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Research Article

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Assessment of Seedling Growth of Deepwater Rice Genotypes under Osmotic Stress Condition

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Abstract

The experiment was conducted to study the early establishment osmotic stress of advanced deep water rice. Severe parameters such as percent germination, seedling height, and number of leaves, leaf length, node length, and root length were studied for *in vitro* study. This study was conducted by MS medium supplemented with two treatments of PEG (1% and 5% PEG) and control (0% PEG). For percent germination, Bazail, HBJ.A.IV, and Birpala showed better performance with control (0%) but Gabura and Lalkhama performed better in both T_1 (1% PEG) and T_2 (5% PEG). The genotype Gabura showed more seedling height under control and HBJ.A.IV with T_1 and T_2. At 5% PEG, more number of leaves was found in BR5915-B-7, Gabura and Lalkhama. HBJ.A.IV and Lalkhama showed more leaf length and node length at T_2. The genotype BR224-2B-2-5 was best and control and T_1. But Birpala showed more root length at T_2. The information generated in this study indicated that there is a close relationship between *in vitro* studies for the growth performance of the rice genotypes although significant variation was observed within the genotypes. Finally, the advanced genotype BR224-2B-2-5 may be a good source to meet the future challenge.

Keywords: Seedling Growth; Deep water; Osmotic Stress; Rice (*Oryza Sativa* L.)

Introduction

Rice (*Oryza sativa* L.) is the most important and extensively cultivated cereal crops in Bangladesh. Rice has been considered as staple food and about 80% of the total cultivated lands in Bangladesh are used for rice cultivation [1]. Bangladesh is the world's fourth largest rice producer. In the last three to four decades, great efforts in rice research and farming innovations were made to boost rice production. The country's total production has also increased to about 33.3 million tons (milled rice) in 2011 whereas Indonesia, India, and China produced 44.31, 103.8 and 135.1 million tons respectively (FAO, 2011). Furthermore, rice alone contributes about 9.5% of the total agricultural GDP in the country. Among all crops, rice is the driving force of Bangladesh agriculture. In fact, food production in Bangladesh is dominated by a single crop (rice) and a single season (Boro, which accounts for over 60% of total rice production) [2].

Rice is considered as major crop in Bangladesh. It is not only the main source of carbohydrate but also provides 69.61% of calories and 56.15% of the proteins in the average daily diet of the people [3]. The rice production also increased steadily along with net food demands (rice and wheat) and reached 32.1 and 33.3 million tons in 2009 and 2010 [4].

Complete submergence annually affects about 16 million ha of rice in South and Southeast Asia, and about one third of the total rice growing area in Africa. The effect of damage caused by transient submergence is dependent on the characteristics of flood waters, including temperature, turbidity, concentration of dissolved gases, and extent of light penetration [5].

Deepwater rice (DWR) is grown in flooded conditions with water more than 50 cm (20 inch) deep. More than 100 million people in South and Southeast Asia rely on deepwater rice for their sustenance. Many districts of Bangladesh are flooded during the rice cultivation season every year and thus, curtail the national rice yield by causing severe damage to the rice cultivated field. Therefore, it is high time to select potential rice cultivars for breeding program to develop submergence tolerant as well as flash flood resistant rice variety [6]. The topographical situation along with availability of water and sub tropical climate constitutes an excellent habitat for rice cultivation in Bangladesh. In fact, there are different rice ecosystems, namely upland (direct seeded pre-monsoon Aus), irrigated (mainly, dry season Boro), rainfed lowland (mainly monsoon season transplanted Aman, medium-deep stagnant water up to 50-100 cm) [7].

Rice is a semi-aquatic plant and one of the most important crops cultivated in both tropical and temperate regions. DWR is grown in more than 50 cm water for one month or longer during the cropping season. Based on stature and depth of water, these are of two types: (i) traditional tall, and (ii) floating. Traditional tall cultivars are tall with long leaves, and grown at water depths between 50 and 100 cm; floating rice is grown in 100 cm or deeper situations. In Bangladesh most of the rice grown in the low lying areas during monsoon are floating rice, generally called as deepwater rice, locally known as broadcast aman, jolidhan, poushdhan etc. [8].

