International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

Comparative Study on Callus Initiation and Regeneration Frequency of Two Salt Tolerant Rice (*Oriza sativa*)

Asif Mahmud[a]*, Shamsul Haque Prodhan[b]

[a,b]*Department of Genetic Engineering & Biotechnology, School of Life Sciences, Shahjalal University of Science and Technology, Sylhet-3114, Bangladesh*

[a]*Email: genasif@gmail.com*

Abstract

The objectives of this study were to find out the *in vitro* callus initiation and regeneration potentiality of two salt tolerant rice cultivars, viz., BRRI dhan47, BRRI dhan53. Mature seeds were used as explants. MS media supplemented with different concentrations of 2,4-D (1.0, 2.0, 3.0, 4.0 mg/l) were used for callus induction. The highest calli frequency was 85 % for BRRI dhan47 on MS media containing 3 mg/l 2,4-D, while other variety BRRI dhan53 showed maximum frequency of 75 % callus induction on MS media containing 3 mg/l 2,4-D. For complete plant regeneration the calli of two cultivars were plated on MS media containing different concentrations of kinetin, NAA (1-Naphthalene acetic acid) and BAP (6-benzyl aminopurine). The best regeneration frequency of BRRI dhan47 was 71.42 % on MS media containing 2 mg/l kinetin, 2 mg/l BAP and 1 mg/l NAA and it was 87.50 % for BRRI dhan53 on MS media containing 2 mg/l kinetin, 1 mg/l BAP and 1 mg/l NAA.

Keywords: 2,4-D; BRRI dhan; callus, in vitro; salt tolerant rice.

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* Corresponding author.

1. Introduction

Salinity is a major obstacle to agriculture practices. High concentration of salinity in soil and water has drawn attention of scientists now-a-days in Bangladesh as well as many countries due to its detrimental effects on rice production. In Bangladesh, coastal areas constitute about 2.5 million hectares which amount to about 25 percent of the total cropland of the country. Of this, nearly 0.84 million hectares are affected by varying intensities of salinity, resulting in very poor land utilization. Most of the southern districts of the country are under saline zones, which cover an area of 25-30 percent of the total arable land [1]. Rice is moderately saline sensitive crop. It exhibits considerable intraspecific variability in resistance to salinity. Several research workers have attempted to increase the salt tolerance of rice species, both at the cellular and at the whole plant levels. *In vitro* selection of plants in salt-stressed culture medium is a potential tool to raise plants tolerant to saline environment. Plant tissue culture techniques have provided a potential and fundamental as well as applied research for the improvement of many crops. Global population is expected to reach around 10 billion by 2050. Thus there is a need to improve upon the yield of the local varieties/cultivars, because loss in production could lead to hunger and famine, especially in the developing countries [9]. So this comparative study was conducted to establish a system for callus initiation and regeneration of two *indica* rice varieties, one is BRRI dhan47 and the other is BRRI dhan53 for saline prone areas in boro and T. Aman seasons, respectively.

2. Materials and Methods

The study was conducted in the Plant Genetic Engineering Laboratory, Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology (SUST), Sylhet, Bangladesh. The detail of materials used and analytical methods employed during this study is given below:

2.1. Callus initiation

Mature seeds of two indica rice (*Oryza sativa*) varieties BRRI dhan47 and BRRI dhan53 were used for callus induction and plant regeneration experiment. Seeds were collected from Bangladesh Rice Research Institute (BRRI). For callus induction, the mature seeds were surface-sterilized and placed on the appropriate medium for that cultivar. The original explants and its entire associated callus are transferred to a fresh medium at the end of 21 days. After that embryogenic callus is isolated and transferred again to a fresh medium. Selected healthy seeds were manually dehusked and were placed in autoclaved beaker and washed with sterile distilled water. The seeds were then kept in 70% ethanol for 3-5 minute, followed by washing with autoclaved distilled water for several times. To remove excess water, the seeds were then placed on the sterilized petridish having sterile filter papers with the help of forceps. Surface sterilized seeds of two varieties were inoculated on solid MS [13] media in a laminar airflow cabinet containing growth regulator 2,4-D. Before inoculation and also in between the work, autoclaved scalpels and forceps were again sterilized by burning in fire. The cultures were incubated in culture room at 25 ± 3ºC, to white florescent light under 16 hour's photoperiods. The basal MS medium was used for callus induction. The proposed medium was supplemented with various concentration of growth hormone 2,4-D (1, 2, 3 and 4 mg/l). The pH of the media was adjusted to 5.8 before autoclaving. After inoculation, the surface sterilized seeds of two varieties were transferred and maintained in an environmentally

controlled growth room for 2-3 weeks for callus induction and growth. The cultures were positioned away from continuous light provided by general electric white florescent tubes. Temperature was maintained at (25 ± 3) ºC throughout the growth period. Callus quality replicated three times and twelve test tubes with twenty seeds were used per replication for each genotype. All the calli originated from a single seed was considered as one. Frequency of callus induction was calculated according to the following formula:

$$\text{Callus induction frequency (\%)} = \frac{\text{No.of seeds produced calli}}{\text{No.of seeds cultured}} \times 100$$

