents were grown in the greenhouse. Fresh leaf tissues from 20-days old seedlings were harvested in mesh bags. DNA was isolated from genotypes according to CTAB method (Murray and Thompson, 1980). DNA concentration was determined by spectrophotometric analysis at 260 and 280 nm wave length.

SSR markers assay and detection of polymorphism

Ten SSR markers (namely, RM3825, RM301, RM55, RM518, RM451, RM553, RM201, RM215, RM228 and RM271) (Table 3) associated with drought tolerance QTLs, were evaluated for their use in marker assisted selection (MAS) in the ten parents and their 24 hybrids.

A total volume of 25 µl PCR reaction containing 40 ng template DNA, 10 pmole of each of the forward and reverse primers, 0.1 mM dNTP's, 2.5 µl from 5× PCR buffer and 0.5 unit *Taq polymerase* was prepared. The volume was brought up to 25 µl by adding autoclaved double distilled H_2O. The template DNA was initially denaturated at 94°C for 5 min followed by 35 cycles of PCR amplification under the following parameters: 1 min denaturation at 94°C, 1 min primer annealing at 55°C and 2 min primer extension at 72°C. A final step of 7 min extension at 72°C was allowed for completion of primer extension on thermalcycler, followed by storage at 4°C. The PCR products were separated electrophoretically in 1.5% (w/v) agarose gel using 0.5 × TBE buffer and visualized under a UV light source in a gel documentation system. Ten SSR DNA markers distributed throughout the rice genome were selected and primer sequences were obtained from Gramene (http://www.gramene.org). The selected SSR DNA primers were tested for polymorphism on the parents and 24 rice hybrids.

Table 1. Cytoplasmic sources, origins and salient features for the parental CMS and restorer lines.

Genotype	Cytoplasmic sources	Origin	Salient features
IR58025A	CMS line (WA)	IRRI	Indica type, late maturing, extra long grain, low amylose content and strong aroma.
IR68885A	CMS line (Mutant IR62829A)	IRRI	Indica type, very early maturing, Med. Long grain and Med. Amylose content.
IR68897A	CMS line (WA)	IRRI	Indica type, Med. Early maturing and Med. Long grain.
IR68902A	CMS line (WA)	IRRI	Indica type, Med. Maturing, long grain, Med. Amylose content and strong aroma.
IR70368A	CMS line (WA)	IRRI	Indica type, Med. Early maturing, Med. Long grain and Med. amylose content.
G46A	CMS line (Gambiaca)	China	Indica type, early maturing, short grain and Med. amylose content.
Giza 178R	Restorer line	Egypt	Indica-Japonica type, early maturing, short statured, tolerance to salinity, short grain and high yielder.
IR25571R	Restorer line	IRRI	Indica type, early growth duration, Med. stature, high yielder and good restorer for CMS lines.
PR1	Restorer line	Egypt	Indica type, Med. maturing, semi dwarf, selender long grain and aromatic restorer.
PR78	Restorer line	Egypt-India	Indica type, Med. maturing, semi dwarf, selender long grain and good aromatic restorer.

Table 2. List of 24 commercial hybrid rice combinations.

No.	Hybrid	No.	Hybrid	No.	Hybrid	No.	Hybrid
1	IR58025A/Giza 178R	7	IR68885A/ PR1	13	IR68902A/Giza 178R	19	IR70368A/PR1
2	IR58025A/IR25571R	8	IR68885A/ PR78	14	IR68902A/IR25571R	20	IR70368A/PR78
3	IR58025A/PR1	9	IR68897A/Giza 178R	15	IR68902A/PR1	21	G46A/Giza 178R
4	IR58025A/PR1	10	IR68897A/IR25571R	16	IR68902A/PR78	22	G46A/IR25571R
5	IR68885A/Giza 178R	11	IR68897A/PR1	17	IR70368A/Giza 178R	23	G46A/PR1
6	IR68885A/ IR25571R	12	IR68897A/PR78	18	IR70368A/IR25571R	24	G46A/PR78

The SSR banding pattern was then scored and used to prepare the matrix. Employing the computer package SPSS Pc, Jaccord's similarity coefficients were calculated and used to establish genetic relationship among the genotypes based on un-weighted pair group method of arithmetic averages (UPGMA) and sequential agglomerative hierarchical nested clustering.