In Bangladesh deepwater rice covers an area of 0.48 million ha [7] where there is no other option to cultivate modern T. aman varieties. In haor and beel areas like Sunamganj, Sylhet, Habiganj, B. Baria, Faridpur, Gopalganj and Pabna, local deepwater rice varieties are cultivated which have low yield potential [8].

The depth of water in some areas can exceed 4 m as in floating rice areas. Apparently each of these types of floods requires specific adaptive traits, which necessitates the development of unique varieties [9]. Though DWR is cultivated in small areas with low yield, attention should be given to achieve breakthrough in yield potential. Many advanced lines of DWR having better yield potential have been developed by Bangladesh Rice Research Institute (BRRI).

Screening genotypes at seedling stages have several benefits, such as low cost, ease of handling, less laborious and getting rid of susceptible genotypes at earliest [10]. *In vitro* selection techniques involving the use of Polyethylene glycol (PEG), is one of the reliable methods for screening desirable genotypes and to study further the effects of water scarcity on plant germination indices [11,12]. PEG is a non-penetrate and non-toxic osmotic substance which can be used to lower the water potential of culture medium [13]. The selected abiotic stress tolerance rice cultivars have a potential of direct introduction in to farmer fields [14] or utilize them in breeding programs to develop abiotic stress tolerance rice cultivars [14,15].

Early establishment of crop is necessary for DWR to face the flood water throughout the growing season (typically one or two months). The crop established before monsoon begins or floodwater enters can withstand better in raising water level situation. Thus, effective crop growth can boost up total grain production at the end of the season. Crop improvement against moisture stress has been difficulty mainly for 1) lack of suitable screening technique that allows large population and 2) complexity in its arrival times and extents, since it occurs with different intensity and extent in different years. Growth study along with different plant structures is of utmost necessity for crop improvement under stress condition. Though DWR area is comparatively low compared to total rice area, there is necessity of working on it because there exist low lying rice areas where it is one and only option during Aman season.

If high yield potential DWR varieties are introduced, there will be significant increase in national rice production. Hence there is need of advanced breeding lines to select for a rice variety suitable for DWR areas. Considering the above facts, the present research studies was undertaken to fulfill the following objectives: To study the osmotic stress tolerance of newly developed advanced DWR genotypes through PEG treatment and to study the extent of variation of growth status of advanced rice genotypes under osmotic stress and actual field conditions.

Materials and Methods

The experiment (*in vitro*) reported herein was carried out during the period from April, 2014 to May, 2014 in the Tissue Culture Laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh. Seeds of seven DWR genotypes were collected from the laboratory of BRRI, Regional Station, Habiganj. Among them Hbj.A.IV and Birpala are local check variety. A description of genotypes used in the present study is presented in Table 1.

The procedure for preparation of *in vitro* media

The procedure for preparation of *in vitro* media with different concentration of PEG 6000 is as follows:

Treatment of PEG: The PEG 6000 was applied at the rate of

T_0=0% i.e. 0 g/100 ml medium (Control)

T_1=1% i.e. 1 g/100 ml medium and

T_2=5% i.e. 5 g/100 ml medium

Designation	Growing season	Origin/Source
BR224-2B-2-5	B. Aman	BRRI, R/S, Habiganj
BR5915-B-7	B. Aman	BRRI, R/S, Habiganj
Bazail-65	B. Aman	BRRI, R/S, Habiganj
Gabura	B. Aman	BRRI, R/S, Habiganj
Lal-khama	B. Aman	BRRI, R/S, Habiganj
Hbj.A.IV (local check)	B. Aman	BRRI, R/S, Habiganj
Birpala (local check)	B. Aman	BRRI, R/S, Habiganj

Table 1: Names, ecotypes and sources of seeds of the rice genotypes/ varieties.

Culture method: Sterilized mature seeds of seven rice genotypes were inoculated into vials with forceps each containing 10 ml MS medium with PEG at different concentration viz., T_0=0% (control/no PEG), T_1=1% PEG, T_2=5% PEG with four replication. Cultures were maintained at 25°C with controlled lighting for a period for a day night cycle of 16 h/8 h (day/night).

Parameters under study

Percent germination:

$$\text{Percent germination}=\frac{\text{Number of seeds germinated and became seedlings}}{\text{Number of seed set for germination}}$$

Here, number of seed set for germination=4

Seedling height, number of leaves, leaf length, root length and node length: At the 21st day, the seedlings from the vial were taken out with the help of forceps and numbers of leaves were counted. Then seedling height, shoot length, root length and node length of three seedlings were measured in centimeter by a graduated scale and total length was calculated from the recorded data.

