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Short Communication

Polyethyleneglycol mediated rapid *in vitro* screening of rice (*Oryza sativa* L.) genotypes for drought tolerance

Sanjib Bhadra, Bidhan Roy[1*] and T. S. Ghimiray[2]

Deptt. of Genetics and Plant Breeding, [1]Deptt. of Seed Science and Technology, Uttar Banga Krishi Viswavidyalaya (UBKV), Pundibari, Cooch Behar 736 165, W.B.; [2]Regional Research Station (New Alluvial Zone), UBKV, Majhian, Dakshin Dinajpur, W.B.

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Abstract

Cultivation of tolerant genotypes in drought prone areas is the right option to augment productivity of rice. Thus, rapid, feasible and cost effective *in vitro* protocol for screening a large number of genotypes was standardized. MS medium fortified with 80 mg/L of PEG was found to be the best concentration for screening under *in vitro* drought situation. Tolerance index was used to classify the genotypes. Based on tolerance index of the individual seedling parameter, varieties like Annada, IET 24171, MTU 1010, Bitti-1 and Tulsibhog were classified as tolerant. Similarly, judging the mean rank score of all the seedling parameters of the genotypes IET 24171, Annada, MTU 1010, Malsira, Tulsibhog and Bitti-1 were categorized as tolerant. In these two methods of classification, IET 24171, Annada, Bitti-1, MTU 1010 and Tulsibhog were common.

Key words: Rice cultivars, *in vitro* screening, drought tolerance

Drought is one of the most important abiotic stresses in the reduction of rice yield under rain-fed condition. Drought stress affects the water relations of plants at cellular, tissue and organ levels, causing specific as well as unspecific reactions, damage and adaptation reactions (Back et al. 2007). To cope with the drought, tolerant plants initiate defense mechanisms against water deficit (Chaves and Oliveira 2004). Drought is the most severe stress and the main cause of significant losses in growth and productivity of crop plants. Drought stress has been reported to severely reduce germination and seedling stand (Harris et al. 2004). However, in rice, drought stress during the vegetative stage greatly reduced the plant growth and development (Tripathy et al. 2000; Manikavelu et al. 2006). Selection of genotypes for any experiment is very important to express the treatment to the level of correct interpretation of the result and to draw a definite conclusion. One of the popular and dependable approaches to identify tolerant genotypes against drought is to screen under stimulated water stress conditions induced by osmotic substances having high molecular weight like PEG (Landjeva et al. 2008; Saktivelu et al. 2008). Polyethylene glycol (PEG) is a non-penetrating inert osmoticum that can lower the water potential of nutrient solutions without being taken up or phytotoxic. This approach has been used to simulate drought stress in plants and selection of tolerant genotypes in different crops (Badiane et al. 2004). Considering the importance of the abovecited references, 18 rice genotypes were taken to develop an *in vitro* protocol for screening of rice genotypes against drought at seedling stage.

The experimental material consisted of 18 varieties of rice. *In vitro* screening for drought tolerance was performed in culture tubes and surface sterilized seeds were inoculated on MS (Murashige and Skoog 1962) medium supplemented with PEG6000 (20, 30, 40 and 80 mg/L). Each treatment had four culture tubes, thus there were 12 seedlings per treatment. Each experiment was repeated tow times. Cultures were kept in control environment in tissue culture chamber at 25 ± 2C under 16/8h light/dark cycle for

*Corresponding author's e-mail: bcroy10@yahoo.com
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21 days. The data were collected onroot length, shoot length, number of primary roots, seedling fresh weight and seedling dry weight. The experimental plan used was factorial complete randomized blocks with two factors, namely genotypes and stress environments in two replications. Eighteen rice genotypes were screened for drought tolerance at the seedling stage based on a standard tolerant variety, Annada. Statistical analyses of data were conducted with absolute values, using PEG6000 concentration and cultivar as variables. The data were subjected to standard statistical methods of analysis of variance using AgRes Statistical Software, (c) 1994 Pascal Intl Software Solutions, Version 3.01 and significant differences were compared by LSD.