Parameters and statistical analysis

Data were recorded for fresh weight, shoot height, root length and number of roots/seedling after 21 days of

Table 3. List of used SSR primers.

No.	SSR loci	Position on LG	Sequences		Position (cM)	Expected PCR product size (bp)
			Forward 5'→ 3'	Reverse 5'→ 3'		
1	RM 3825	1	AAAGCCCCCAAAAGCAGTAC	GTGAAACTCTGGGGTGTTCG	143.7	147
2	RM 301	2	TTACTCTTTGTGTGTGTGTGAG	CTACGACACGTCATAGATGACC	59.3	153
3	RM 55	3	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG	168.2	226
4	RM 518	4	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC	25.5	171
5	RM 451	4	GATCCCCTCCGTCAAACAC	CCCTTCTCCTTTCCTCAACC	115.5	207
6	RM 553	9	AACTCCACATGATTCCACCC	GAGAAGGTGGTTGCAGAAGC	76.7	162
7	RM 201	9	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA	81.2	158
8	RM 215	9	CAAAATGGAGCAGCAAGAGC	TGAGCACCTCCTTCTCTGTAG	99.4	148
9	RM 228	10	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC	130.3	154
10	RM 271	10	TCAGATCTACAATTCCATCC	TCGGTGAGACCTAGAGAGCC	59.4	101

sowing date. One gram of shoot sample from each treatment for all varieties was taken and subjected to SDS-PAGE for protein bands separation. The data were subjected to Minitab for analysis of variance. The obtained mean values ± standard error were compared using the least significance difference (LSD) method to compare the significance of differences between all the parents for control and mannitol stress; the results were considered significant at $P < 0.05$ and 0.01.

RESULTS AND DISCUSSION

Parental phenotyping for drought tolerance

After seven days of incubation, fresh weight, shoot height, root length and number of roots/seedling for each seedling treated with or without mannitol are demonstrated in Table 4. Significant variations in physio-morphological traits associated with drought tolerance were observed among all the studied parents.

Fresh weight

Growth parameters including fresh weight of rice grown under iso-osmotic water deficit were increased relating to osmotic pressure in the culture media. Among the ten rice parents, all of them recorded higher fresh weight under mannitol stress, except the CMS line G46A. These results are not similar to previous documents in rice growth reduction when exposed to iso-osmotic stress (Bahaji et al., 2002; Hien et al., 2003; Ahmad et al., 2007).

Shoot height

It was decreased with mannitol stress for all parents, except the CMS line IR68885A and the restorer line IR25571R. The highest values (31.00 and 29.94 cm) were recorded under control and 100 mM mannitol treatment for the restorer line PR78, respectively, while the lowest values (5.50 and 4.33 cm) were noticed for control and 100 mM mannitol concentration for IR25571R and G46A, respectively.

Root length

Root systems have an important role to play in contributing to crop performance. Improvement in yield requires efficient uptake of water and nutrients that must be captured from the soil via roots. Minimum root lengths (2.33 and 2.39 cm) were noticed for control of G46A and IR25571R, respectively as compared to the maximum values recorded by control (11.17 and 11.44 cm) for PR78 and IR68902A, respectively. It is evident from Table 4 that the root length strongly varies among all parents.

Number of roots/seedling

The maximum number of roots/seedling (23.56) was recorded for the restorer line Giza 178R under control condition, while the minimum root numbers/seedling (5.56 and 5.67) were recorded for G46A under mannitol treatment and IR25571R under control, respectively.

From the above mentioned data, significant variations in the four studied physiomorphological drought tolerance traits were observed among the parents (Table 4). For fresh weight, the CMS parent G46A gave the lowest mean value under mannitol treatments (0.03 gm). Regarding shoot height trait, under the drought stress (mannitol) treatment, the CMS parent G46A and the restorer parent IR25571R recorded the lowest mean values (4.33 and 8.33 cm), respectively. By studying root length trait, it was surprising that the both mentioned above two parents (G46A and IR25571R) recorded the lowest mean values under mannitol treatment (4.33 and 3.44 cm), respectively. Data in Table 4 presented also that both parents (G46A and IR25571R) recorded the lowest mean values for number of roots/seedling.

This can be explained by the findings of previous researchers who reported that differences of stress tolerance efficiency among seedling cultivars are usually

Table 4. Changes in the studied growth characters of rice cultivars under photoautotrophic system with 100 mM mannitol for 7 days compared to mannitol free condition.