Statistical analysis: The recorded data were analyzed using Microsoft Statistical (MSTAT) program and Microsoft Excel wherever applicable. The experiment was arranged in Completely Randomized Design (CRD) and the collected data were analyzed following one way analysis of variance (ANOVA) technique and the mean differences were adjusted by Duncan's Multiple Range Test (DMRT) and the ranking was indicated by letters.

Result and Discussion

The results of studies related to performance of rice genotypes growing under *in vitro* conditions and field conditions are presented and discussed in this chapter.

Percentage of germination

The results of analysis of variance for the characters per cent of germination is presented in Table 2. The genotype varied significantly

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for the characters per cent of germination and media supplemented with different concentration of PEG in the medium for above parameter was also significant (Table 2). The analysis of variance showed that genotypes and different moisture stress condition had conspicuous effect on per cent of germination.

Response among the genotypes/varieties for germination

The mean squares of genotypes for per cent of germination are highly significant indicating the presence of adequate variability among the varieties for this parameter (Table 2). The results of genotype responses to PEG (for germination) are presented in Figure 1. Among the genotypes, Bazail-65, HBJ.A.IV, and Birpala showed better performance without PEG in the medium (T_0) but Gabura and Lal-khama performed better in both T_1 (1% PEG) and T_2 (5% PEG) condition (Figure 2). Similar declines in seed germination have been reported in the literature [16,17].

Sources of variance	Degree of freedom	Mean Square					
		Percent germination	Seedling height	Number of leaves	Leaf length	Root length	Node length
Factor A (Genotype)	6	431.55*	70.57**	1.08**	53.50**	15.85**	0.52**
Factor B (Treatment)	2	2239.58**	855.59**	16.29**	393.97**	89.43**	16.10**
AB	12	312.5	25.44**	0.46**	10.65**	16.75**	0.49**
Error	83	183.53	6.27	0.03	3.87	2.16	0.03

Table 2: Summary of analysis of variance on per cent germination and some plant characters. *Significant at 5% level of probability, **Significant at 1% level of probability.

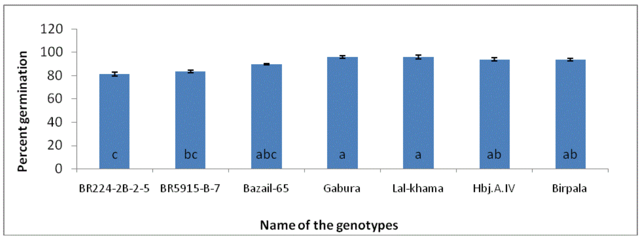

Figure 1: Genotypes mean for per cent germination. Mean (± SE) was calculated from four replicates from each treatment. Bars with different letters are significantly different at P<0.05 applying LSD test.

PEG concentration	Per cent germination
T_0=0 g/100 ml	93.75^a
T_1=1 g/100 ml	97.32^a
T_2=5 g/ 100 ml	80.36^b
LSD (0.05)	**7.235**
S.E. (±)	**2.56**

Table 3: Mean effect of different PEG concentration on per cent of germination. * The values bearing different letters in the same column are significantly different at 5% level of probability.

Per cent of germination varied from 81.35 to 95.83. The highest per cent of germination was in varieties Gabura and Lal-khama (95.83) and the lowest in BR224-2B-2-5 (81.25) and there were significant differences among the genotypes (Figure 1).

Response of genotypes/varieties to different treatments

Mean squares due to different PEG treatments (T_0=0% PEG, T_1=1% PEG and T_2=5% PEG) were highly significant (Table 2). The effect of different moisture stress level of medium is presented here (Table 3). The highest per cent of germination was in varieties Gabura and Lal-khama and the lowest in BR224-2B-2-5 and there were significant differences among the genotypes (Table 3).

Among the treatments, MS media containing 1% PEG showed best result for per cent germination i.e. 97.32, there was significant difference between T_0 (control) and T_2, and no significant difference was found between T_0 (control) and T_1 (Table 3). The treatment T_1 better than control (T_0) indicates osmotic stress response of the deepwater genotypes at certain level and it declines when concentration is high (5% PEG).

