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SCREENING AND MOLECULAR ANALYSIS OF SOME RICE (*Oryza sativa* L.) GENOTYPES FOR DROUGHT TOLERANCE AT SEEDLING STAGE

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ABSTRACT

Drought is one of the major abiotic constraints for rice grown under the rain-fed condition and causes a substantial reduction in yield. It has now become a severe threat to food security in the developing world including Bangladesh. We used 30 rice genotypes of diverse geographical origins including 2 drought-tolerant check varieties, NERICA-1 and NERICA-10, to screen under drought stress and to evaluate simple sequence repeat (SSR) markers for identifying drought-tolerant rice genotypes at the seedling stage. The screening was done by a hydroponic method using polyethylene glycol (PEG 6000). Three drought stress levels, -0.66, -1.03 and -1.48 bars of water potential ware imposed using 6%, 8% and 10% PEG, respectively. Control was maintained using the only nutrient solution. Genotypes were scored based on IRRI standard evaluation score for visual stress injury and data were collected on root length, shoot length and root/shoot ratio at three different levels of treatments along with control. A total of 86 alleles were detected among 30 rice genotypes by using 20 SSR markers. The polymorphic information content (PIC) values ranged from 0.11(RM28502) to 0.84 (RM562) with an average of 0.58 and gene diversity value ranged from 0.88 (RM25022) to 0.23 (RM28502) with an average of 0.63. The SSR markers successfully separated drough-tolerant varieties in four different groups. Combining molecular assessment with morphological findings it was accomplished that FR13A was highly drought-tolerant, Sadamota was moderate drought-tolerant and Baichmon, Q-31, BRRI dhan48, Binadhan-17 and BRRI dhan46 were low drought tolerant genotypes. This information could be used for the selection of suitable parents to develop drought-tolerant rice varieties using marker-assisted back crossing program.

Keywords: Drought; *In vitro* screening; PEG; Rice; SSR markers
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INTRODUCTION

Bangladesh has been cultivating rice for a very long time as its agro-climatic conditions are suitable for growing rice year-round. However, compared to the other rice-growing countries, the national average rice yield is much lower (2.74 to 3.74 t/ha) in Bangladesh (BRKB, 2019). Bangladesh has a tropical climate with considerable variation in climatic factors like temperature and rainfall (BBS, 2018). As a result, abiotic stresses resulting from drought, cold and heat are some of the major environmental factors which affect plant growth and production. In Bangladesh, rice production largely depends on monsoon rains. Therefore, drought under rain-fed conditions causes a considerable reduction in crop production and quality on a large scale. However, as rice is direct-seeded and grown under rain-fed upland conditions and it could suffer drought any time from the seedling to reproductive stages, but the yield potential of these rice varieties is very low (Shelley *et al*., 2016). Developing high yielding rice plants tolerant to drought could increase crop productivity. To develop a high yielding rice plant, effective selection approach like proper drought screening, is necessary which clearly distinguishes drought-susceptible lines from drought-tolerant lines. Polyethylene glycol (PEG), a high molecular weight osmoticum, can create osmotic pressure which imitates soil drying. The *in vitro* screening of rice seedlings using PEG is a simple, rapid and preliminary bioassay and can be used in mass screening for evaluating seedling of rice genotypes under drought stress (Sabesan and Saravanan, 2016). The positive adaptive responses of rice varieties towards drought stress induced by PEG 6000-8000 can be used in the genetic improvement of drought resistance rice breeding programs (Swapna and Shylaraj, 2017). Phenotypic markers are influenced by the environmental factors and expose low polymorphism amongst the genotypes (Fufa *et al*., 2005). But screening using a hydroponic system could efficiently reduce environmental effects, as it is free from soil associated stress problems (Bhowmik *et al*., 2009). However molecular markers, a gift of modern biotechnology is independent of environmental factors, show high polymorphism amongst the genotypes and allow a swift analysis of a huge number of loci scattered

throughout the plant genome and so best suited for efficient selection and evaluation of plant materials (Chakravarthi and Naravaneni, 2006).

Among various molecular markers, simple sequence repeat (SSR) markers have been proved to be ideal for making genetic maps (McCouch *et al*., 2002; Kirungu *et al*., 2018), studying genetic diversity in germplasm (Rahman *et al*., 2012; Islam *et al*., 2018b), DNA fingerprinting (Ma *et al*., 2011), cultivar identification (Ping *et al*., 2012; Li *et al*., 2013), plant breeding application (Ahmad *et al*., 2015), etc. Combined information gained through phenotypic characterization using screening and molecular characterization using SSR markers could be a superior approach for the planning of rice breeding programs aimed to develop high yielding rice plants tolerant to stress (drought, salinity, heat, etc.). The objective of this study was to screen rice genotypes under drought stress and to evaluate SSR markers to identify drought-tolerant rice genotypes at the seedling stage.

MATERIALS AND METHODS

Genetic materials: Experimental material consisted of 30 rice genotypes involving 5 modern varieties, 8 advance breeding lines and, 17 land races including two drought-tolerant lines (NERICA-1 and NERICA-10; Table 1). All rice genotypes were collected from Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh and Bangladesh Rice Research Institute (BRRI), Gazipur.

Table 1: List of genotypes with their origin and Source of collection.