Cultivar	Treatment (mM mannitol)	Fresh weight (gm)	Shoot height (cm)	Root length (cm)	Number of roots/seedling
IR58025A	0	0.18±0.009	25.72±0.736	7.50±0.333	10.89±1.073
	100	0.23±0.023	24.67±1.255	7.33±0.768	11.89±1.006
IR68885A	0	0.22±0.034	21.39±1.647	7.28±0.565	14.22±1.681
	100	0.26±0.023	23.11±1.589	7.28±0.833	14.56±1.015
IR68897A	0	0.21±0.016	26.72±0.817	10.33±0.645	10.56±0.647
	100	0.24±0.018	24.72±0.692	10.11±0.605	11.22±0.618
IR68902A	0	0.20±0.014	28.17±0.513	11.44±0.863	13.67±0.288
	100	0.21±0.018	23.44±2.266	10.00±1.010	8.11±0.771
IR70368A	0	0.16±0.011	24.67±0.661	6.33±0.408	9.89±0.824
	100	0.23±0.021	23.44±0.891	7.50±0.485	10.11±0.633
G46A	0	0.08±0.012	7.44±0.647	2.33±0.204	7.78±0.954
	100	0.03±0.001	4.33±0.583	4.33±0.950	5.56±0.818
Giza 178R	0	0.30±0.021	25.44±0.925	5.56±0.281	23.56±0.647
	100	0.31±0.026	23.44±0.742	5.94±0.574	20.33±0.971
IR25571R	0	0.34±0.041	5.50±0.250	2.39±0.351	5.67±0.577
	100	0.59±0.051	8.33±0.527	3.44± 0.294	7.67±0.577
PR1	0	0.22±0.017	27.33±1.381	6.28±0.500	11.89±0.611
	100	0.33±0.032	21.72±1.654	5.56±0.549	12.00±0.687
PR78	0	0.21±0.015	31.00±1.500	11.17±0.485	12.78±0.572
	100	0.30±0.028	29.94±1.220	10.94±0.523	9.56±0.647
$L.S.D._{0.05}$		0.068	3.186	1.686	2.318
$L.S.D._{0.01}$		0.090	4.206	2.226	3.061

determined by the inhibition of growth characteristics at the whole plant level (Rampino et al., 2006). The seedling of cultivars that showed lower reduction of growth characteristics could be drought-tolerant than those which showed higher reduction of growth characteristics. This could suggest that seedlings of drought-tolerant cultivars may have better adaptive responsibility such as the controlling stomatal pore and the stability of organelles within the cell (Setter and Flannigan, 2001).

Protein profile

Concerning biochemical studies and according to SDS-PAGE analysis (Figure 1), the protein band with M.W. of 70 KDa appeared in all parents under control and mannitol treatments. Most of the protein bands with M.W. less than 70 KDa (55, 45, 35, 25 and 10 KDa) appeared in all parents under control and drought stress treatment, except in the case of the two parents G46A (number 6) and IR25571R (number 8); these bands were absolutely absent in the mannitol treatments. This result was of specific interest while the two parents (G46A and IR25571R) which failed to give good results or valuable mean values in the physiomorphological traits related to drought stress under study were also not able to express specific protein bands under drought stress treatment. This means that these absent protein bands could be expressed only under control of specific genes; these genes may lost their function in both parents as a result of point or chromosomal mutations. But it is very