Shoot length, root length, number of primary roots per seedling, seedling fresh weight and seedling dry weight showed highly significant differences among the rice varieties at all levels of PEG6000 stresses and their interactions. It is notable that the genotypes whose shoot lengths were longer in control and at lower concentrations of drought stress (PEG6000 @ 20, 30 and 40 mg/L) could not produce longer shoot in a higher concentration of stress (PEG6000 @ 80 mg/L). It may be due to the genotypic potentiality in respect of *ideotype* and the tolerance ability towards drought stress. Statistically the mean values of five treatments of PEG6000 across the genotypes for shoot length were significant (Tables 1 and 2). Decrease in shoot length in the stress conditions at 20, 30 and 40 mg/L were not noteworthy. However, there was a remarkable difference in shoot length between control and stress environment created by treating with PEG6000 @80mg/L.

The importance of root systems in acquiring water has long been recognized. Differences in root length could confer tolerance to drought by some varieties. Increase in number of primary roots per seedling in stress medium as compared to the non-stressed medium was observed in four rice cultivars, namely Malsira, Bonnidhan, Tulsibhog, and IET 24171 (Table 3). A prolific root system can confer the advantage to support accelerated plant growth during the early crop growth stage and extract water from shallow soil layers to tackle abiotic stress situation (Bhadra and Roy 2014). Another two genotypes (KaloNunia and Bitti-1) retained the same number of primary roots per seedling in stress condition as compared to the non-stress environment.

On an average the seedling freshand dry weight significantly increased in stressed medium fortified with PEG6000 @ 20 mg/L but significantly decreased in stressed medium fortified with PEG6000 @ 30 mg/

Table 1. Mean values of the genotypes against drought stress (PEG6000) across five treatments

Variety/ characters	Shoot length	Root length	No. of primary roots	Fresh weight	Dry weight
IET 24171	21.42c	6.81f	6.80de	140.72e	23.71e
Annada	19.82d	8.06c	5.31f	154.40d	18.31h
MTU-1010	18.16e	6.58fg	8.00c	152.60d	26.40c
MTU-1075	18.26e	7.16e	6.80de	133.00f	23.68e
Nobin	16.94f	5.00kl	7.00d	139.20e	22.50f
Kalo Nunia	22.14bc	4.96l	6.40e	110.12ij	20.94g
Kataribhog	20.06d	9.30a	5.20fg	87.56l	15.52i
Bonnidhan	19.98d	8.54b	5.00fg	117.32g	24.26de
KNS-2-D-3	21.59c	5.52ij	4.80g	95.72k	17.56h
Jashoya	21.88bc	7.64d	5.00fg	108.06j	20.82g
Kalodhayapa	23.12a	6.00h	7.00d	113.20hi	23.80de
Mungamuthi	22.56ab	7.14e	8.80b	177.60b	31.92a
Bitti-1	14.06h	6.42g	3.20h	116.40gh	18.06h
Tulaipanji	18.72•e	5.44ij	7.20d	173.00c	21.52fg
Tulsibhog	23.38a	5.32jk	9.40a	216.80a	24.80d
Malsira	15.96g	7.14e	5.00fg	80.20m	17.90h
Fulpakri	16.96f	5.26jkl	7.00d	82.20m	17.73h
Jaldhyapa	20.00d	5.70hi	3.20h	111.60ij	29.70b
Range	23.38-14.04	9.30 4.96	9.40-3.20	216.80-82.20	31.92-17.73

****:**values bearing same letter in the column are not significantly different at P = 0.01 of LSD

Table 2. Mean values of five treatments of drought stress (PEG6000) across the genotypes

Variety/ characters	Shoot length	Root length	No. of primary roots	Fresh weight	Dry weight
Control	22.36a	7.25a	7.00a	147.22b	25.58a
PEG 20 mg/L	20.85b	6.92b	6.41b	152.06a	24.56b
PEG 30 mg/L	20.62b	7.06bc	6.50b	129.34c	22.90c
PEG 40 mg/L	20.40c	6.84c	5.80c	122.81d	21.25d
PEG 80 mg/L	14.36d	4.70d	5.14d	90.13e	16.55e
Range	22.36-14.36	7.25-4.70	7.00-5.14	147.32-90.13	25.58-16.55