S. No.	Name of genotypes	Origin of genotypes	Source of collection
1	NERICA-1	Africa	BINA
2	Bourani	Bangladesh	BINA
3	Puita-aizon	Bangladesh	BINA
4	Depu	Bangladesh	BINA
5	PNR-519	India	BINA
6	Bashad	Bangladesh	BINA
7	THDB	Vietnam	BINA
8	PNR-166	India	BINA
9	Surjojuni	Bangladesh	BINA
10	FR13A	India	BINA
11	Ab. Hai	Bangladesh	BINA
12	Moulota	Bangladesh	BINA
13	Gochi	Bangladesh	BINA
14	Sadamota	Bangladesh	BINA
15	Balam	Bangladesh	BINA
16	Baichmon	Bangladesh	BINA
17	Mokbul	Bangladesh	BINA
18	BRRI dhan46	Bangladesh	BRRI
19	Loknath	Bangladesh	BINA
20	Pokkali	India	BINA
21	NERICA-10	Africa	BINA
22	Binadhan-17	Bangladesh	BINA
23	Boira-3	Bangladesh	BINA
24	R-3027	China	BINA
25	E-02	Bangladesh	BINA
26	MV-40	Malaysia	BINA
27	MV-20	Malaysia	BINA
28	Q-31	Malaysia	BINA
29	Lakhai	Bangladesh	BINA
30	BRRI dhan48	Bangladesh	BRRI

BINA: Bangladesh Institute of Nuclear Agriculture
BRRI: Bangladesh Rice Research Institute

Plant culture: Screening experiment was conducted in 2017 in the glass house of Biotechnology Division, Bangladesh Institute of Nuclear Agriculture (Longitude: 24.7232ºN, Latitude: 90.4316ºE) using a hydroponic system following the standard protocol of International Rice Research Institute (Gregorio *et al*., 1997). To

prepare nutrient solution, 1.0 g Yoshida solution (Yoshida *et al*., 1976) and 200mg/L ferrous sulphate were mixed carefully per liter of fresh water. The pH was adjusted to 5.1 using HCl and NaOH. The pH of the solution was monitored daily and maintained around 5.1. We changed the nutrient solution after every seven days as solution volume was reduced due to evaporation and transpiration. At 21 days after sowing, drought stress was applied.

Phenotypic screening of rice genotypes: Drought stress was imposed using PEG 6000 according to Govindaraj *et al*. (2010) and continued for 15 days. Drought stress was imposed at three different concentrations, viz., 6%, 8% and 10% PEG which induced -0.66, -1.03 and -1.48 bars of water potential, respectively. A control (0 bar) was maintained using only nutrient solution.

After 15 days of imposing drought stress, visual score for drought stress symptoms was recorded at mid-day using IRRI standard evaluation system for rice (IRRI, 1980) in 0 to 9 scale (Table 2). Stress data on root length, shoot length and root/shoot ratio were recorded at three different levels of treatment along with the control. The experiment was laid in a Randomized Complete Block (RCB) design with 3 replications.

Table 2. Standard evaluation system for drought stress injury score according to IRRI, (1980).

Drought score	Description
0	No symptoms of stress effects
1	Slight leaf rolling and tip drying
2	Leaf rolling and tip drying extended to 1/4 length in 25% of all leaves (normally the older leaves)
3	Leaf rolling and tip drying extended to 1/4 length or more in at most 50% of all leaves
4	Leaf rolling and tip drying extended to 1/4 length or more in 50% of all leaves with 25% of leaves fully rolled and dried
5	50% of all leaves fully rolled and dried
6	More than 50% but less than 70% of all leaves fully rolled and dried
7	Seventy percent of all leaves fully rolled and dried
8	More than 70% of all leaves fully rolled and dried
9	All plants dead

Genotyping of SSR markers: The DNA extraction was done from the leaf tissues of 21-day old seedlings using acetyltrimethyl ammonium bromide (CTAB) method (Stein *et al*., 2001). Twenty-four SSR markers were initially taken. Among them, 20 microsatellite primers, covering all 12 chromosomes, were considered suitable (based on polymorphism) for Polymerase chain reaction (PCR) amplification and analysis. Detailed information of the selected primers is given in Table 3. PCR was done using, G-STROM, GSI, England, thermo cycler. The PCR cocktail volume was 10 µl which contained, template DNA (diluted) 3 µl, Mg^{2+} free PCR buffer (10X) 1.00 µl, *Taq* DNA polymerase 0.10 µl, dNTPs (10mM) 0.20 µl, Mg^{2+} ions 1.30µL, forward primer and reverse primer 0.50 µL of each and ddH_2O 3.40 µl. The PCR profile used an initial denaturation step for 5 minutes at 94°C followed by three cyclic steps (35 cycles) - 1-minute denaturation at 94°C, 1-minute annealing at 55°C and 2-minute primer elongation at 72°C followed by a single cycle of final elongation at 72°C for 5 minutes and then stored at -20°C. To separate the amplified products, electrophoreses were done at 70V for 1.5-2 hours in 1X Tris-borate-ethylenediaminetetraacetic acid (TBE) buffer using Polyacrylamide gels 10% (w/v). After staining gels in ethidium bromide (25-30 min in dark), gels were visualized in a UVPRO (Uvipro platinum, EU) gel documentation unit.

Table 3. Primer name, chromosomal position, repeat motif, major allele size, allelic variation, number of polymorphic alleles, PIC values and gene diversity values for selected SSR markers used in the study.