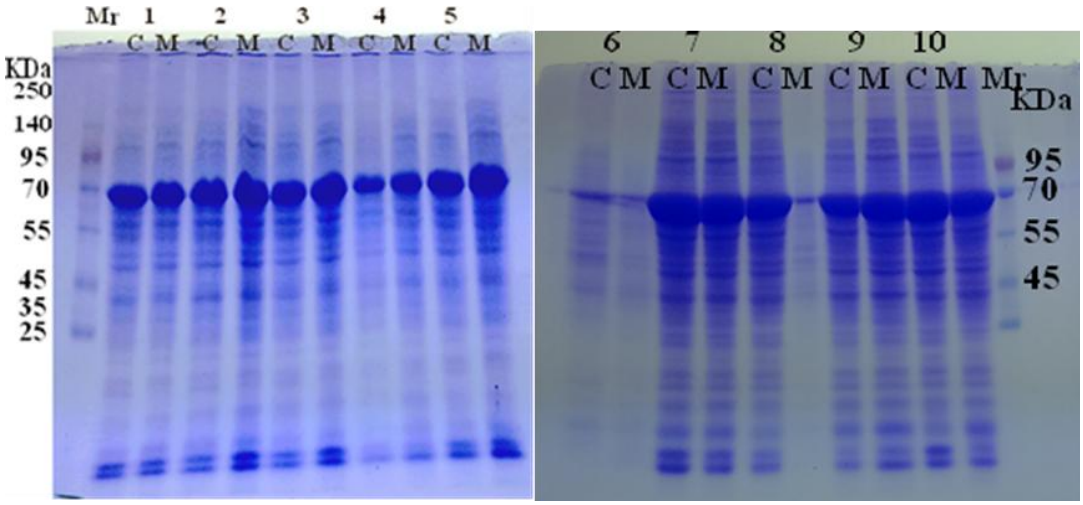

Figure 1. SDS-PAGE pattern of proteins in seedlings of ten rice cultivars (1-10) under different levels of drought stresses: (Mr) Molecular mass marker, C (control) and M (100 mM mannitol).

important to mention that these absent genes may play a main role in drought tolerance in rice.

On the other hand, the main protein band of 70 KDa, which was strongly expressed in all parents under control and mannitol treatments, very weakly appeared in the case of the two parents (G46A and IR25571R) under drought stress treatments. This means that the gene(s) which control the expression of this band were suppressed as a result of drought stress in both parents; also, this gene(s) may play essential role in drought tolerance in rice. Meanwhile, the protein bands which have more than 70 KDa (250, 140 and 95 KDa) in Figure 1 showed highly differences under control and drought stress treatments.

The raise of seedlings protein content can be due to induction of specific proteins involved in stress tolerance/response. Beside their specific functions, proteins which are accumulated in the plants by stress exposure may provide a storage form of nitrogen that is reutilized when stress is over and probably play a role in osmotic adjustment (Niknam et al., 2006; Ahmad et al., 2007). The observed differences between the intensity of a protein band with molecular mass ranging from 70 to 250 kDa in all cultivars, suggest a probable role of this protein in drought tolerance. Also, the induction of some protein bands under stress treatments may play a role in higher osmotic stress tolerance of this parent. The results are in line with those obtained by Shobbar et al. (2010), who concluded that drought imposed adverse effect on the protein profile of rice genotypes. Also, Kamal et al. (2010) however, identified 9 drought stress responsive proteins in wheat genotype China-108, 15 in Yeonnon-78, 20 in Norin-61, and 26 in Kantou-107 during their study.

Molecular diversity assessment

A total of ten SSR DNA markers evenly distributed in the rice genome were chosen to screen a 24 hybrid populations derived from line x tester mating design and their parents. Out of the ten SSR DNA markers used, six of them namely, RM301, RM518, RM451, RM553, RM201 and RM215 were found to exhibit polymorphism among all genotypes. The rest four markers showed no polymorphism among hybrids and their parents. These four including markers RM3825 and RM271 which showed one band appeared in all tested genotypes; while the other two, RM55 and RM228 did not show any bands in all tested genotypes. A total of 15 alleles for 10 SSR loci were detected among 34 rice genotypes. Number of alleles produced by the ten markers ranged between 1 and 3, so that the highest number of alleles was related to marker RM451.

Data in Figure 2 presented the polymorphic banding pattern of RM 201 marker for the tested parental and hybrid genotypes. The expected band size for this marker is 158 bp (Table 3). This band is related to drought tolerance trait in rice by using this primer. This band was missed in the case of G46A parent (number 6) but was found in most other parents. Additional band with molecular size of 100 bp was found in all parents, except G46A and PR78 (number 10). This means that the two bands which appeared by using this marker were absent in the parent G46A. Some hybrids which contain the parent IR25571R (number 8) such as IR68897A/IR25571R (3×8) (number 20 in Figure 2) lost both DNA bands, and the hybrid number 34 in Figure 2 (G46A/PR78) which contain the parent G46A (6×10) lost also the expected DNA band with molecular size of 158 bp. It is possible that the two DNA bands with molecular sizes of 158 and 100 bp which appeared by applying RM 201 marker in the majority of parents and hybrids play an