****:** values bearing same letter in the column are not significantly different at P = 0.01 of LSD

Table 3. Tolerance index for different characters (tolerance index was calculated based on the performance of the seedling at PEG6000 @ 80 mg/L

Variety	Shoot length	Root length	No. of primary roots/ seedling	Seed-ling fresh weight	Seed-ling dry weight
IET 24171	90.00*	82.39*	116.67*	82.28*	77.12*
Annada	89.29*	97.00*	91.80*	80.86*	58.27
MTU-1010	75.00*	73.79*	36.36	64.85	82.14*
MTU-1075	51.81	53.92	66.67	57.32	64.81
Nobin	58.06	78.41*	85.71	57.23	69.29*
KaloNunia	67.86	58.87	100.00*	39.84	47.10
Kataribhog	58.16	68.00	71.43	43.74	66.67
Bonnidhan	61.11	65.78	120.00*	52.71	58.62
KNS-2-D-3	55.17	69.77*	50.00	62.73	57.14
Jashoya	53.95	49.61	42.86	56.67	58.33
Kalodhayapa	67.65	63.57	75.00	60.29	60.71
Mungamuthi	55.56	73.88*	55.56	58.08	66.67
Bitti-1	61.54	61.54	100.00*	79.60*	68.10*
Tulaipanji	84.13*	57.47	50.00	59.49	65.04
Tulsibhog	80.00*	49.83	120.00*	61.63	76.79*
Malsira	62.50	78.57*	150.00*	63.64	60.00
Fulpakri	66.67	46.67	30.00	69.47*	57.14
Jaldhyapa	35.71	43.94	66.67	50.76	65.63
Range	35.00-90.00	43.94-97.00	30.00-150.00	39.84-82.28	47.10-82.14

*=Best performing genotypes with respect to different characters per tolerance index

L and PEG6000 @ 40 mg/L.Very high and significant difference in seedling fresh and dry weight was reported between control and stressed environment fortified with PEG6000 @ 80 mg/L. Highest seedling fresh weight at has been observed in Tulsibhog followed by Annada, IET 24171, Tulaipanji, Mungamuthi and MTU 1010. Low seedling fresh weight was observed for Kataribhog, Malsira and KaloNunia. Highest seedling dry weight at 20 mg/L of PEG6000 was observed for Mungamuthi and Jaldhyapa. Other genotypes with high seedling dry weight were Tulsibhog, MTU 1075 and Bonnidhan. Comparatively, low seedling dry weight was observed for Kataribhog, Annada, KNS-2-D-3 and Fulpakri. ASimilar trend was observed at 30 mg/L and 40 mg/L of PEG6000. Highly significant reduction in seedling dry weight was recorded at 80 mg/L of PEG6000. Highest seedling dry weight was observed for Mungamuthi followed by MTU 1010, Tulsibhog, Jaldhyapa and IET 24171. Low seedling dry matter yielders at 80 mg/L of PEG6000 were Fulpakri, Kataribhog, KNS-2-D-3 and Annada.Greater plant fresh and dry weights under water deficit conditions are desirable characters (Sikuku et al. 2012).

In the present study, modern high yielding rice genotypes, namely, IET 24171, Annada and MTU 1010 performed better under PEG6000 mediated *in vitro* drought conditions, which could be attributed to their relatively better root length, number of primary roots, seedling fresh weight and seedling dry weight. The Root system is considered as one of the important component to fighting against the drought environment. The improvement of rice through a deeper root system is thought by many to be a promising way to increase water uptake, and ultimately grain yield under drought condition. A deeper and thickened root system has been shown to allow rice varieties to extract more water from the soil, resulting in a higher yield potential under drought situation.

