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Effect of Different Concentrations of Plant Growth Hormones for *in Vitro* Regeneration of Rice Varieties BRRI Dhan 28 and BRRI Dhan 29

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Abstract

A method for *in-vitro* propagation of BRRI dhan 28 and BRRI dhan 29 was developed by using seed embryos as explants on MS media and half strength MS media containing different concentrations of plant growth regulators and hormones. In case of BRRI dhan 28, approximately 100±0% callus were observed on both media composition when media was supplemented with 2, 4- dichlorophenoxyacetic acid (2, 4-D) at 3 mg/l and 4 mg/l. But BRRI dhan 29 showed approximately 80±2.254% callus formation on both media containing 2, 4-D at 4 mg/l. Good and healthy calli were transferred into regeneration media containing different concentrations of Benzyladenine (BA) and naphthalene acetic acid (NAA). BRRI dhan 28 showed 87.5±3.365% regeneration on MS media containing 2 mg/l BA + 0.5 mg/l NAA but 100±0% regeneration was observed on half strength MS media containing 1.5 mg/l BA + 0.5 mg/l NAA. BRRI dhan 29 showed 62.5±4.059% and 100±0% regeneration on both MS and half strength MS media when 1.5 mg/l BA + 0.5 mg/l NAA were used. The regenerated plantlets were successfully acclimatized under *ex-situ* condition.

Keywords: Plant Tissue Culture; Callus; MS Media; BRRI dhan 28; BRRI dhan 29.

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1. Introduction

Rice (*Oryza sativa* L.) belongs to the family Poaceae. Rice contributes the world's most important food crop after wheat and maize [1] and it is the staple food for the people of Bangladesh [2]. More than 90% of the world's rice is grown and consumed in Asia, where 60% of the earth's people live. In Asian countries, it provides half of total dietary carbohydrates supplying 50-80% of their daily calorie need for more than three billion people [3]. As rice is the staple food for most of the developing world, there is urgent need to double the production to reduce malnutrition. During the past few decades, tremendous progress has been made in the area of plant biotechnology through the techniques of tissue culture like protoplast fusion, another culture, leaf culture, root culture and dehusked grain culture and these are employed in the rice breeding to exploit somaclonal variation to create novel rice varieties [4]. *In vitro* techniques constitute an efficient tool in rice breeding. These have the potential not only to improve cultivars but also for the synthesis of novel varieties and early release of high yielding plants that are resistant to various stresses. The rice production has increased worldwide by the large scale adoption of modern high-yielding rice varieties. BRRI dhan 28 and BRRI dhan 29 are such two varieties developed in Bangladesh Rice Research Institute (BRRI). These varieties are important for their productivity, small grain size and high yield. Tissue culture of these varieties is a need of time because of their economic significance. Suitable plant regeneration methods are required for the successful application of tissue culture techniques for crop improvement. Micropropagation protocol for BRRI dhan 28 and BRRI dhan 29 are considered to be developed for their further improvement through genetic engineering or somaclonal variation for pest, disease and drought resistance. Very high frequencies of modifications are induced in *in vitro* cultures and some of them have been proved very useful [5]. The standardization media for the callus induction and regeneration are very important in tissue culture technique. The different growth hormones are used for the different crops. Slight variation in the doses is necessary for different genotypes of the same crop also. Hence, the study was carried out to standardize the effect of growth hormones in rice embryo culture both for callus induction as well as regeneration and also to establish an efficient technique for high yield and improved quality of the crop.

2. Materials and Methods

The study was conducted at Plant Genetic Engineering Laboratory of Dept. of Genetic Engineering and Biotechnology in Shahjalal University of Science and Technology, Sylhet. Two High Yielding Varieties (HYV) if rice viz. BRRI dhan 28 and BRRI dhan 29 were collected from the farmers of Feni district in Bangladesh. Mature and healthy rice grain embryos were used as explants. To ensure aseptic condition *in vitro*, all instruments, glassware and culture media were sterilized by autoclaving with 121^{o}C for 20 minutes. Seeds were surface sterilized with 70% ethanol for 5-6 minutes and washed with sterile distilled water. Then the explants were treated with 1N NaOCl for 2 minutes assisted with 1 drop of Tween 20. After sterilization, the seeds were washed 6-7 times with sterile distilled water and allowed to dry in laminar airflow. Sterilized explants were inoculated on MS and half strength of MS media supplemented with various concentrations of 2,4-D (1 mg/l, 2 mg/l, 3 mg/l, 4 mg/l, and 5 mg/l) for callus induction. The inoculated samples were sub cultured on same media after 2 weeks. For shoot regeneration, a healthy portion of the callus was taken and cut into pieces and these pieces were placed on shoot regeneration media. MS and half strength of MS media were supplemented with

different concentrations of BA (0.5 mg/l, 1 mg/l, 1.5 mg/l, 2 mg/l, and 2.5 mg/l) and 0.5 mg/l NAA for shoot regeneration. MS media was supplemented with different concentrations of IBA and NAA for root induction. The culture vessels with inoculated explants were incubated both in dark and light in a temperature controlled culture room (25±1^{o}C) with a light intensity of 2000-3000 lux and relative humidity (60-70%). Visual observations were taken every three days and the effect of different treatments was quantified on the basis of the percentage of calli showing response for shoot and root regeneration. A completely randomized design with three replications per treatment for each genotype was followed in this study and standard deviation (SD) was calculated.

