IDENTIFICATION OF SSR MARKERS FOR DROUGHT STRESS INDUCED BY MANNITOL IN THREE DIFFERENT GRAMINEAE PLANT GENERA

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Stress is known as all of the biological and environmental factors which lead to troubles in the function and structure of the body of living organisms. Plant in the normal conditions is considered one of the organisms which exposed to various stress factors. Drought stress affects strongly plant growth and productivity. This stress causes many problems during plant life cycle such as inhibition of water transport and limitation of the proper functioning of cell membranes, in addition to the disorganization of the cooperation between cellular organelles (Hsiao *et al.*, 1976). The negative effects of drought at the cellular level change the growth and development of plants (Olszewska *et al.*, 2010).

Drought is considered a complex phenomenon than other stresses such as salinity, pests and diseases and occurs at any crop production period. On the other hand, drought has a large effect on many physiological, biochemical and molecular processes in plants. Until now the nature of genetic mechanisms controlling drought tolerance expression in rice is not well understood but it is known that this process is very complex and under control of polygene and is dependent on the phenotype evaluated. It is of great interest to understand and interpret the molecular genetic basis of drought tolerance in rice to help the breeders and molecular biologists to develop new varieties with more drought tolerance characters (Nguyen and Buu, 2008).

Plant response to drought conditions occurs as a result of altering the expression of stress inducible genes and appears through changes at the physiological and developmental levels (Philippe *et al.*, 2010). It was reported that drought tolerance responsible genes strongly interact with the environmental conditions, therefore the ability to improve rice drought tolerance go slowly (Lin *et al.*, 2007).

Mannitol is an osmotic adjustment and has the ability to control the osmotic potential in the culture media to induce water deficit conditions for protein expression (Zang and Komatsu, 2007). It also used as a causative agent of drought osmotic stress in plants (Soetaert *et al.*, 1999). Drought stress induced by mannitol is widely used in many plants. It was proposed that increasing mannitol

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levels decrease the moisture content and seedling length (Seong *et al.*, 1988).

Traditionally, drought tolerant cultivars have been evaluated in field based on phenotypic and physiological traits observed under drought conditions (Reynolds and Tuberosa, 2008). With the advancement of molecular techniques, a diverse group of molecular markers have been developed. Among different types of molecular markers, simple sequence repeats (SSRs) or microsatellite markers have proven to be the markers of choice for marker-assisted selection (MAS) in breeding and genetic diversity studies. SSR markers have their advantage of high polymorphic information content, multi-allelic nature, codominant inheritance, random distribution within the genome, simplicity and inexpensive developmental methodology (Powell *et al.*, 1996). These markers have high potential for identification, estimation and discovery of gene(s) related to specific traits like drought. This allows fast screening at an early growth stage; independent of environmental conditions, that speed up breeding (Tester and Langridge, 2010). These molecular markers have been extensively used in identification and characterization of important traits related QTLs (Bernier *et al.*, 2007; Wang *et al.*, 2012), genetic mapping (Agrama *et al.*, 2007; Zhang *et al.*, 2014) and in phylogenetic relationship (Das *et al.*, 2013; Babu *et al.*, 2014).

Whereas, strategies to identify drought-tolerant genotypes are of major interest, the present investigation aimed to: (1) phenotypic evaluation of root and shoot traits as well as germination% under drought conditions, (2) identification of SSRs polymorphism among rice, wheat and barley cultivars for the development of common markers for screening the three genera of gramineae for drought tolerance.

MATERIALS AND METHODS

This study was carried out at the Laboratories of Genetics Department, Faculty of Agriculture, Kafrelsheikh University, Egypt.

Plant materials

Three different plant genera of gramineae (rice, wheat and barley) obtained from Sakha Agriculture Research Station (ARC), Egypt were used in this experiment (four cultivars from each genus) as shown in Table (1) to study the effect of drought stress induced by mannitol.

Samples preparation

The grain samples from the three different plant genera were rinsed under running tap water for 20 min and then washed with distilled water. Grains were sterilized in 10% commercial bleach (Clorox; 6.0% sodium hypochlorite) for 5 min and washed three times with sterile distilled water. Grains from each sample were cultured and lined up in sterile Petri dishes with filter paper (10 grains/Petri

dish) and placed with equal distance from each other and replicated three times for each treatment.

Mannitol treatment preparation

A stock of 1 M mannitol solution was prepared by adding 18.217 gm of mannitol to 100 ml distilled water and vortex for dissolving mannitol. Three different concentrations of mannitol (125, 250 and 500 mM) in addition to the control (0) were used in this study. The concentrations were prepared by adding 12.5, 25 and 50 ml of 1 M mannitol stock solution to 87.5, 75 and 50 ml distilled water, respectively, to make 125, 250 and 500 mM mannitol solutions. The dilutions were prepared and kept in flasks for watering the experimental material.

Layout and experimental design

Completely Randomized Design (CRD) with two factors was used as statistical design with three replicates for each treatment to minimize the experimental error. Ten grains of each genotype from all plant materials were sown in one Petri dish and after three days watered with 2 ml of mannitol solutions for each treatment on the alternate days for 21 days. For germination, Petri dishes were incubated in growth chamber in the dark for three days and then with 16/8 h photoperiod at 25±2°C. The factors used were genotypes (four genotypes/genus) and mannitol treatments (0, 125, 250 and 500 mM).

Statistical analysis

After three weeks of sowing date, seedlings were observed and recorded for root length, shoot length and germination percentage for all treatments and tested genotypes. Analysis of variance and mean performance for the obtained data were carried out using SX program (Analytical Software, Statistix Version 3.5). The data of germination percentage were arcsine transformed prior to statistical analysis.

Molecular analysis

DNA extraction

Genomic DNAs of the 12 genotypes under study were extracted from leaf tissues of seedlings (100-150 mg) by using Cetyl trimethyl ammonium bromide (CTAB) method according to Murray and Thompson (1980). DNA concentrations were recorded and diluted to a final concentration of 40 ng/µl for PCR amplification.