Effects of genotype×PEG treatment on germination

Mean squares due to interaction (genotype×treatment) were not significant (Table 2). Interaction effects of genotype and treatment on both the character are presented in Figure 2. Among the genotypes, Bazail-65, HBJ.A.IV, and Birpala showed better performance without PEG in the medium (T0). Gabura and Lalkhama performed better in both T1 (1% PEG) and T2 (5% PEG) condition (Figure 3). Among the genotypes, Bazail-65, HBJ.A.IV, and Birpala showed better performance without PEG in the medium (T0) but Gabura and Lal-khama performed better in both T1 (1% PEG) and T2 (5% PEG) condition (Figure 4). Similar declines in seed germination have been reported in the literature [16,17].

Plant Characters

The results of analysis of variance for plant characters have been presented in Table 2.

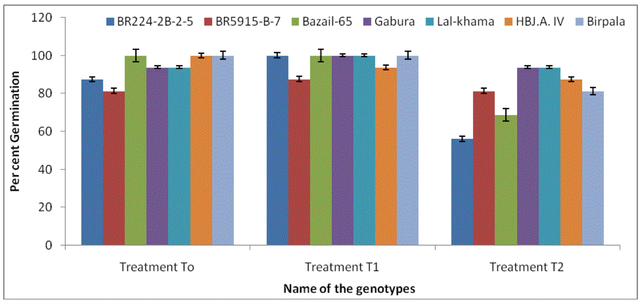

Figure 2: Per cent germination of different rice genotypes at different treatments. Mean (±SE) was calculated from four replicates for each treatment. T_0, T_1 and T_2 indicate control (0%PEG), 1% PEG and 5% PEG respectively.

Response among the genotypes/varieties

The variety mean square was highly significant (Table 2) indicating the presence of adequate variability among the genotypes/varieties. Mean performance of the genotypes/varieties are presented in Table 4.

The seedling height varied from 17.45 to 24.03 cm and maximum seedling height (24.03 cm) was found for the variety Hbj.A.IV and minimum (17.45 cm) seedling height for BR224-2B-2-5 whereas Gabura, Lal-khama, Hbj.A.IV, and Birpala showed no significant difference. In case of number of leaves, the data showed a variation from 1.83 to 2.67 and many of the genotypes showed significant difference whereas maximum live leaves were found for the genotype BR5915-B-7, Gabura, Lal-khama and a few leaves in Bazail-65. Leaf length varied from 10.77 to 15.83 cm, the variety Hbj.A.IV & Birpala showed the maximum leaf length and Bazail-65 showed the minimum leaf length. Root length was not varied much like other parameters. It varied from 5.87 to 8.43 cm (Table 4). Node length was also varied significantly from 2.17 to 2.68 cm and the lowest node length was recorded for Birpala and the highest for Lalkhama (Table 4). Maximum seedling height was found for the variety Hbj.A.IV and minimum seedling height for BR224-2B-2-5 whereas Gabura, Lal-khama, Hbj.A.IV, and Birpala showed no significant difference (Table 4). Maximum leaves were found for the genotype BR5915-B-7, Gabura, Lal-khama, and minimum leaves in Bazail-65. Hbj.A.IV and Birpala showed highest leaf length and lowest leaf length for Bazail-65 (Table 4). Root length was not varied much like other parameters. The lowest node length was found for Birpala and the highest for Lalkhama (Table 4). Sandhu et al. [18] also reported that lowland *indica* were more sensitive to PEG stress by reducing seedling and root length.

Name of the genotype/ variety	Seedling height (cm)	Number of leaves	Leaf length (cm)	Root length (cm)	Node length (cm)
BR224-2B-2-5	17.45^{c}	2.42^{b}	10.87^{c}	8.02^{a}	2.59^{a}
BR5915-B-7	18.67^{bc}	2.67^{a}	11.93^{bc}	8.43^{a}	2.33^{b}
Bazail-65	19.71^{b}	1.83^{c}	10.77^{c}	5.87^{b}	2.33^{b}
Gabura	22.19^{a}	2.67^{a}	12.58^{b}	6.40^{b}	2.66^{a}
Lal-khama	22.02^{a}	2.67^{a}	13.25^{b}	6.78^{b}	2.68^{a}
Hbj.A.IV	24.03^{a}	2.33^{b}	15.83^{a}	5.89^{b}	2.65^{a}
Birpala	22.99^{a}	2.33^{b}	15.80^{a}	8.41^{a}	2.17^{c}
LSD (0.05)	2.04	0.13	1.60	1.20	0.15
S.E. (±)	0.72	0.05	0.57	0.42	0.05

Table 4: Mean performance of different genotypes/varieties for different plant characters. *The values bearing different letters in the same column are significantly different at 5% level of probability.