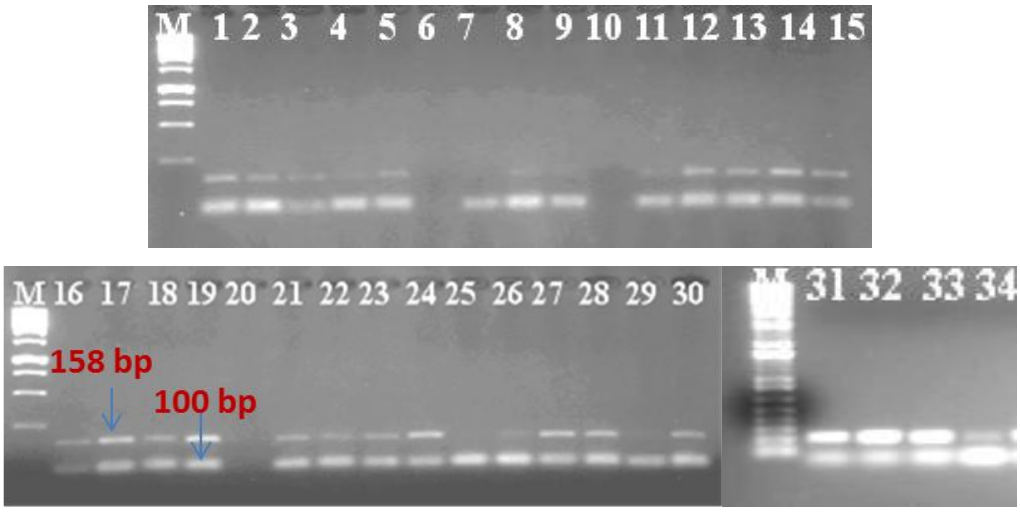

Figure 2. Polymorphic banding pattern of RM201 for the tested ten parents (1-10) and 24 commercial hybrid line genotypes (as shown in Table 2). Expected size = 158 bp.

important role in drought tolerance in rice and their absence affect negatively drought tolerance ability of some rice genotypes. These bands should be isolated and sequenced for further studies.

These results proved that the two rice parents, G46A and IR25571R are susceptible to drought stress, but the other eight parents were able to exhibit different levels of drought tolerance under the conditions used in this experiment. Results also appeared that the two susceptible parents failed to express some protein bands which may be related to drought tolerance in rice. Meanwhile some DNA bands were absent in G46A parent by using RM 201 marker and some hybrids which contain G46A and IR25571R parents. Probably these DNA bands may be expressed under control of specific genes responsible for drought tolerance in rice.

On the other hand, by using the SSR DNA marker RM451 (Figure 3), the expected band with molecular size of 207 bp which is related to drought tolerance in rice (Table 3) was presented in all parental and hybrid genotypes. An additional band with molecular size of 100 bp also appeared in all 34 studied genotypes by using this marker. It was of specific interest that the four hybrids which contain the parent G46A (number 6) with numbers 21, 22, 23 and 24 as shown in Table 3 (G46A/Giza 178R, G46A/IR25571R, G46A/PR1 and G46A/PR78), respectively contained a second additional band with molecular size of 80 bp. Moreover, this band was strongly expressed in the hybrid number 22 (G46A/IR25571R) which contains the two drought susceptible parents (numbers 6 and 8) as reported above. To determine the role of the gene(s) responsible for expression of this specific additional band (80 bp) in drought response in the used rice genotypes, further molecular studies such as DNA sequencing should be done.

The high amount of primers that were able to detect clear polymorphism reflects the genetic diversity among the tested genotypes. The results also demonstrate the power of SSR primers in detecting polymorphism with higher numbers of alleles. Results obtained here were in full agreement with those of Ammar et al. (2009), who used 52 STMS primers to estimate the genetic diversity in ten Egyptian commercial rice varieties. They added the existence of significant level of molecular diversity among the commercial varieties and the number of detected alleles ranged from one to three. Moreover, they used 28 distinct primers to construct a specific profile for each variety to be used as a reference in seed purity assessment and IPR related issues. Assessment of genetic diversity of Philippine rice cultivars using SSR markers was carried out by Lapitan et al. (2007). They reported that twenty-four rice cultivars carrying good quality traits were evaluated for the genetic diversity using 164 SSR markers. A total of 890 alleles were detected by 151 polymorphic markers.