SSR markers and PCR amplification

Thirteen SSR primer pairs (iNtRON Biotechnology, Inc, Korea); listed in Table (2), were used to determine genetic diversity among the 12 tested genotypes.

The PCR amplification was performed in 10 µl reaction mixture contained 1 µl genomic DNA as a template, 5 µl of 2X PCR Master mix solution [(i-TaqTM) iNtRON Biotechnology], 1 µl of each of the

forward and reverse primers (10 pmol/µl) and 2 µl double distilled water. PCR was carried out in a thermal cycler (Perkin Emer Cetus) programmed as the following; one cycle of pre-denaturation at 94°C for 5 min., then 45 cycles consisting of 1 min. at 94°C for denaturation, 1 min. at 62°C for primer annealing and 1.5 min. at 72°C for extension, and then 7 min. at 72°C for final extension.

The amplification products were determined by electrophoresis on 3% agarose gels and visualized on Benchtop UV-transilluminator, then photographed using photo Doc-ItTM Imaging System. The molecular size of the amplified products was determined against 1 Kb plus DNA ladder (TIANGEN).

Data analysis

For molecular marker analysis, data of SSR amplification were introduced as binary values of (1) and (0) for the presence and absence of an amplification product, respectively. The presence or absence of specific bands was scored and used to determine number of alleles per primer and polymorphic information content (PIC) values. Polymorphic information content (PIC) value of each primer pair was calculated according to the formula reported by Roldan-Ruiz *et al*. (2000) as follow: *PICi = 2fi(1-fi)*, where *PICi* is the polymorphic information content of the locus i, *fi* is the frequency of the present bands, and (1-*fi*) represents the frequency of the absent bands. The PIC of each primer was calculated using the average PIC value from all loci of each primer. A dendrogram was constructed using the unweighted pair-group method with arithmetic average (UPGMA) to determine the genetic relationship among the 12 genotypes.

RESULTS AND DISCUSSION

Analysis of variance for all studied seedling traits for the three different plant genera (rice, wheat and barley)

Analysis of variance for rice and barley genotypes showed highly significant differences for root length, shoot length and germination% traits (Table 3). Also, mannitol treatments revealed highly significant differences for root length and shoot length in the three plant genera (rice, wheat and barley) cultivars and germination% in rice and barley only, while the interaction between genotypes and mannitol treatments showed significant differences only in rice for shoot length trait (Table 3). Genotypes, however, showed no statistical differences for root and shoot lengths in wheat cultivars. No variation between wheat genotypes for shoot length in this experiment which agreed with the result of Simova-Stoilova *et al.* (2008) who concluded that physiological response to drought was similar among the genotypes. The significant variations between genotypes, for mannitol concentrations and their interaction demonstrated that the genotypes responded differently to stress condition.

Mean performances of seedling traits for rice, wheat and barley genotypes under normal and stress conditions

Root length (cm)

Root systems play a significant role in contributing to plant performance. Enhancement yield requires efficient water and nutrients uptake that must be captured *via* roots (Thorup-Kristensen *et al.*, 2009). In the case of root length in rice cultivars, the overall mean performances of mannitol induced drought stress treatments recorded the highest values in control and decreased by increasing mannitol stress (Table 4 and Fig. 1A). On the other hand, the highest overall mean value (3.66 cm) was recorded for the drought tolerant genotype E. Hybrid 1, while the lowest mean value (0.98 cm) was recorded for the sensitive genotype Giza-177. For all the studied rice genotypes, the mean performance showed linear decrease with increasing mannitol concentration, except Giza-179 which recorded the highest mean value for 125 mM mannitol treatment.

The effect of mannitol concentrations on root length in wheat cultivars as shown in Table (4) and Fig. (1B) indicated that mannitol reduced the root lengths to a great extent. The highest overall mean value (3.68 cm) of root length was recorded for Sids-12 cultivar followed by Sakha-95 cultivar (3.57 cm). Minimum root length (1.35 cm) was observed at 500 mM mannitol treatment as compared to the maximum root length in control (5.60 cm).

Assessment of barley cultivars response to different concentrations of mannitol generated informative results. A strong negative relationship was noted between mannitol concentrations and root length trait which showed decrease of root length when mannitol concentration was increased as shown in Table (4) and Fig. (1C). Significant differences in root length between the studied barley cultivars were observed (Table 4). Among all the studied barley cultivars at 500 mM mannitol treatment, root length recorded the lowest mean values, except Giza-2000 which recorded the lowest mean value at 250 mM.

Shoot length (cm)

The results in Table (4) and Fig. (1A) illustrated that, shoot length showed the same results as root length for all studied rice cultivars and for all treatments of mannitol induced drought stress, except in the case of 500 mM in Sakha-105 and the overall mean values were in the range of 0.87 cm for Giza-177 cultivar to 2.81 cm for E. Hybrid 1 cultivar.

Effects of mannitol induced drought stress on shoot length of wheat cultivars showed that it was decreased gradually with increasing mannitol induced drought stress as recorded for root length for cultivars and treatments (Table 4 and Fig. 1B). The control treatment recorded the highest mean value (7.08 cm) of shoot length while, 500 mM mannitol treatment recorded the lowest mean value (1.87 cm).

All the studied barley cultivars recorded negative relationship between mannitol concentration and shoot length while increasing mannitol concentration led to decrease of shoot length in most treatments. These results were in the same line of root length trait and clearly indicated that increasing root length led to increasing shoot length. All studied barley cultivars showed slow growth in shoot length at 500 mM mannitol in comparison with control (Table 4). The cultivar Giza-126 had the best growth rate (9.13 cm) when compared to the other genotypes followed by Giza-121 (8.07 cm). On the other side, the two cultivars Giza-2000 and Giza-118 showed inhibition of growth at 250 mM and 500 mM in comparison with control.