Response of genotypes to MS medium supplemented with different treatment

Mean squares due to different treatments of PEG were significant for seedling height, node length, number of leaves, leaf length and root length (Table 2). The effect of media containing different treatment is presented in Table 5.

PEG concentration	Seedling height (cm)	Number of leaves	Leaf length (cm)	Root length (cm)	Node length (cm)
T_0=0 g/100 ml	23.81^{a}	2.85^{a}	14.94^{a}	8.31^{a}	2.95^{a}
T_1=1 g/100 ml	24.57^{a}	2.85^{a}	15.39^{a}	7.96^{a}	2.89^{a}
T_2=5 g/ 100 ml	14.64^{b}	1.54^{b}	8.68^{b}	5.06^{b}	1.61^{b}
LSD (0.05)	1.33	0.09	1.05	0.78	0.1
S.E. (±)	0.47	0.03	0.37	0.28	0.03

Table 5: Mean effect of different treatments of PEG on plant characters. *The values bearing different letters in the same column on the table are significantly different at 5% level of probability.

Among the three treatments, seedling height and number of leaves are significantly different from each other. For all characters T0 and T1 did not differ significantly (Table 5).

Effect of genotype×PEG treatment on plant characters

Mean squares due to interaction (genotype×treatment) were significant (Table 2). Interaction effects of genotype and treatment on plant characters are presented in Figures 3-7. The genotype Gabura showed best performance in seedling height under control (T_0) and HBJ.A.IV with T_1 and T_2 but Birpala recorded maximum seedling

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height with T_2 (Figure 3). In case of number of leaves all the genotypes except Bazail-65 showed better performance with control (T_0) and 1% PEG whereas BR5915-B-7, Gabura, and Lal-khama recorded the highest number of leaves at 5% PEG (Figure 4). The cultivar Birpala showed better performance in respect of leaf length at control (T_0) whereas HBJ.A.IV and Lal-khama cultivar recorded the highest leaf length at T_1 and T_2 respectively (Figure 4).

The genotype Gabura showed best performance in seedling height when under control (T_0) whereas HBJ.A.IV recorded the highest seedling height at T_1 and T_2 (Figure 4). In case of number of leaves all the genotypes except Bazail-65 showed better performance with control (T_0) and 1% PEG (T_1) but maximum number of leaves were found in BR5915-B-7, Gabura, and Lalkhama at 5% PEG (T_2) treatment (Figure 4). The cultivar Birpala showed better performance in respect of leaf length at control (T_0) but HBJ.A.IV and Lal-khama cultivar recorded maximum leaf length at T_1 and T_2 respectively(Figure 5).

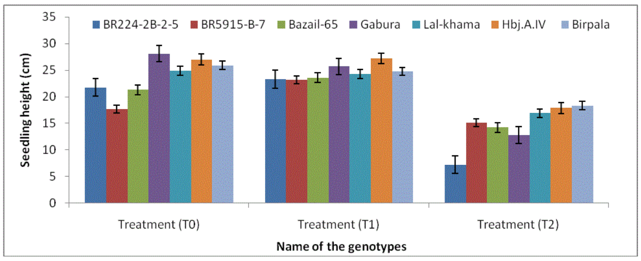

Figure 3: Seedling height of different rice genotypes at different treatments. Other details as in Figure 2.

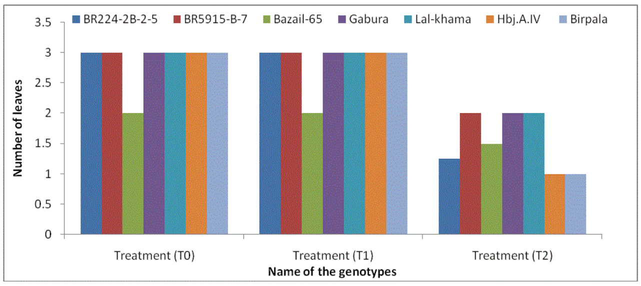

Figure 4: Number of leaves of different rice genotypes at different treatments. Other details as in Figure 2.