The banding patterns (the presence or absence) of DNA bands for the ten SSR primers were used to calculate the similarity index among each pair of genotypes using hierarchical nested cluster analysis SPSS software. The dendrogram explaining the genetic relatedness among the tested genotypes using UPGMA is presented in Figure 4.

The results revealed that at 25% of the genetic similarity, the set of genotypes tested was divided into two main groups: A and B. The A group contained only one genotype (H19), while the B group consisted of 33 genotypes and represented all the parents. Nevertheless, the clustering largely depended on drought tolerance according to absence or presence of the banding by SSR markers rather than genetic background *per se*. This is

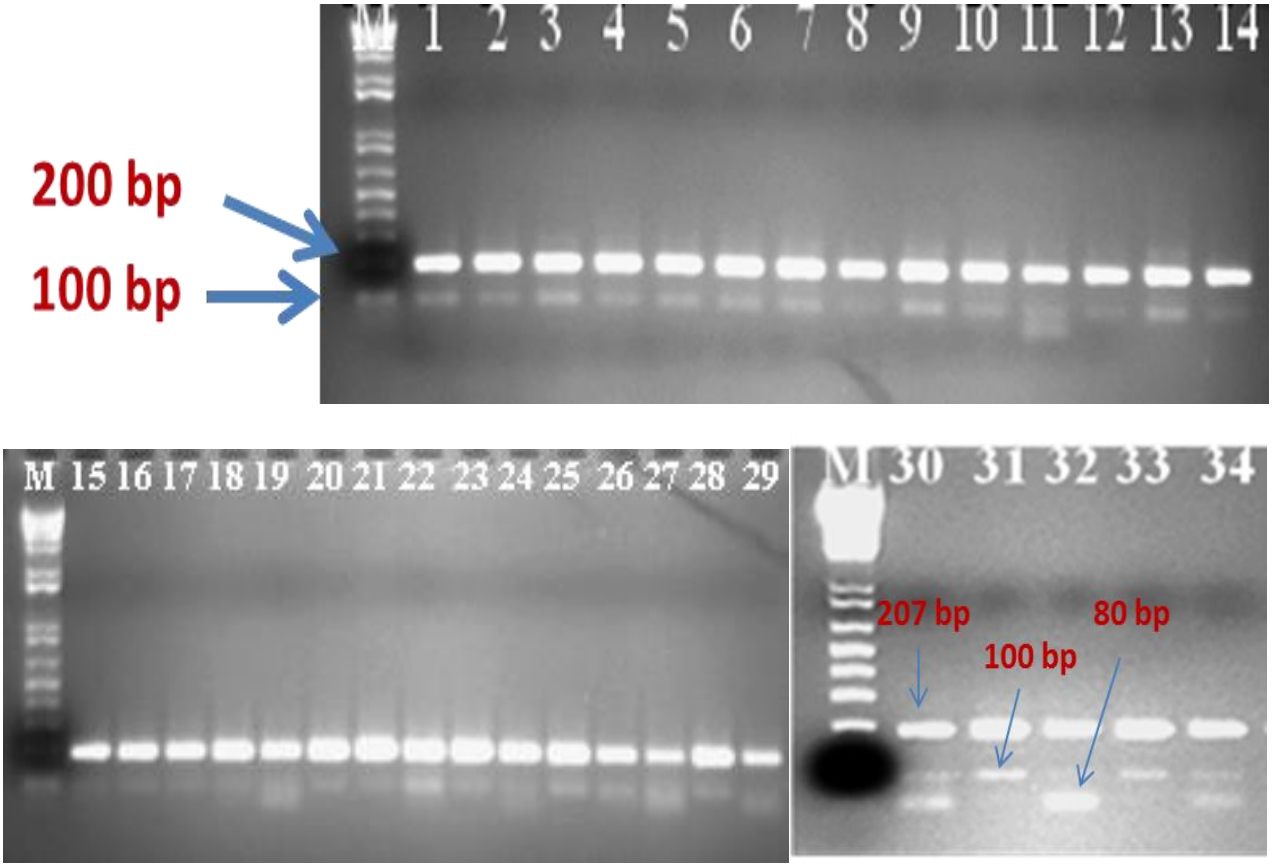

Figure 3. Polymorphic banding pattern of RM451 for the tested ten parents (1-10) and 24 commercial hybrid line genotypes (as shown in Table 2.). Expected size = 207 bp.

primarily due to the presence of different resistance genes operating on those genotypes and the use of many randomly selected SSR primers for diversity assessment. The second group (B) was further divided into two main subgroups B1 and B2 (Figure 4). The B1 subgroup had three parents and H10 genotype, while the B2 subgroup included 29 genotypes that further diverged into two subgroups at 15% level of genetic similarities.