Germination (%)

For germination% in rice as shown in Table (4), the highest level of mannitol (500 mM) showed the lowest germination percentage (51.67%) in comparison with control which showed the highest germination percentage (91.67%). As the results obtained from root length and shoot length, the drought tolerant E. Hybrid 1 cultivar recorded the highest germination percentage (92.50%) and the lowest mean value (53.33%) was recorded for the drought sensitive cultivar Giza-177.

On the other side, data in Table (4) illustrated that germination% in wheat cultivars recorded the highest value for the two studied cultivars Sakha-95 and Misr-2 in the control treatment, while Gemmiza-11 and Sids-12 recorded the highest values at 125 mM mannitol treatment. For genotypes, Gemmiza-11 showed the highest percentage of germination (54.17%) followed by Sakha-95 (49.17%) while, Misr-2 and Sids-12 recorded the lowest germination percentage (31.67%).

Meanwhile, the effects of drought stress induced by mannitol on the germination% of barley cultivars showed that germination of grains and seedlings development in lab conditions have been recognized as testing procedure in barley and it is well identified that the increase of mannitol concentrations, inhibits the germination rate and growth of seedlings (Table 4). Giza-126 cultivar showed the highest germination rate at the highest concentration of mannitol (500 mM) in comparison with control and the other treatments. Also, among the investigated cultivars, Giza-126 showed low germination rate reduction with increasing mannitol concentration than the other genotypes.

Under mannitol induced drought stress conditions, rice seedlings showed reduction in all studied traits. This can be explained by the findings of previous researchers who reported that differences of stress tolerance efficiency among seedling cultivars are usually determined by the inhibition of growth characteristics at the whole plant level (Rampino *et al.*, 2006; Aboulila, 2015). This result was also recorded for wheat and barley cultivars in this study. The seedling of cultivars that

showed lower reduction of growth characters could be drought tolerant than those which showed higher reduction of growth characters under drought stress condition. 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). Drought stress can cause seedling damage by different mechanisms related to osmotic and oxidative damages at cellular levels. In drought stressed plants, several kinds of stress inducible genes are differently expressed (Seki *et al.*, 2002). The gene expression depends on the genetic content inside each of the rice cultivars and effect on drought tolerance efficiency of whole seedling. Under the condition of drought stress, chloroplast ultra-structures are the first targets to be damaged in the cellular levels since it is the major site of ROS production (Munné-Bosch and Peñuelas, 2003).

The result of this study can be explained by the findings of different researchers (Alfoecea and Larher, 1994; Kerepesi and Galiba, 2000) who reported increase accumulation of carbohydrates in roots under condition of drought stress. Decrease in vigor of wheat cultivars due to drought stress have been reported previously by Jajarmi (2009) and Alaei *et al.* (2010).

Also, these results are in agreement with earlier studies of Rouhi *et al.* (2011) and Ansari and Sharif-Zadeh (2012), who reported that significant inhibition in the germination rate as well as root and shoot growth was induced by mannitol as drought stress agent.

Almaghrabi (2012) reported that environmentally confined seedlings in laboratory experiments would appear to be suitable for screening large population to improve drought tolerance prior to yield testing. Usually the drought tolerant genotypes will have the highest germination rate and better survival due to their capability to absorb water under mannitol induced water stress condition. Turk *et al.* (2004) reported that water stress at germination stage delay or reduce or hinder germination completely.

Genetic diversity analysis

To evaluate the genetic relationship among the twelve genotypes representing the three diverse cultivated genera of gramineae (rice, wheat and barley), genetic diversity analysis was done using thirteen SSR primers designed for drought in rice.

All the SSR fragments were scored for allele polymorphism showing a high level of polymorphism (100%). The possible reason of the high level of polymorphism is the use of diverse genotypes representing diverse plant genera for assessment of diversity. Polymorphic information content (PIC) values ranged from 0.22 to 0.47 with an average of 0.33 per marker (Table 5). In this respect, the previous studies of Jain *et al.* (2006) and Das *et al.* (2013) reported higher PIC values

for SSR primers from genomic sequences of rice.

A total of 73 DNA fragments with an average of 5.62 alleles per primer and size differences from 113 to 692 bp were amplified among the 12 genotypes as shown in Table (5). As expected, number of alleles for each genus revealed that a higher average number of alleles was observed for rice (23 alleles per cultivar) compared to those for wheat and barley (17 and 12 alleles per cultivar, respectively). The average number of alleles per marker was 5.62 in the present study. This average value is higher in comparison with the average values published in earlier studies (Babu *et al.*, 2012; Das *et al.*, 2013).

Out of the 13 SSR primers, 7 yielded PCR amplicons of the expected size. Primer RM201 showed only one allele with an expected size of 158 bp, while six primers showed larger sizes than the expected product size ranged from two alleles with expected size of 207 bp (RM451) to 9 alleles with expected size of 226 bp (RM545). The remaining six primers possessed multiple products ranged from 5 (RM302) to 12 (RM228) alleles but did not show the expected product size. However, producing more alleles than the expected by SSR primers was reported previously by Li *et al.* (2014).

Four SSR primers (RM451, RM518, RM3825 and RM553) yielded PCR amplicons in all the tolerant and sensitive cultivars of the three genera, while the remaining primers failed to amplify bands for some genotypes (Table 5 and Fig. 2). All the 13 primers yielded PCR amplicons in all the tolerant and sensitive rice cultivars, except RM201 which did not amplify any PCR products in the two tolerant cultivars and RM9 which did not amplify any bands in the sensitive cultivar Giza-177. Primer RM201 did not amplify any PCR products in all wheat and barley cultivars. Thus this primer can be considered as a specific marker for the sensitive rice cultivars; Giza-177 and Sakha-105. Also, the four primers RM261, RM9, RM301 and RM302 failed to amplify any bands in all barley cultivars.