Primer	Chromosome	Repeat motif	Major allele bp.	Number of alleles	Polymorphic alleles	PIC value	Gene diversity
RM493	1	$(CTT)_9$	211	5	5	0.70	0.72
RM562	1	$(AAG)_{13}$	243	6	6	0.84	0.86
RM526	2	$(TAAT)_5$	121	3	3	0.62	0.65
RM300	2	$(GTT)_{14}$	240	4	4	0.62	0.64
RM211	2	$(TC)_3A(TC)_{18}$	161	4	4	0.75	0.78
RM5639	3	$(AAG)_{13}$	123	5	5	0.76	0.80
RM6659	4	$(GTT)_{14}$	101	4	4	0.65	0.68
RM252	4	$(CT)_{19}$	216	7	7	0.73	0.85
RM131	4	$(CT)_9$	215	3	3	0.45	0.47
RM334	5	$(CTT)_{20}$	182	6	6	0.79	0.85

RM314	6	$(GT)_8(CG)_3(GT)_5$	118	3	3	0.58	0.61
RM445	7	$(AG)_{12}$	251	3	3	0.27	0.33
RM342	8	$(CAT)_{12}$	141	8	8	0.30	0.44
RM7175	9	$(ATAG)_6$	105	2	2	0.46	0.50
RM25022	10	$(TA)_{45}$	225	7	7	0.75	0.88
RM228	10	$(CA)_6(GA)_{36}$	154	3	3	0.30	0.34
RM26456	11	$(AT)_{11}$	282	3	3	0.80	0.84
RM28502	12	$(GA)_{26}$	155	3	3	0.11	0.23
RM27639	12	$(AT)_{11}$	244	4	4	0.69	0.72
RM277	12	$(GA)_{11}$	124	3	3	0.40	0.47
Total	-	-	-	86	86	-	-
Average	-	-	-	4.3	4.3	0.58	0.63

Data analysis: Molecular weight of each unambiguous band was determined by comparing their migration distance with the 100 base pair DNA ladder using Alpha-Ease FC 5.0 software (Alpha Innotech, USA). The band profiles for each SSR primer were scored for distinct and reproducible bands as present (1) or absent (0). Dendrogram based on Jaccard's similarity coefficient was generated using UPGMA by the dendrogram assembly utility DendroUPGMA (https:// genomes.urv.es/UPGMA/) (Garcia-Vallvé *et al*., 1999). The PIC value was calculated using the formula proposed by Anderson *et al*. (1993).

Analysis of variance (ANOVA) tables were generated from quantitative data and significant means (For root length, shoot length and root/shoot ratio at different level of stresses and control) were compared using LSD test. MSTATE-C software was used to perform all the statistical analysis.

RESULTS

Analysis of variance (ANOVA) of different morphological traits: Statistically significant interaction between drought stress and genotypes was found for all three seedling parameters (shoot length, root length and root/shoot ratio) recorded at 15 days, after imposing drought stress to the 21 days old seedlings (Table 4). The result indicated that genotypes response to different levels of drought stresses were statistically different. Relative drought tolerance among the genotypes varied in terms of our measured morphological parameters.

Table 4: Mean squares values from analysis of variance for 3 morphological traits at different drought stress levels and control among 30 rice genotypes.

Variation	DF	Root	Shoot	Root/Shoot
Replication	2	258.133	94.164	0.002
Stress level(A)	3	437.807**	4311.127*	0.011**
Genotypes(B)	29	79.263**	1045.760**	0.015**
A× *B*	87	5.910*	21.023**	0.003**
Error	238	4.871	23.968	0.001

* P≥ 0.05; **p≥0.01

Visual score of drought stress injury: Significant difference in drought stress injury was observed between the genotypes (Table 5). In the present study, genotypes NERICA-1, NERICA-10 and FR13A showed highly tolerant to tolerant drought injury scores in all the stress conditions; genotype Sadamota showed tolerant to moderately tolerant drought injury scores in all the stress conditions; genotypes Baichmon, BRRI Dhan46, Binadhan-17, Q-31 and BRRI Dhan48 showed tolerant to moderately tolerant drought injury scores at 6% and 8% PEG stress but susceptible scores at 10% PEG stress condition. Whereas, genotypes Puita-aizon, THDB, Balam, Mokbul, Loknath, Boira-3, E-02 and Lakhai showed susceptible to highly susceptible drought injury scores in all the stress conditions (Fig.1).

Effect of water stress on root length: Crop response under drought stress is largely determined by the structure and development of the root system. In the current study, we found that the root length considerably varied with the increase of drought stress and subsequently all of the genotypes (accept NERICA-1 in 6% PEG stress) showed a decrease in root elongation in all the treatments compared to their control (Table 6). The mean root length varied from 11.5 cm (THDB) to 22.16 cm (NERICA-10) in the control condition. In PEG treatments, the root length varied from 8.16 cm (Bashad)

to 20.83 cm (NERICA-10). Though the genotypes PNR-166, Surjojuni, Gochi showed higher root length in control but their root length drastically decreased after PEG stresses. The genotypes NERICA-1, FR13A, Sadamota, Pokkali and NERICA-10 experienced a lower root length reduction at all three stress conditions. The genotypes Baichmon, Q-31 and BRRI dhan48 had lower root reduction rates in 6% PEG and 8% PEG stress conditions

Effect of water stress on shoot length: Shoot response under drought stress plays a vital role in identifying drought-tolerant genotypes. In our study, all rice genotypes showed a decrease in shoot length with an escalation in drought stress (Table 6). The shoot length varied from 45.5 cm (BRRI dhan48) to 89.5 cm (Moulota) in the control condition. In PEG treatments, shoot length varied from 32 cm (BRRI dhan48) to 72.5 cm (Sadamota). The genotypes NERICA-1, PNR-519, FR13A and NERICA-10 showed lower shoot length reduction in all three stress conditions. The genotypes Gochi, Sadamota and Binadhan-17 showed a lower reduction in 6% and 8% PEG stress conditions. The genotypes Bourani, Puita-aizon, Depu, Bashad, Surjojuni, Baichmon, Boira-3, MV-20, MV-40 and Q-31 showed lower shoot length reduction in only 6% PEG stress condition.