2.2. Plant regeneration

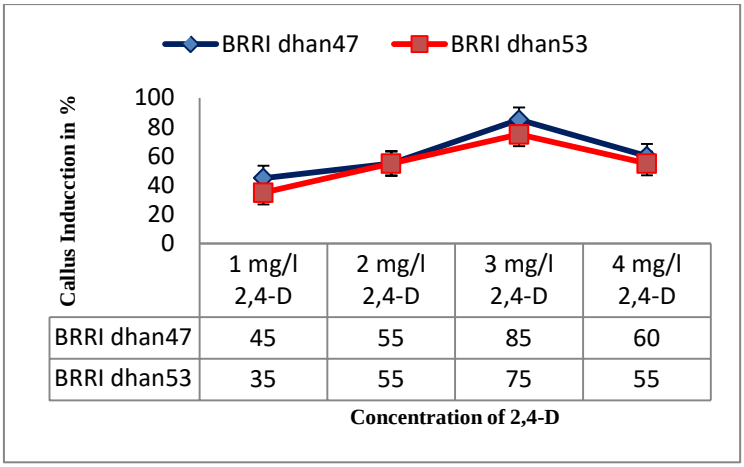
After inoculation, the callus of two varieties were transferred and maintained in an environmentally controlled growth room for 4 weeks. For plant regeneration, calli were then inoculated on regeneration media. The pH of media was adjusted to 5.8 before autoclaving. The culture was performed at (25 ± 3) ºC under a cycle of 16 hour's light/8 hour's dark for 4 weeks. After which the frequencies of plant regeneration were calculated, based on the appearance of shoots. Plant regeneration from plated calli was calculated with the following formula:

$$\text{Plant regeneration (\%)} = \frac{\text{No.of calli produced plants}}{\text{No.of calli plated}} \times 100$$

3. Results and Discussions

3.1. Callus induction and growth

Mature dehusked rice seeds were used as an explants because calli initiated from scutellum of mature seeds of all rice varieties have high embryogenic potential [7, 14] and was excellent material for transformation of rice by *Agrobacterium* [2, 3, 4, 5, 10]. Embryogenic calli obtained from mature seed explant have high regeneration capacity [8]. MS and N6 were the most commonly used basal media [3, 10, 12]. To determine the optimum level of plant growth regulator, different concentrations of 2,4-D (1mg/l, 2mg/l and 3mg/l, 4 mg/l) were used with MS media for callus induction and growth (Figure 1).



	1 mg/l 2,4-D	2 mg/l 2,4-D	3 mg/l 2,4-D	4 mg/l 2,4-D
BRRI dhan47	45	55	85	60
BRRI dhan53	35	55	75	55

Figure 1: Callus initiation frequency on different 2,4-D concentration

Mostly 2,4-D has been used as the only growth regulator in callus induction media . The results from our study revealed that both varieties gave better callus induction response on MS media supplemented with concentrations of 2, 4- D 3.0 mg/l (Figure 2, 3, 4, 5).

Figure 2: Callus of BRRI dhan47

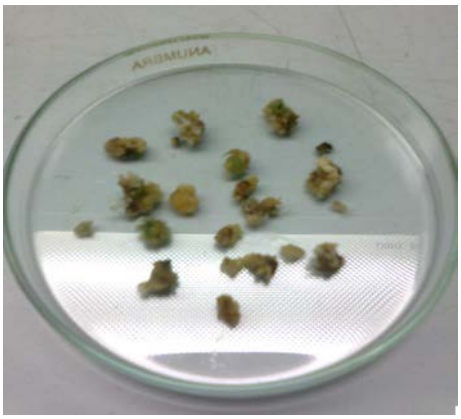


Figure 3: Callus of BRRI dhan53

Other researcher [8] found better callusing frequency at a concentration of 2.5 mg/l 2, 4-D. The two varieties BRRI dhan47 and BRRI dhan53 showed higher callus induction frequency on 3mg/l 2,4-D that is 85 % and 70.59 %, respectively. These results are confirmatory to the findings of other researchers [4, 14]. The response of the explants to different concentrations of 2,4-D in terms of callus induction was genotype dependent. These findings are in agreement with previous reports of other researcher [8]. The present study revealed that both genotype and media composition and their interaction largely effect on callus induction.