On the other hand, four primers (RM55, RM451, RM09 and RM301) showed differences between wheat tolerant and sensitive cultivar. Bands with sizes of 326 bp and 256 bp (RM55), 200 bp (RM451), 348 bp (RM301) were amplified only in the two tolerant cultivars (Sakha-95 and Misr-2) and did not amplify in the sensitive cultivars of wheat (Gemmiza-11 and Sids-12). In addition, bands with sizes of 207 bp (RM451) and 136 bp (RM09) amplified only in the two sensitive cultivars and failed to amplify any product in the two tolerant cultivars.

Likewise, five primers (RM55, RM3825, RM545, RM228 and RM20A) showed differences between the tolerant and sensitive cultivars of barley. All of these primers showed distinct band in the

two tolerant genotypes (Giza-126 and Giza-2000) and failed to amplify this band in the sensitive cultivars (Giza-118 and Giza-121). Primer RM55 exhibited band size of 278 bp only in the two tolerant cultivars and a different band size of 326 bp in the two sensitive cultivars of barley.

Although the appearance of distinct bands was in wheat and barley, the highest number of amplified bands was in rice genus. A possible explanation for the lack of amplification could be due to that these primers are specific to drought genes in rice. Generally, only RM55 primer succeeded in showing distinct differences between tolerant and sensitive cultivars in all the three genera; rice, wheat and barley. Thus, it could be considered the most important marker associated with drought in the three genera and could be considered as the potential marker for use in marker-assisted breeding.

Figure (2) presents agarose gel profiles showing allelic variations between the tolerant and sensitive cultivars for the three different genera; rice, wheat and barley, with RM55, RM451, RM518, RM201, RM261, RM3825, RM228, RM301, RM302 and RM20A SSR primers.

Results of our study, which showed that SSR markers are useful in detecting a high level of polymorphism among 12 different gramineae cultivars, are in agreement with previous diversity studies of Das *et al.* (2013) and Babu *et al.* (2014) on rice, El-Maghraby *et al.* (2005) on wheat, and Abou-Elwafa (2016) on barley. Results of Hellal *et al.* (2017), also support our finding about high polymorphism using SSR markers. They reported that; out of six SSR primers used for tolerant stress in barley, three (Bmac603, Ebmac84 and Bmag770) showed clear fragment patterns with 100% polymorphism and the other primers were monomorphic. Bands size varied from 110 to 220 bp (primers Ebmac84 and Bmag770, respectively) with two alleles for Bmag770 and three alleles for Bmag84. Some of these bands were found only in barley tolerant cultivars using the specific primer Bmag770.

Molecular markers which reveal many polymorphisms at the DNA level have been shown to be a very powerful tool for cultivar characterization and detection of gene(s) related to specific traits which considered one of the primary and important steps in breeding programs.

SSR markers have been used as a valuable tool in the characterization and evaluation of genetic diversity within and between species and populations. Eviatar and Guoxiong (2010) studied genetic diversity and the variations of drought tolerance in wild relatives of wheat (*Triticum dicoccoides*) and barley (*Hordeum spontaneum*) emphasizing that the interspecies variations are more profound than intraspecies variations. These results are also supported by Raju *et al.* (2010) who used 15 EST-SSRs to study the genetic diversity of 32 cultivars and

eight accessions of two *Cajanus* species; *C. platycarpus* and *C. scarabaeoides*. In this respect, Scott *et al.* (2000) reported that SSR markers can facilitate better cross-genome comparisons due to target protein-coding regions that are more likely to be conserved between related species.

Cluster analysis

Jaccard's similarity coefficients were calculated for pair-wise combinations of all the genotypes and a dendrogram was constructed (Fig. 3). Neighbor-joining dendrogram showed distinct separation of the 12 gramineae genotypes into two main clusters at a genetic similarity of 18.5%. Cluster I was divided into two sub-clusters; Ia and Ib, at genetic similarity of 19.4%. It included the two tolerant cultivars of rice (Giza-179 and E. Hybrid 1) in sub-cluster Ia, while sub-cluster Ib was corresponded to the four wheat genotypes which were classified into two groups with 39.2% similarity. The first group represented the tolerant wheat cultivars (Sakha-95 and Misr-2) and the second group consisted of the two sensitive cultivars (Gemmiza-11 and Sids-12).

Cluster II included the two sensitive rice cultivars (Giza-177 and Sakha-105) in sub-cluster IIa and the four barley cultivars in a different sub-cluster (IIb) which was divided into two distinct groups (33.9% similarity). The first group included the tolerant barley cultivars (Giza-126 and Giza-2000) and the second group included the sensitive barley cultivars (Giza-118 and Giza-121). Each of the sensitive and tolerant cultivars belonged to barley shared 33.9% similarity between them and 24.1% similarity with sensitive rice cultivars (Giza-177 and Sakhs-105).

Interestingly, all wheat cultivars as well as barley cultivars were closely related and placed in the same sub-clusters (Ib for wheat and IIb for barley), suggesting that these genotypes were grouped according to their gene pools. This result was supported by Dutta *et al.* (2011), who used 20 highly polymorphic genic-SSR markers to assess the genetic relationship among species of the genus *Cajanus*. The results showed that the UPGMA-based cluster analysis grouped the genotypes belonging to the same gene pool in the same sub-clusters.

SSR markers have been extensively used in phylogenetic relationship and diversity analysis among rice genotypes (Das *et al.*, 2013; Babu *et al.*, 2014). Based on investigation of ten barley cultivars, Hellal *et al.* (2017) found four main clusters; the first cluster included only cultivar Giza 135. However, the second and third clusters consisted of most of the tolerant cultivars (Giza 126, 134, 2000, 130 and 127) and the last cluster included susceptible cultivars. Moreover, authors reported that the highest similarity coefficient value was 1.0 (100% similarly) between tolerant barley cultivars Giza 130 and Giza 127, while it was 60% similarly between the susceptible cultivars Giza 125 and Giza 129.