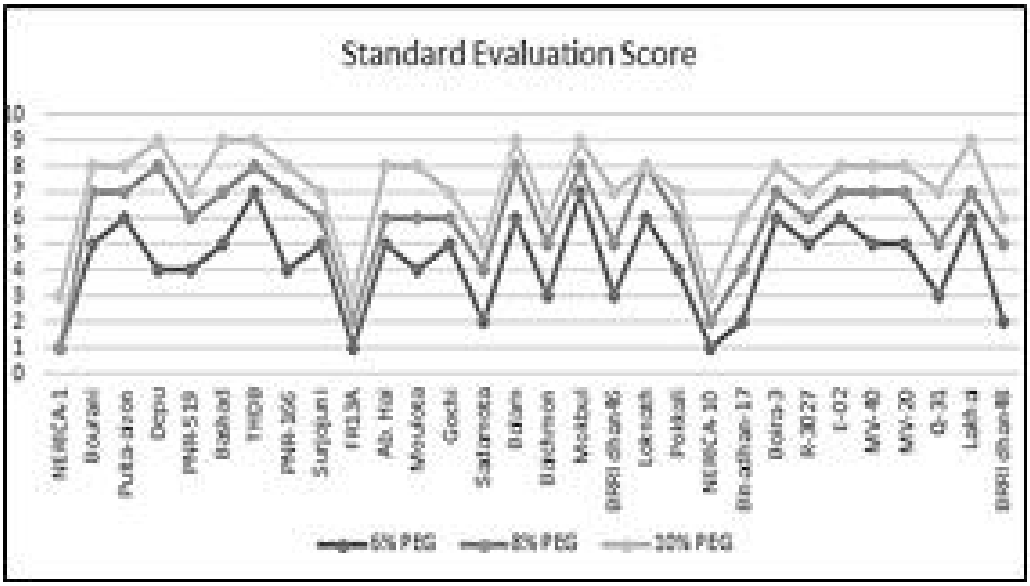

Fig. 1. Visual score of 30 rice genotypes for drought stress injury. Here, score 1 specify highly tolerant, 2-3 specify tolerant, 4-5 specify moderately tolerant and 6-9 specify susceptible to highly susceptible.

Table 5: Mean squares values from analysis of variance for drought injury scores among 30 rice genotypes at 3 different PEG treatments.

Source	DF	6% PEG stress	8% PEG stress	10% PEG stress
Replication	2	1.15	0.14	0.29
Genotypes (A)	29	16.16**	10.55**	12.84**
Error	58	0.60	0.96	0.73

* P≥0.05; **p≥0.01

Effect of water stress on root/shoot ratio: Root/shoot ratio also plays an important role in identifying drought-tolerant genotypes. In the current study, we found substantial variations of the root/shoot ratio among the 30 rice genotypes (Table 6). It was found that in most cases root/shoot ratio increased with the increase of external water stress. The ratio ranged from 0.16 (THDB) to 0.34 (PNR-519) in the control condition. In the stress conditions, root/shoot ratio ranged from 0.17 (Bashad) to 0.39 (NERICA-1) in 6% PEG treatment, 0.17 (PNR-519) to 0.35(NERICA-1) in 8% PEG treatment and 0.17 (PNR-166) to 0.35 (NERICA-1) in 10%PEG treatment. In the control condition, a high root/shoot ratio was exhibited by several genotypes but failed to maintain the same under drought stress conditions. However, the genotypes NERICA-1, NERICA-10, Binadhan-17, FR13A, Baichmon, BRRI dhan46, BRRI dhan48 had higher root/shoot ratio under all three treatments along with the control condition. The genotypes Loknath had a high root/shoot ratio in 8% and 10% PEG treatments

along with control condition while genotype R-3027 had a high root/shoot ratio in 8% and 10% PEG treatments but not in the control condition. The genotypes Sadamota and Q-31 had a high ratio in only 10% PEG stress condition whereas, genotype MV-20 had a high ratio in 10% PEG treatment and control condition. The genotype Puita-aizon had a high root/shoot ratio in 6% PEG treatment and in control condition.

Overall allelic diversity: The 20 primers used in the study generated 86 unambiguous bands with an average of 4.3 bands per primer pair (Table 3). Polymorphic alleles generated by each primer varied from 3 (primer RM314, RM445, RM28502) to 8 (primer RM342) alleles. Average number of alleles was 4.3. The PIC value of the primers varied from 0.11(RM28502) to 0.84 (RM562) and average value was 0.58. Gene diversity value of the primers ranged from 0.88 (RM25022) to 0.23 (RM28502) with an average of 0.63. A linear relationship was observed between gene diversity value and the number of alleles per locus. The DNA profile of RM252 primer, associated with root length and root thickness (Zheng *et al*., 2000) is shown in Figure 2.