The obtained results reflect the existence of considerable amount of molecular diversity among the tested genotypes and hence demonstrate the feasibility of genetic improvement of drought tolerance as well as other morphological traits using those genotypes in the breeding program.

Similar trends were also reported by many authors. 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 cultivars in Argentina was evaluated at the DNA level (Giarrocco et al., 2007). They surveyed 69 accessions with 26 simple sequence repeat (SSR) markers revealing the genomic relationship among cultivars. Herrera et al. (2007) 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 was done with nine primer pairs which produced ten genotype specific alleles. Although the genetic diversity was low, SSR proved to be an efficient tool in assessing the genetic diversity of rice genotypes.

Conclusion

The most significant application of the so far identified major QTLs for drought tolerance is to pyramid those favorable alleles into an elite local rice line through marker assisted breeding. Thus, the markers RM 201 and RM 451 will be useful for more efficient way for selecting drought tolerant lines through MAS approach,

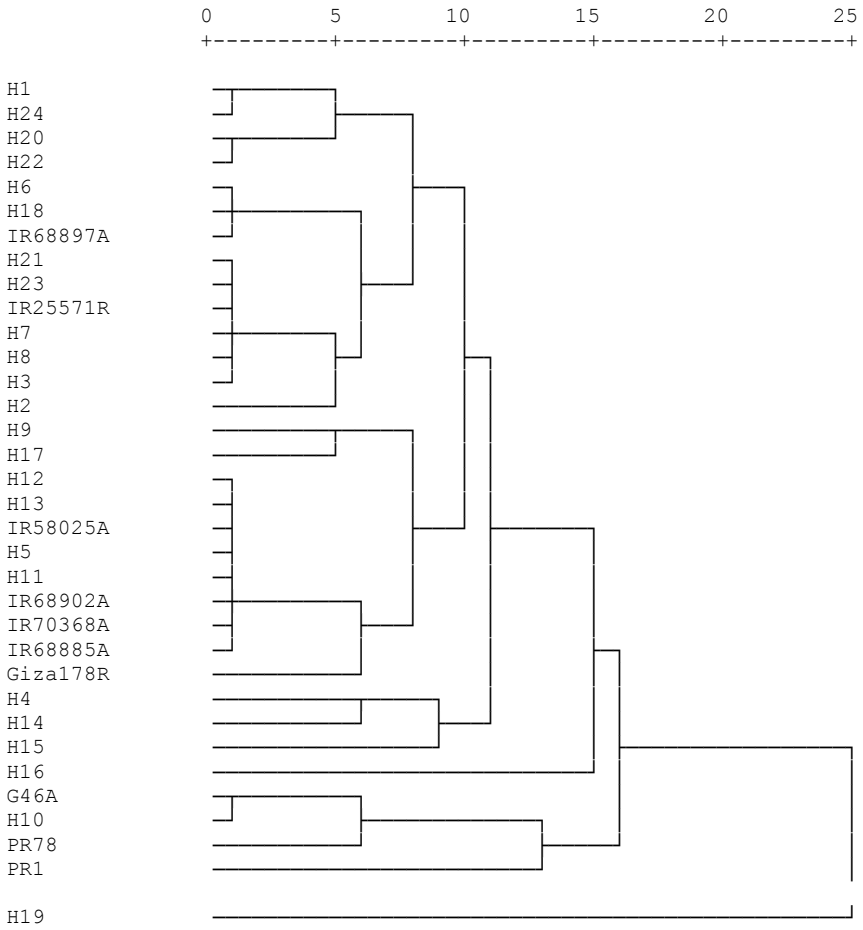

Figure 4. Dendrogram based on Jaccard's coefficients. Dendrogram generated from SSR data.

especially in those regions of growing rice with irrigated ecosystem. Thus, the drought tolerance genes or other important genes isolated in rice can be used to facilitate the isolation of corresponding genes in other crops such as maize or wheat.

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