From the present study, it could be concluded that shoot and root lengths of the cultivars of the three different plant genera decreased as the level of mannitol stress increased. Also, the effect of mannitol induced drought stress condition on shoot and root lengths were linear. On the other hand, as reported before better growth under stress condition as a trait to select germplasm to improve the yield so, the most tolerant rice cultivars were E. Hybrid 1 and Giza-179, while the sensitive rice cultivars were Giza-177 and Sakha-105. For the studied wheat cultivars, Sids-12 and Sakha-95 recorded the best results for growth characters so they were identified as drought tolerant cultivars, while Misr-2 and Gemmiza-11 were identified as drought sensitive cultivars because they recorded the lowest values of growth rate. For the studied barley genotypes, Giza-126 and Giza-121 were found to be tolerant for drought stress and the two sensitive cultivars were Giza-2000 and Giza-118. Also, our study represents a significant addition that can play important role in the discovery of new common drought SSR markers available for diversity analysis among the three different gramineae genera. The availability of molecular markers that are tightly linked to drought trait in different genera is a prerequisite for undertaking molecular breeding in plants which remarkably speeds up the efficiency and preciseness of breeding program over the traditional breeding. Generally, only RM55 primer succeeded in showing distinct differences between tolerant and sensitive cultivars in all the three genera; rice, wheat and barley. Thus, it could be considered the most important marker associated with drought in the three genera and could be considered as the potential marker for use in marker-assisted breeding.

SUMMARY

The negative effects of drought stress induced by mannitol on three different plant genera of gramineae were studied. Identification of SSR markers for drought tolerance in gramineae was an important goal for this study. Four cultivars from three different plant genera of gramineae (rice, wheat and barley), two tolerant and two sensitive from each genus were treated with three different mannitol concentrations (125, 250 and 500 mM) in addition to control and incubated for three weeks in room temperature. Data were collected for three drought related important seedling traits (root length, shoot length and germination%). Significant differences for genotypes were revealed by analysis of variance for root length and shoot length in rice and barley cultivars. While in rice, wheat and barley, significant differences were observed in mannitol treatments. On the other hand, significant differences for shoot length only in rice cultivars were recorded for the interaction between genotypes and treatments. Data showed that mean performances of the studied traits decreased linearly in the three plant genera with increasing mannitol concentrations. The highest values of root length, shoot length and germination% were rec-

orded for the control treatments in rice and barley, while the lowest values for these traits were identified in the highest concentration of mannitol treatment (500 mM) in rice and wheat. Based on the molecular analysis using 13 SSR primers associated with drought tolerance in rice, evaluation of genetic diversity of 12 studied cultivars produced clear and polymorphic banding patterns. In general, 73 polymorphic alleles with an average of 5.62 alleles per primer and sizes varied from 113 to 692 bp were amplified among the 12 studied cultivars. Polymorphic information content (PIC) values ranged from 0.22 to 0.47 with an average of 0.33 per primer. Only RM55 primer succeeded in showing distinct differences between tolerant and sensitive cultivars in all the three genera; rice, wheat and barley. Using cluster analysis, the 12 studied genotypes were divided into two main clusters which relatively corresponding with drought response. This study represents a significant addition that can play important role in the discovery of new common drought SSR markers available for diversity analysis among the three different plant genera.

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Table (1): The used genotypes from three different plant genera of gramineae and their reaction for drought stress.

Genotypes	Genus		
	Rice	Wheat	Barley
1	Giza-179 (T)	Sakha-95 (T)	Giza-126 (T)
2	E. Hybrid 1 (T)	Misr-2 (T)	Giza-2000 (T)
3	Giza-177 (S)	Gemmiza-11 (S)	Giza-118 (S)
4	Sakha-105(S)	Sids-12 (S)	Giza-121 (S)

T: drought tolerant, S: drought sensitive

Table (2): Name and nucleotide sequence of SSR primers used in this study.

Sr. No.	Primer	Sequence (5'→3')	
		Forward	Reverse
1	RM55	CCGTCGCCGTAGTAGAGAAG	TCCCGGTTATTTTAAGGCG
2	RM451	GATCCCCTCCGTCAAACAC	CCCTTCTCCTTTCCTCAACC
3	RM518	CTCTTCACTCACTCACCATGG	ATCCATCTGGAGCAAGCAAC
4	RM201	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA
5	RM261	CTACTTCTCCCCTTGTGTCG	TGTACCATCGCCAAATCTCC
6	RM09	GGTGCCATTGTCGTCCTC	ACGGCCCTCATCACCTTC
7	RM3825	AAAGCCCCCAAAAGCAGTAC	GTGAAACTCTGGGGTGTTCG
8	RM545	CAATGGCAGAGACCCAAAAG	CTGGCATGTAACGACAGTGG
9	RM228	CTGGCCATTAGTCCTTGG	GCTTGCGGCTCTGCTTAC
10	RM301	TTACTCTTTGTGTGTGTGTGAG	CTACGACACGTCATAGATGACC
11	RM553	AACTCCACATGATTCCACCC	GAGAAGGTGGTTGCAGAAGC
12	RM302	TCATGTCATCTACCATCACAC	ATGGAGAAGATGGAATACTTGC
13	RM20A	ATCTTGTCCCTGCAGGTCAT	GAAACAGAGGCACATTTCATTG

Table (3): Mean squares for root length, shoot length and germination percentage (G %) of the three different plant genera.

SOV	DF	MS								
		Rice			Wheat			Barley		
		Root length	Shoot length	G%	Root length	Shoot length	G%	Root length	Shoot length	G%
Genotypes (G)	3	15.42 **	10.39 **	2328.6 **	4.16	2.70	761.54	11.94 **	51.72 **	1815.4 **
Treatments (T)	3	19.29 **	24.64 **	1899.4 **	41.49 **	66.30 **	611.31	17.27 **	73.59 **	2338.3 **
G×T	9	1.73	6.64 *	252.98	5.52	7.79	106.70	1.09	5.44	592.83
Error	32	1.11	0.24	157.16	4.35	5.11	255.70	1.34	2.71	313.40

*,** significance differences at 5% and 1% levels of probability, respectively.