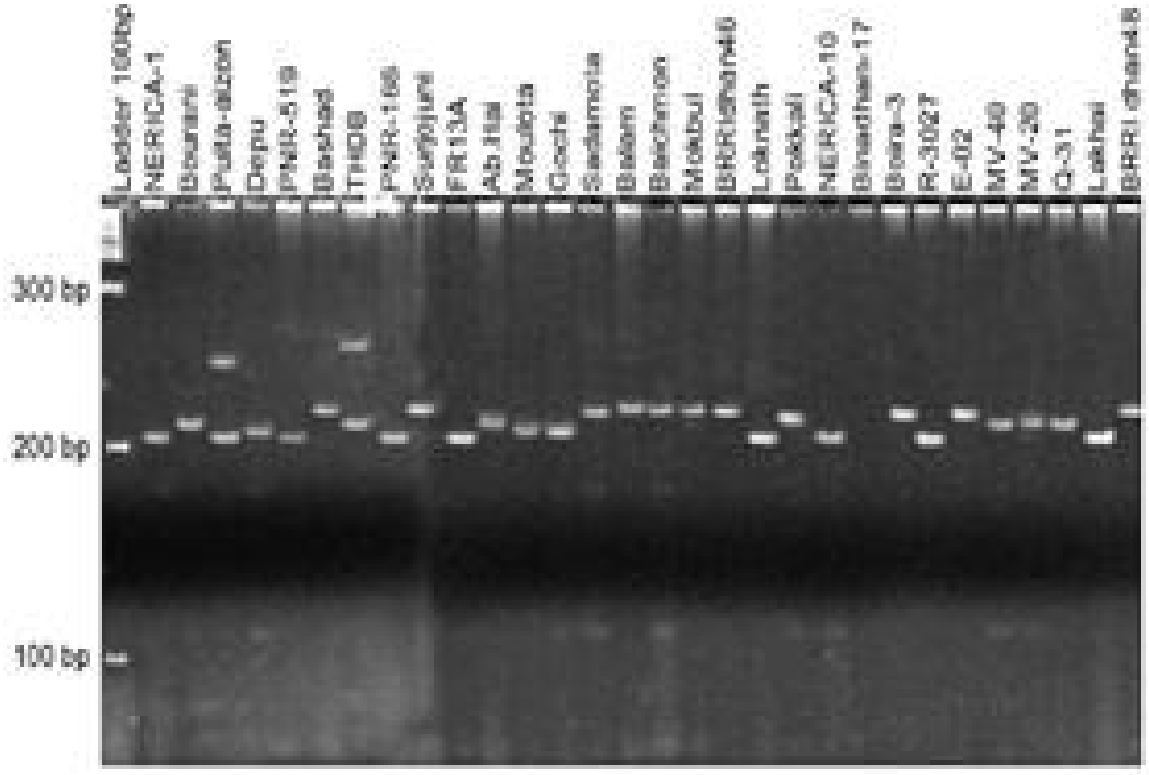

Fig. 2. DNA profile of RM252 primer (associated traits- root length and root thickness) for 30 rice genotypes in 10% (w/v) polyacrylamide gel stained with ethidium bromide.

UPGMA cluster of 30 rice genotypes based on SSR analysis: The UPGMA-based dendrogram was constructed based on the binary data obtained from the SSR marker-based DNA profiles of the sample analyzed (Fig. 3). The correlation coefficient value used to construct the dendrogram was 0.78. The genotypes with genetic similarity clustered together in the dendrogram. We observed two major clusters where cluster II contained most of the genotypes. Our two check varieties NERICA-1, NERICA-10 along with FR13A formed Cluster I. Other 27 genotypes formed cluster II which was divided into eight sub-clusters. Sub-cluster I contained only one genotype, Sadamota. Sub-cluster II consisted of five genotypes namely, Puita-aizon, Bourani, Loknath, Mokbul and Depu. Sub-cluster III consisted of four genotypes namely, PNR-519, PNR-166, Surjojuni and Bashad. Sub-cluster IV consisted of six genotypes namely, Baichmon, Boira-3, BRRI dhan46, E-02, MV-20 and MV-40. Sub-cluster V consisted of two genotypes namely, R-3027 and Gochi. Sub-cluster VI consisted of five genotypes namely, Binadhan-17, Pokkali, BRRI dhan48, Lakhai and Q-31. Sub-cluster VII consisted of two genotypes namely, THDB and Ab. Hai and finally Sub-cluster VIII consisted of two genotypes namely, Balam and Moulota.

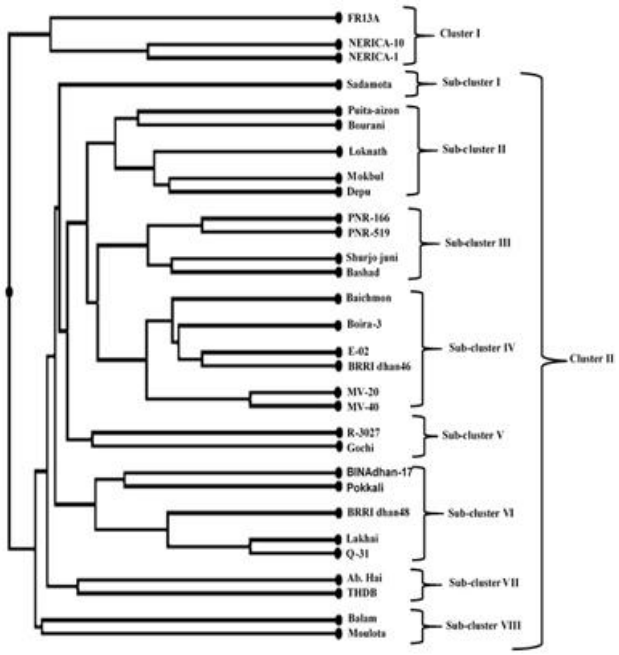

Fig. 3. UPGMA cluster dendrogram showing the genetic relationship among 30 genotypes based on 20 SSR markers. Jaccard's similarity coefficient was used to construct the dendrogram and Cophenetic Correlation Coefficient value was 0.783.