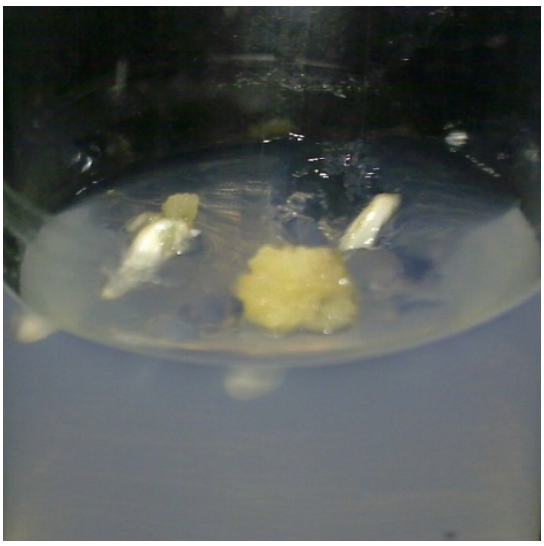


Figure 4: Callus induction of BRRI dhan47 on MS media containing 3 mg/l 2,4-D

Figure 5: Callus induction of BRRI dhan53 on MS media containing 3 mg/l 2,4-D

3.2. Regeneration

In vitro complete plant regeneration was performed on MS medium supplemented with combinations of growth regulators i.e., kinetin, BAP and NAA (Table 1).

Combinations of auxin and cytokinin along with the effect of basal salts played an important role for plant regeneration [6, 11].

It was observed that plant regeneration ability of plated calli depends on the genotypes and the callus induction media.

After about a period of two weeks, the calli plated on regeneration medium produced green spots and at about the same time some of them became brown. Green shoots were observed within 10 days of transfer of calli into regeneration medium (Figure 6 and 7).

Table 1: Regeneration frequency of BRRI dhan47 and BRRI dhan53 on MS media supplemented with different hormonal combinations and concentrations

Variety	Media type	Concentration of kinetin+ BAP+ NAA (mg/L)	Frequency of Regeneration (%)
BRRI dhan47	MS	2 + 2+ 1	71.42
BRRI dhan53	MS	2 + 2+ 1	75
BRRI dhan47	MS	2 + 1+ 1	54.54
BRRI dhan53	MS	2 + 1+ 1	87.5
BRRI dhan47	MS	2 +1 + 0.5	53.33
BRRI dhan53	MS	2 +1 + 0.5	63.64
BRRI dhan47	MS	2 +2 + 0.5	36.36
BRRI dhan53	MS	2 + 2 + 0.5	52.94

Highest regeneration frequency was produced by BRRI dhan47 that is 71.42 % and it was 87.50 % for BRRI dhan53.

The two varieties BRRI dhan47 and BRRI dhan53 showed higher regeneration frequencies at MS media containing 2 mg/l kinetin, 2 mg/l BAP and 1 mg/l NAA and MS media containing 2 mg/l kinetin, 1 mg/l BAP and 1 mg/l NAA respectively.

Figure 6: Shoot regeneration of BRRI dhan47 on MS+ 2 mg/l kinetin, 2 mg/l BAP and 1 mg/l NAA

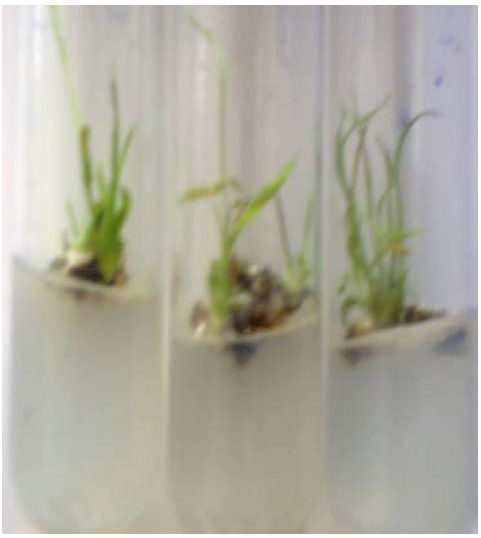


Figure 7: Shoot regeneration of BRRI dhan53 on MS+ 2 mg/l kinetin, 1 mg/l BAP and 1 mg/l NAA

4. Conclusion

It can be concluded that callus from the mature seed embryos is a very good source of material for efficient *in vitro* plant regeneration in rice. Highest frequency of callus induction from the seeds of variety BRRI dhan47 was 85 %, followed by 70.59 % for BRRI dhan53. It can be revealed that 3.0 mg/l 2, 4- D is suitable for callus induction for both BRRI dhan47 and BRRI Dhan53. These two varieties BRRI dhan47 and BRRI Dhan53 showed optimum shoot regeneration on MS media containing 2 mg/l kinetin, 2 mg/l BAP and 1 mg/l NAA and MS media containing 2 mg/l kinetin, 1 mg/l BAP and 1 mg/l NAA respectively. So, this study will provide the basis for future studies on improvement of callus initiation and regeneration and Agrobacterium-mediated transformation to transfer gene of interest to salt tolerant rice to increase rice production which is an increasing demand of present world due to the rapid growth of population and environmental changes.

Acknowledgements

The authors gratefully acknowledge to the authority of Bangladesh Rice Research Institute (BRRI) to give us permission to use their released rice variety seeds as experimental materials and MOST (The Ministry of Science and Technology), Bangladesh, for their financial support.

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