Table (4): Mean performance of root length, shoot length and germination % for seedlings of the studied rice, wheat and barley cultivars treated with 125, 250 and 500 mM mannitol compared to control treatment after 21 days.

Trait	Genus	Genotype	Treatments (mannitol concentration)				Overall mean
			Control	125 mM	250 mM	500 mM	
Root length (cm)	Rice	Giza-179	2.43	4.09	2.31	0.79	2.40^{b}
		E. Hybrid 1	5.46	4.72	3.10	1.36	3.66^{a}
		Giza-177	2.18	1.31	0.39	0.04	0.98^{c}
		Sakha-105	3.74	1.87	0.92	0.49	1.76^{bc}
	Overall mean		3.46^{a}	3.00^{a}	1.68^{b}	0.67^{c}	2.20
	Wheat	Sakha-95	8.05	2.62	2.04	1.58	3.57^{a}
		Misr-2	5.26	3.29	0.37	1.36	2.57^{a}
		Gemmiza-11	3.70	2.34	3.11	1.48	2.66^{a}
		Sids-12	5.38	5.44	2.96	0.96	3.68^{a}
	Overall mean		5.60^{a}	3.43^{b}	2.12^{bc}	1.35^{c}	3.12
	Barley	Giza-126	5.76	2.76	3.23	2.33	3.52^{a}
		Giza-2000	2.62	1.48	0.37	0.63	1.27^{c}
		Giza-118	3.19	2.04	0.98	0.23	1.61^{bc}
		Giza-121	3.50	3.08	2.46	0.48	2.38^{b}
	Overall mean		3.77^{a}	2.34^{b}	1.76^{bc}	0.92^{c}	2.20
Shoot length (cm)	Rice	Giza-179	2.57	3.32	2.32	0.84	2.26^{b}
		E. Hybrid 1	3.81	3.65	2.66	1.12	2.81^{a}
		Giza-177	1.46	1.16	0.56	0.29	0.87^{c}
		Sakha-105	2.14	1.18	0.51	0.53	1.09^{c}
	Overall mean		2.50^{a}	2.33^{a}	1.51^{b}	0.70^{c}	1.76
	Wheat	Sakha-95	6.95	5.23	4.68	2.61	4.87^{a}
		Misr-2	7.27	7.17	0.98	3.13	4.64^{a}
		Gemmiza-11	6.73	5.85	4.63	1.09	4.57^{a}
		Sids-12	7.35	7.47	6.94	0.67	5.61^{a}
	Overall mean		7.08^{a}	6.43^{a}	4.31^{b}	1.87^{c}	4.92
	Barley	Giza-126	9.13	5.85	6.90	3.45	6.33^{a}
		Giza-2000	5.08	2.57	0.53	0.74	2.23^{b}
		Giza-118	6.58	2.52	0.69	0.27	2.51^{b}
		Giza-121	8.07	7.48	5.85	0.57	5.49^{a}
	Overall mean		7.21^{a}	4.60^{b}	3.49^{b}	1.26^{c}	4.14
Germination (%)	Rice	Giza-179	90.00	83.33	90.00	56.67	80.00^{b}
		E. Hybrid 1	96.67	96.67	100.0	76.67	92.50^{a}
		Giza-177	86.67	66.67	43.33	16.67	53.33^{c}
		Sakha-105	93.33	73.33	60.00	56.67	70.83^{b}
	Overall mean		91.67^{a}	80.00^{ab}	73.33^{b}	51.67^{c}	74.17
	Wheat	Sakha-95	66.67	50.00	50.00	30.00	49.17^{ab}
		Misr-2	36.67	30.00	26.67	33.33	31.67^{b}
		Gemmiza-11	60.00	66.67	46.67	43.33	54.17^{a}
		Sids-12	33.33	53.33	26.67	13.33	31.67^{b}
	Overall mean		49.17^{ab}	50.00^{a}	37.50^{ab}	30.00^{b}	41.67
	Barley	Giza-126	76.67	53.33	50.00	83.33	65.83^{a}
		Giza-2000	60.00	43.33	3.33	13.33	30.00^{b}
		Giza-118	86.67	60.00	23.33	3.33	43.33^{b}
		Giza-121	46.67	43.33	30.00	16.67	34.17^{b}
	Overall mean		67.50^{a}	50.00^{a}	26.67^{b}	29.17^{b}	43.33

Values followed by different letters are significantly differd at 0.05 probability level .

Table (5): Details of thirteen SSR primers including expected size, number and size of presented alleles, range of allele sizes and PIC values among 12 gramineae genotypes.

No.	Primers	Expected size (bp)	Presented alleles (bp)	Range of size (bp)	Number of alleles	Size of presented alleles (bp)												PIC value
						Rice				Wheat				Barley				
						Giza-179	E. Hybrid 1	Giza-177	Sakha-105	Sakha-95	Misr-2	Gemmiza-11	Sids-12	Giza-126	Giza-2000	Giza-118	Giza-121	
1	RM55	226	326	108	8				326	326	326					326	326	0.36
			316					316				316		316				
			309												309	309	309	
			300			300	300			300								
			296								296							
			278					278	278	278				278	278			
			256							256	256							
			218												218	218	218	
2	RM451	207	207	7	2			207	207			207	207	207	207	207	207	0.47
			200			200	200			200	200						200	
3	RM518	171	230	59	5			230	230									0.27
			221				221			221	221	221	221	221	221	221	221	
			206			206												
			179					179	179									
			171			171	171											
4	RM201	158	158	-	1			158	158									0.22
5	RM261	125	125	3	2			125	125				125					0.33
			122			122	122											
6	RM09	136	158	25	4						158	158	158					0.31
			149			149												
			136			136	136					136	136					
			133						133	133								

Table (5): Cont.