DISCUSSION

The present study was conducted for the screening of thirty rice genotypes from diverse geographical origin to evaluate drought tolerance at the seedling stage, and molecular analysis based on simple sequence repeat (SSR) markers, was used to identify drought-tolerant rice genotypes at the seedling stage. In the past, for measuring drought linked physiological traits, field trials had been used, which was labor and time-intensive process. But PEG can induce water stress in the plant in a comparatively controlled manner by changing the osmotic potential of nutrient solution, suitable for experimental protocols (Lagerwerff *et al*., 1961). The *in vitro* screening of rice seedlings using PEG is a simple, rapid and preliminary bioassay and used in the previous plant studies conducted by El-Tayeb and Hassanein (2000); Al-Bahrany (2002); Biswas *et al*. (2002); Joshi *et al*. (2011). Screening at the seedling stage is a rapid process based on the simple criterion and devoid of difficulties associated with the vegetative and reproductive stage and so is acceptable (Gregorio *et al*., 1997).

Visual scores of drought stress injury are considered as alternative and reliable approach to measure drought tolerance and oxidative damage in plants, as they reflect the correlation of plant tissue dehydration with its relative water content **(**Ingram and Bartels, 1996; Cabuslay *et al*., 2002; Fen *et al*., 2015). Leaf rolling, an acclimation response of rice, is thought to be one of the best criteria to estimate the levels of drought tolerance (Pandey and Shukla, 2015). Plants use leaf rolling to create a microclimate to reduce the transpiration rate and withstand drought stress (Kadioglu and Terzi, 2007; Kadioglu *et al*., 2012). There is also a positive correlation of leaf rolling with drought score, proline accumulation and chlorophyll content (Swapna and Shylaraj, 2017; Islam *et al*., 2018a). According to the standard evaluation score, Genotypes NERICA-1, NERICA-10 and FR13A showed high tolerance to tolerance and Genotype Sadamota showed tolerance to moderate tolerance depending on high (8% and 10% PEG) to low (6% PEG) stress levels. Genotype BRRI Dhan48, BRRI Dhan46, Binadhan-17, Q-31 and Baichmon showed tolerance to moderate tolerance in 6% and 8% PEG stress, respectively but susceptibility in 10% PEG stress.

In the current study, we found that most of the genotypes showed a decrease in root elongation in all the treatments compared to their control (Table 6). The genotypes having the capability of deep rooting with large xylem size can acquire much water from deeper soil (Yoshida and Hasegawa, 1982). So, genotypes with deep root system is a desirable trait of drought tolerance. In the present study, compared to the tolerant check NERICA-1 and NERICA-10 genotypes, FR13A, Sadamota, Pokkali showed lower root length reduction rate at all three stress conditions and genotypes Baichmon, Q-31 and BRRI dhan48 showed lower root length reduction rate in 6% PEG and 8% PEG stress

conditions. Which implies that through profuse and elongated root system of these genotypes uptake more nutrients from the growth medium, and thus helps to withstand under ambient drought condition. Reduction in the root length with the increase of the external water stress was also reported by Pandey and Shukla (2015); Akte *et al*. (2016). Shoot length variation under water stress can also assist the breeder to select the superior rice genotypes against drought. In our study, we observed shoot length decreased with the escalation of drought stress (Table 6). Which might be an adaptive strategy for plant to avoid transpiration-induced water loss. Though shoot and the aerial portions of the rice plant contain the most profitable parts of the crops, these parts are mostly affected by water stress. Because of some modifications such as restoration of water potential gradient via osmotic shift and escalation in loosening capability of the cell wall, root growth is usually maintained while shoot growth is inhibited under low to mild water deficit (Hsiao and Xu, 2000). Compared to the tolerant check NERICA-1 and NERICA-10, the genotypes PNR-519 and FR13A showed lower shoot length reduction in all three stress conditions whereas the genotypes Gochi, Sadamota and Binadhan-17 showed a lower shoot length reduction in 6% and 8% PEG stress conditions. A similar reduction rate with the increase of drought stress was also reported by Sokoto and Muhammad (2014); Madabula *et al*. (2016). Under laboratory condition shoot and root length reduction in response to drought stress had also been reported by Sabesan and Saravanan (2016). In the contrary, we found that in most cases root/shoot ratio increased with the increase of external water stress (Table 6). Govindaraj *et al*. (2010); Xu *et al*. (2015) also reported a reversal relationship of root/shoot ratio with external water stress. Compared to the tolerant check NERICA-1 and NERICA-10, the genotypes Binadhan-17, FR13A, Baichmon, BRRI dhan46, BRRI dhan48 showed higher root/shoot ratio under stress conditions along with control condition. In our observation some genotypes, viz., THDB, Balam, Boira-3, Pokkali showed comparatively a lower root reduction rate but got high drought injury scores compared to the other studied genotypes. The reason for this fact maybe they comparatively had a low root/shoot ratio than the other genotypes. So, rice genotypes having a higher root/shoot ratio upon drought stress are much desired for breeding purposes. A higher root/shoot ratio was stated as an indicator of drought-tolerant genotypes by Samson *et al*. (2002); Wang and Yamauchi (2006); Gowda *et al*. (2011).