In case of root length, the genotype BR224-2B-2-5 performed better at control and T_1 but Birpala recorded maximum root length at T_2 (Figure 6). Node length was similar among the varieties in case of control and 1% PEG (T_1) treatment but BR224-2B-2-5, Lal-khama, and HBJ.A.IV showed better performance at T_2 (Figure 7). In case of root length the genotype BR224-2B-2-5 performed well at control and T_1 but Birpala showed maximum root length at T_2 (Figure 6). Node length was similar among the varieties in case of control and 1% PEG (T_1) treatment but BR224-2B-2-5, Lal-khama, and HBJ.A.IV showed better performance at T_2 (Figure 7). Rapid establishment is accelerated by root growth and root length in seedlings under osmotic stress can perform better in field establishment [13]. The present study is similar with this observation.

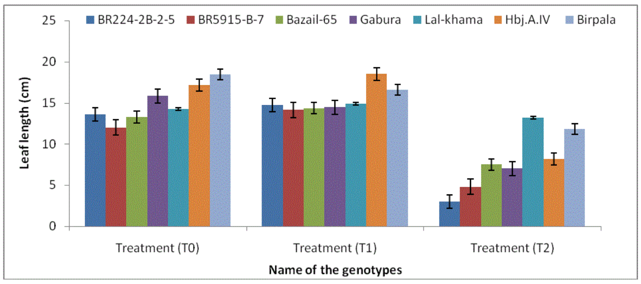

Figure 5: Leaf length of different rice genotypes at different treatments. Other details as in Figure 2.

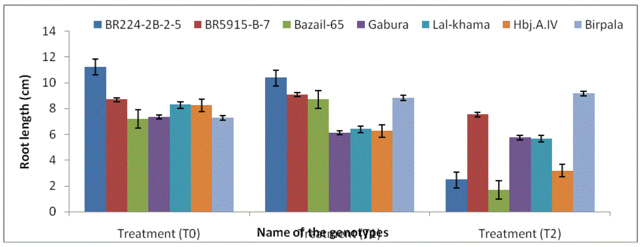

Figure 6: Root length of different rice genotypes at different treatments. Other details as in Figure 2.

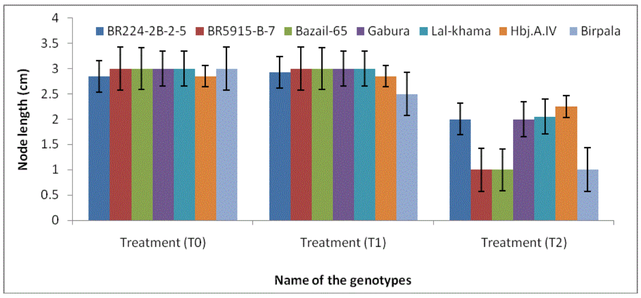

Figure 7: Node length of different rice genotypes at different treatments. Other details as in Figure 2.

Conclusion

In vitro study was conducted under different moisture stress conditions with seven advanced DWR genotypes. The results indicated that in case of emergence the genotype Gabura and Lal-khama performed well even under high level of osmotic stress (5% PEG). In case of seedling height HBJ.A.IV showed good performance with T_1 (1% PEG) and T_2 (5% PEG). Birpala performed best for seedling height with 5% PEG. All the genotypes except Bazail-65 showed maximum leaves with control and T_1. At 5% PEG, maximum number of leaves was recorded in BR5915-B-7, Gabura, and Lal-khama. In case of leaf length HBJ.A.IV and Lal-khama genotype was good performing at T_1 and T_2 respectively. Node length was similar at control and T_1 but BR224-2B-2-5, Lal-khama and HBJ.A.IV showed the highest node length at T_2. In respect of root length the genotype BR224-2B-2-5 performed better at control and T_1 but Birpala recorded the highest root length at T_2 we summarize that the advanced genotype BR224-2B-2-5 is able to establish significantly at early stage as it showed vigorous growth against stress condition present at early stage. The information generated in this study indicated that there is a close relationship between *in vitro* study for the growth performance of the rice genotypes although significant variation was observed within the genotypes. Finally, the advanced genotype BR224-2B-2-5 may be a good source to meet the future challenge.

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