7	RM3825	147	283	131	8					283	283	283	283					0.33
			263			263	263	263	263							263		
			229												229			
			225														225	
			200					200	200							200		
			165												165	165		
			156			156	156							156	156			
			152					152	152									
8	RM545	226	257	91	9				257			257		257	257			0.31
			241			241	241	241										
			233			233	233		233						233			
			226					226			226	226						
			204								204	204						
			197									197			197			
			180											180				
			172										172					
			166								166							
9	RM228	154	692	577	12					692		692					692	0.30
			474					474				474						
			355						355					355	355			
			327							327		327				327		
			300													300		
			267							267		267						
			234							234							234	
			213													213		
			182									182						
			159			159	159					159						
			121					121	121							121		
			115			115	115			115								
10	RM301	153	348	195	3					348	348							0.28
			156			156	156											
			153					153	153									

Table (5): Cont.

11	RM553	162	627 580 431 422 407 389 154	473	7	431 407 154	627 580 431 407 154	422 389 154	627 580 422 389 154	580 431 407	422 389	627 580 431 407	431	580	580	627 580	580	0.43
12	RM302	156	198 181 165 128 126	72	5	165 126	165 126	128	128	198		181						0.23
13	RM20A	140	274 260 232 214 203 163 113	161	7	260 232	260 232	274, 203	274 203	163 113	163 113	163 113	163 113	260	260			0.36
Total		-	692-113	-	73	21	22	23	26	20	15	23	10	10	14	14	10	-
Average		-	-	-	5.62	23				17				12				0.33

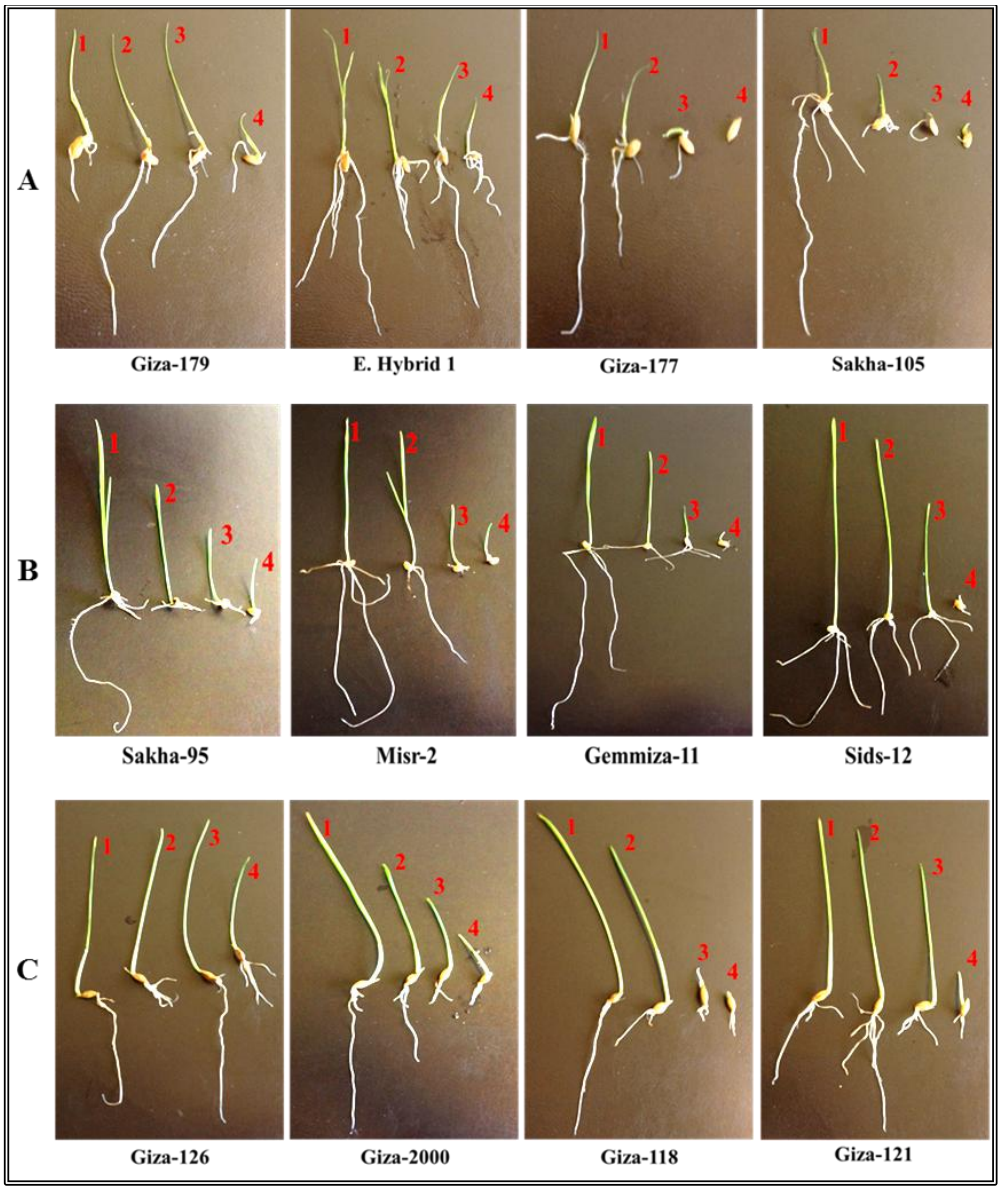

Fig. (1): Effect of mannitol induced drought stress on root and shoots lengths of the studied rice (A), wheat (B) and barley (C) cultivars.