Among the 24 SSR primers 20 showed polymorphism and unambiguous bands among genotypes. The mean PIC value recorded was 0.58. Although this value was higher than that determined by Seetharam *et al*. (2009) in 30 rice genotypes in India (0.46), but it was lower than 0.77 determined by Afiukwa *et al*. (2016) in 30 rice varieties and landraces cultivated in Nigeria and 0.75 determined by Borba *et al*. (2009) in 242 rice lines and cultivar accessions. According to DeWoody *et al*. (1995) the marker having PIC value higher than 0.5 is informative. As the mean PIC value (0.58) of our study was higher than 0.5 we could deduce that most of the marker used in the current study was highly informative. On the other hand, average gene diversity value (0.63) observed in our study correlates with the findings of Shakil *et al.* (2015); Pérez-Almeida *et al.* (2019). We also found a linear relationship between gene diversity value and allele number, which was also supported by the findings of Herrera *et al.* (2008); Rana *et al*. (2018). Based on PIC value and gene diversity value, marker RM562 would be best in screening our 30 studied genotypes followed by RM26456, RM334, RM5639, RM211, RM25022, RM252, RM493, RM27639, RM6659, RM314, RM526, and RM300.

The UPGMA-based dendrogram was constructed from SSR markers successfully separated different drought-tolerant genotypes. Cluster I contained genotype FR13A which showed a high level of drought tolerance in morphological screening along with our two tolerant checks NERICA-1 and NERICA-10. Genotype Sadamota which showed a moderate level of drought tolerance, alone formed sub-cluster I. The genotype Baichmon in sub-cluster IV showed moderate drought tolerance while the genotypes Boira-3 and BRRI dhan46 in this sub-cluster showed low drought tolerance in the morphological study. Genotypes BRRI dahn48 and Q-31 in sub-cluster VI showed moderate drought tolerance while the genotypes Binadhan-17 and Pokkali showed low drought tolerance in the morphological study. In the current study, comparing these two diversity analysis methods, the highest level of diversity between the studied genotypes was provided by molecular analysis. SSR markers utilized in this study presented adequate power of resolution to categorize between genotypes which could serve as a potential tool in the identification and characterization of drought-tolerant cultivars from various sources. Beyene *et al*. (2005); Ali *et al*. (2014) also reported that molecular diversity gives a significantly higher estimation of genetic diversity compared to physiological or morphological methods.

Table 6. Performance of root length, shoot length with their variation (%) compared to control and root/shoot (ratio) of 30 rice genotypes under drought stress and in control condition at seedling stage using a modified hydroponic system.