It is noteworthy that the genotypes whose seedling parameters were desirable or better in control and at lower concentrations of drought stress (PEG6000 @ 20, 30 and 40 mg/L) could not produce desirable or better seedling parameters in a higher concentration of stress (PEG6000 @ 80 mg/L). It may be attributed to the genotypic potentiality in respect of *ideotype* and the tolerance ability towards drought stress. A remarkable difference in seedling parameters between control and stress environment created by treating with PEG6000 @ 80 mg/L. Thus, the concentration 80 mg/L of PEG6000 was used to work out tolerance index of genotypes (Table 3). It is not justified to conclude the tolerance ability simply based on the performance of seedling parameters in stressedenvironment, because the seedling parameters are highly variable across the genotypes and seedling parameters are the phenotype of expression of *ideotype* of an individual genotype and the genotypic potential. The tolerance index is the appropriate parameter for classification of genotypes into different classes of tolerance as susceptible, moderately tolerant and tolerant. Ahadiyat et al. 2012 had used tolerance index for selection of genotypes for drought tolerance following the equation outlined by IRRI (IARI 2002).

Character-wise best performing genotypes based on tolerance. The genotypes, namely IET 24171, Annada, Tulaipanji, Tulsibhog and MTU 1010 showed tolerance index for shoot length. Root length showed higher tolerance index for Annada, IET 24171, Malsira, Nobin, Mungamuthi, MTU 1010, KNS-2-D-3. Higher tolerance index for number of primary roots was observed in Malsira, Bonnidhan, Tulsibhog, IET 24171, KaloNunia, Bitti-1 and Annada. The genotypes, such as IET 24171, Annada, Bitti-1 and Fulpakri, exhibited high tolerance index for fresh weight, whereas, the genotypes MTU 1010, IET 24171, Tulsibhog, Nobin and Bitti-1 showed higher tolerance index for seedling dry weight. IET 24171 was found to be the best performing genotype comparing tolerance index of all the five characters (Table 4). When we took at least four characters, the genotypes Annada was found to be common. If we consider at least three characters at a time, another three genotypes- Bitti-1, MTU 1010 and Tulsibhog were found better performer under stress environment. All those five cultivars, such as IET 24171, Annada, MTU 1010, Bitti-1 and Tulsibhog may be treated as drought tolerant. This study indicated that selection based on drought index will result in the identification of genotypes with significantly higher performance under moderate to severe *in vitro* drought stress environment.

According to Ahadiyat et al. (2012), the rice genotypes having tolerance index more than 70% may be classified as tolerant. Tolerant genotypes for one and more than one traits are indicted by a superscript star(*) in Table 3. Considering the comparative performance of rice genotypes with Annada in respect of ranking of genotypes based on tolerance index of all the seedling parameters, all the genetic materials under study were classified into three levels of tolerance, namely Tolerant, Medium tolerant and susceptible (Table 5). The ranking was given from 1-18 on the performance basis for the individual parameter. The mean of the ranking scores were finally considered for classification of genotypes, such as, genotypes having average scoring of 1-5 were classified as 'Tolerant', similarly genotypes having average score of > 5 to 10 were categorized as 'Medium tolerant' and genotypes havingto mean score of > 10 were classified as 'Susceptible'.

Based on the above criteria, the genotypes IET 24171, Annada, MTU 1010, Bitti-1 Malsira and Tulsibhog were classified as drought tolerant. Out of those six genotypes, five genotypes, namely, IET 24171, Annada, MTU 1010, Bitti-1 and Tulsibhog were also classified as tolerant when they were chosen based on the performance without scoring. Nobin, Bonnidhan, Mungamuthi, Kalodhyapa and Tulaipanji were found to be medium tolerant. However, Kataribhog, KNS-2-D-3, KaloNunia, Fulpakri, MTU 1075, Jaldhyapa and Jashoya were found to be drought susceptible. Ahadiyat et al. (2012) also used tolerance index for identification of tolerant rice genotype. This scoring method may be used for classification of rice genotypes for drought tolerance.

Authors' contribution

Conceptualization of research (SB, BR); Designing of the experiments (SB, BR, TSG); Contribution of experimental materials (BR, TSG); Execution of field/ lab experiments and data collection (SB, BR); Analysis of data and interpretation (BR, SB, TSG); Preparation of manuscript (BR, SB, TSG).

Declaration

The authors declare no conflict of interest.

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