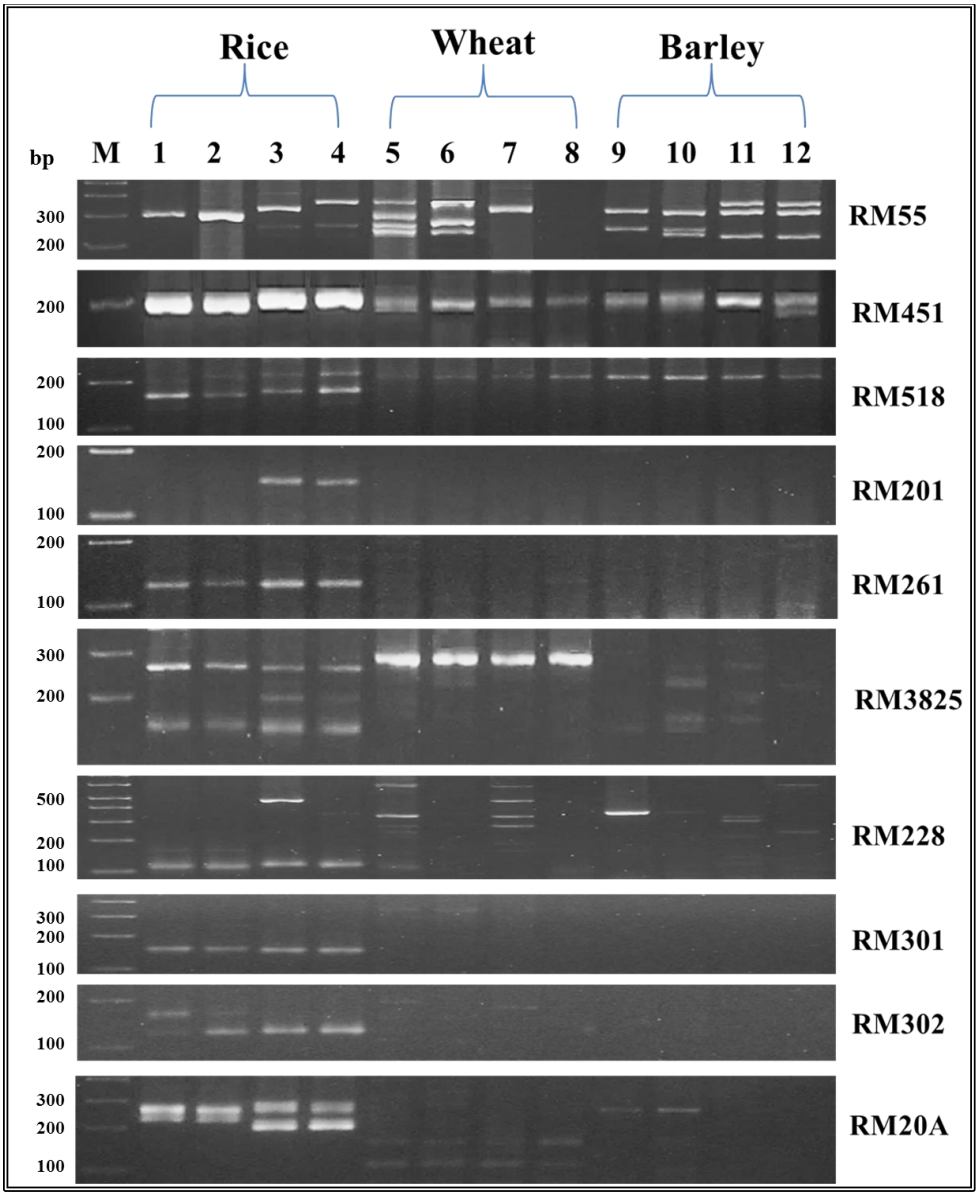

Fig. (2): Agarose gels showing allelic variations among the 12 gramineae genotypes with RM55, RM451, RM518, RM201, RM261, RM3825, RM228, RM301, RM302 and RM20A SSR primers. M: 100 bp DNA ladder, 1: Giza-179, 2: E. Hybrid 1, 3: Giza-177, 4: Sakha-105, 5: Sakha-95, 6: Misr-2, 7: Gemmiza-11, 8: Sids-12, 9: Giza-126, 10: Giza-2000, 11: Giza-118 and 12: Giza-121.

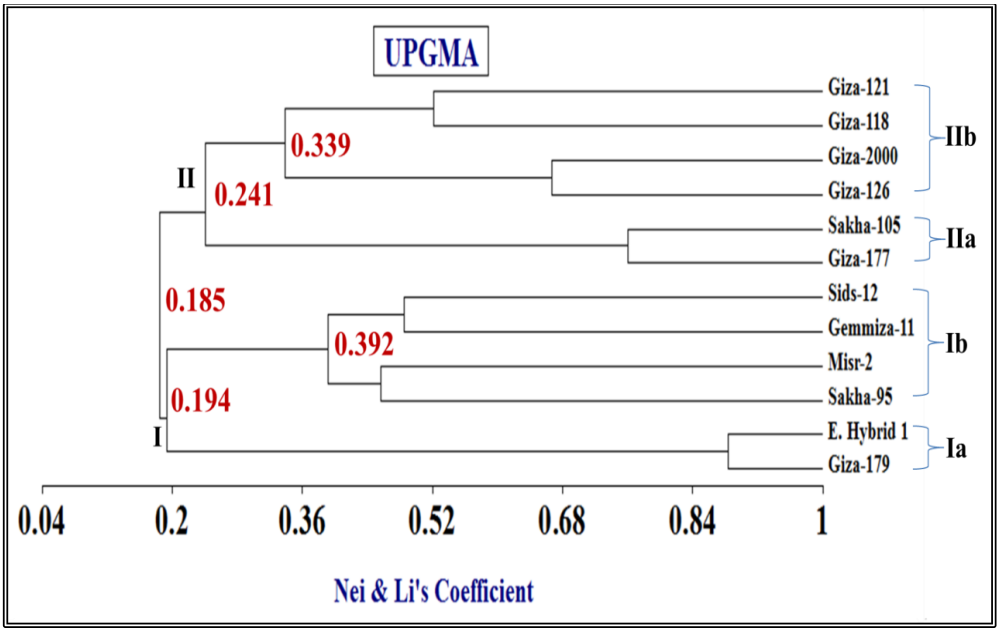

Fig. (3): Dendrogram generated from 13 SSR primers showed phylogenetic relationship among 12 different cultivars belonged to three diverse plant genera; rice, wheat and barley.