Genotypes	Root							Shoot						Root/Shoot			
	Cont.*cm	6% PEG stress		8% PEG stress		10% PEG stress		Cont. cm	6% PEG stress		8% PEG stress		10% PEG stress		Cont.	6% PEG stress	8% PEG stress	10% PEG stress
		RL* cm	Var*%	RL_{cm}	Var%	RL_{cm}	Var%		SL* cm	Var%	SL_{cm}	Var%	SL_{cm}	Var%				
NERICA-1	15.67	17.33	+11.00	15.50	-01.00	14.83	-05.00	47.50	45.00	-05.00	43.83	-08.00	42.50	-11.00	00.33	00.39	00.35	00.35
Bourani	16.67	11.67	-30.00	10.33	-38.00	08.33	-50.00	52.83	44.50	-16.00	41.16	-22.00	40.50	-23.00	00.32	00.26	00.25	00.21
Puita-aizon	16.83	14.33	-15.00	11.33	-33.00	10.67	-37.00	56.50	49.50	-12.00	45.83	-19.00	41.50	-27.00	00.30	00.29	00.25	00.26
Depu	18.33	14.33	-22.00	14.67	-20.00	15.33	-16.00	77.83	65.50	-16.00	60.83	-22.00	58.50	-25.00	00.24	00.22	00.24	00.26
PNR-519	18.33	11.67	-36.0	08.33	-55.00	09.33	-49.00	54.33	47.00	-13.00	48.50	-11.00	46.50	-14.00	00.34	00.25	00.17	00.20
Bashad	17.33	09.67	-44.00	07.83	-55.00	08.16	-53.00	64.16	55.83	-13.00	44.33	-31.00	43.50	-32.00	00.27	00.17	00.18	00.19
THDB	11.50	09.67	-16.00	09.50	-17.00	09.00	-22.00	70.83	52.50	-26.00	50.83	-28.00	45.33	-36.00	00.16	00.18	00.19	00.20
PNR-166	20.67	12.33	-40.00	11.67	-44.00	09.00	-56.00	64.33	52.33	-19.00	51.50	-20.00	51.83	-19.00	00.32	00.24	00.23	00.17
Surjojuni	20.50	12.67	-38.00	10.00	-51.00	09.00	-56.00	66.50	59.50	-11.00	50.83	-24.00	47.50	-29.00	00.31	00.21	00.20	00.19
FR13A	19.33	18.33	-05.00	17.50	-09.00	17.33	-10.00	70.83	66.50	-06.00	64.33	-09.00	61.50	-13.00	00.27	00.28	00.27	00.28
Ab. Hai	14.50	09.00	-38.00	10.00	-31.00	8.83	-39.00	58.50	43.50	-26.00	42.50	-27.00	39.00	-33.00	00.25	00.21	00.24	00.23
Moulota	17.50	16.00	-09.00	14.16	-19.00	13.50	-23.00	89.50	71.50	-20.00	62.83	-30.00	60.33	-33.00	00.20	00.22	00.23	00.22
Gochi	20.67	14.00	-32.00	12.50	-40.00	12.00	-42.00	73.16	65.50	-10.00	63.83	-13.00	56.50	-23.00	00.28	00.21	00.20	00.21
Sadamota	18.83	17.16	-09.00	17.50	-07.00	16.50	-12.00	80.83	72.50	-10.00	68.50	-15.00	60.33	-25.00	00.23	00.24	00.26	00.27
Balam	17.33	14.50	-16.00	14.16	-18.00	13.83	-20.00	82.50	66.50	-19.00	64.33	-22.00	60.83	-26.00	00.21	00.22	00.22	00.23
Baichmon	19.50	17.50	-10.00	16.00	-18.00	15.50	-21.00	68.50	61.33	-10.00	56.33	-18.00	52.50	-23.00	00.28	00.29	00.28	00.30
Mokbul	15.33	10.50	-32.00	09.50	-38.00	08.50	-45.00	52.83	43.33	-18.00	40.83	-23.00	39.33	-26.00	00.29	00.24	00.23	00.22
BRRI dhan46	15.67	13.00	-17.00	12.33	-21.00	12.00	-23.00	53.16	42.50	-20.00	40.50	-24.00	37.33	-30.00	00.29	00.31	00.30	00.32
Loknath	16.50	11.50	-30.00	11.50	-30.00	10.83	-34.00	55.33	45.16	-18.00	43.16	-22.00	38.50	-30.00	00.30	00.25	00.27	00.28
Pokkali	14.33	12.83	-10.00	12.16	-15.00	11.83	-17.00	64.33	52.83	-18.00	50.50	-21.00	46.33	-28.00	00.22	00.24	00.25	00.26
NERICA-10	22.16	20.83	-06.00	19.50	-12.00	18.33	-17.00	67.50	64.25	-05.00	61.16	-09.00	59.50	-12.00	00.33	00.32	00.32	00.31
Binadhan-17	17.83	14.00	-21.00	13.83	-22.00	13.16	-26.00	60.33	53.50	-11.00	50.50	-16.00	47.50	-21.00	00.30	00.28	00.29	00.28
Boira-3	15.50	13.16	-15.00	12.33	-20.00	12.00	-23.00	72.33	60.50	-16.00	57.83	-20.00	54.50	-25.00	00.21	00.21	00.21	00.22
R-3027	13.16	11.00	-16.00	10.83	-18.00	10.00	-24.00	51.83	41.83	-19.00	38.50	-26.00	36.33	-30.00	00.25	00.26	0.028	00.28
E-02	14.33	10.75	-25.00	11.50	-20.00	11.50	-20.00	65.50	52.16	-20.00	48.50	-26.00	45.83	-30.00	00.22	00.21	00.24	00.25
MV-40	14.16	10.50	-26.00	10.00	-29.00	09.83	-31.00	55.33	46.50	-16.00	44.33	-20.00	40.50	-27.00	00.26	00.23	00.23	00.24
MV-20	15.33	11.33	-26.00	11.00	-28.00	10.83	-29.00	53.83	46.33	-14.00	44.50	-17.00	39.50	-27.00	00.28	00.24	00.25	00.27
Q-31	12.33	11.16	-09.00	10.00	-19.00	08.33	-32.00	51.16	45.83	-10.00	40.50	-21.00	37.50	-27.00	00.24	00.24	00.26	00.27
Lakhai	13.33	10.50	-21.00	10.50	-21.00	10.16	-24.00	64.50	50.50	-22.00	49.50	-23.00	46.83	-27.00	00.21	00.21	00.21	00.22
BRRI dhan48	12.83	11.33	-12.00	10.83	-16.00	09.50	-26.00	45.50	37.83	-17.00	35.50	-22.00	32.00	-30.00	00.28	00.30	00.31	00.30
Mean	16.54	13.09	-	12.23	-	11.59	-	63.07	53.40	-	50.20	-	47.07	-	00.27	00.25	00.25	00.25
CV%	05.10	05.29	-	04.13	-	05.57	-	02.05	02.59	-	02.79	-	03.42	-	01.91	02.06	02.26	02.17
$LSD_{(0.05)}$	01.38	01.13	-	00.83	-	01.05	-	02.12	2.27	-	02.29	-	02.64	-	00.05	00.07	00.06	00.06

* cont. = Control, RL = Root length, SL = Shoot length, Var. = Variation as compared to control

Conclusion: Combining molecular assessment with morphological findings and comparison with two drought-tolerant checks, the genotypes viz. FR13A, Sadamota, Baichmon, Q-31, BRRI dhan48, Binadhan-17 and BRRI dhan46 may be suggested as true drought-tolerant genotypes, in which genotype FR13A was highly drought-tolerant, genotype Sadamota was moderate drought-tolerant and genotypes Baichmon, Q-31, BRRI dhan48, Binadhan-17 and BRRI dhan46 were low drought-tolerant. In general, this information will be beneficial for not only the plant breeders but also for farmers of drought-prone areas. The identified seven genotypes could be used for selection of suitable parents for developing of drought-tolerant rice verities using marker-assisted back-crossing program.

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