3. Results and Discussion

The present study was designed to identify the ideal conditions for micro propagation of BRRI dhan 28 and BRRI dhan 29. In most cases, 2, 4-D as a strong synthetic auxin was sufficient to initiate and sustain embryogenic callus growth in rice, thus, 2, 4-D has been used as the only growth regulator for callus induction in this study. However, the optimum concentration of 2, 4-D varied depending on the explants source and genotype of rice [6]. The present study showed that the MS and half strength MS media supplemented with different concentrations of 2,4-D produced good number of calli (Figure 1 and Figure 2) from the seeds of BRRI dhan 28 and BRRI dhan 29 but calli induction frequency differ with the concentrations of 2,4-D. In case of BRRI dhan 28, approximately 100±0% calli were observed on both media when supplemented with 2, 4-D at 3 mg/l and 2,4-D at 4 mg/l. But BRRI dhan 29 showed approximately 80±2.254% callus formation on both MS and half strength MS media containing 2, 4-D 4 mg/l (Figure 6 and Figure 7). The authors in [7,8,9,10] have been reported significant differences in callus induction by using different concentrations of 2,4-D. Nearly similar results were also found in rice [11]; where they found that different rice varieties produced high amount of callus on MS media supplemented with 2.0-4.0 mg/l 2, 4-D.

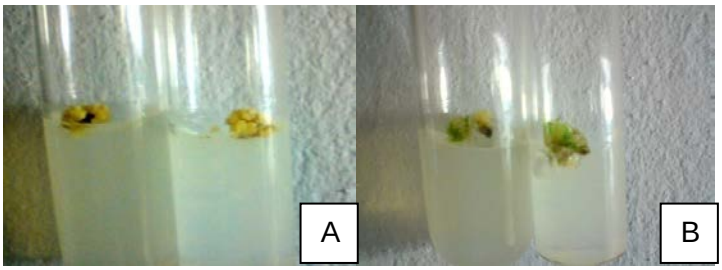

Figure 1: Calli of BRRI dhan 28 (A) and BRRI dhan 29 (B) on MS media

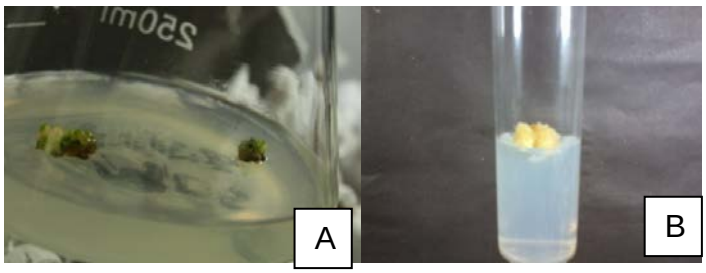

Figure 2: Calli of BRRI dhan 28 (A) and BRRI dhan 29 (B) on half strength MS media

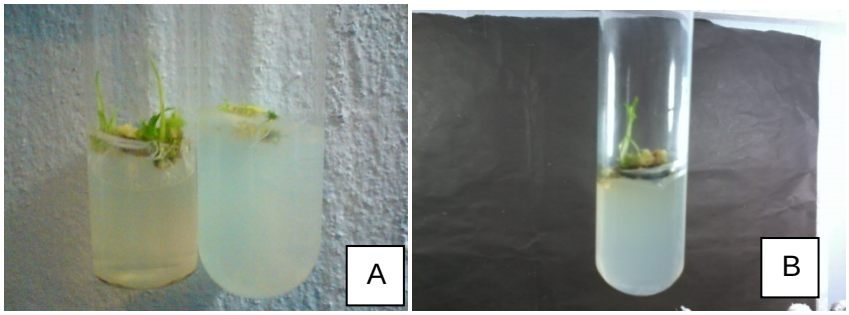

Figure 3: Regeneration of BRRI dhan 28 (A) and BRRI dhan 29 (B) on half strength MS media

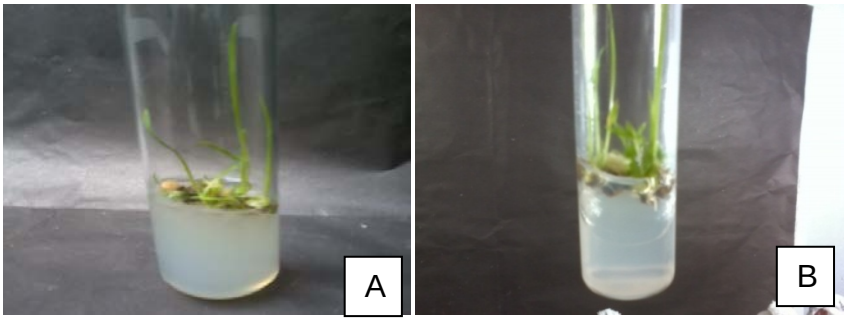

Figure 4: Regeneration of BRRI dhan 28 (A) and BRRI dhan 29 (B) on half strength MS media

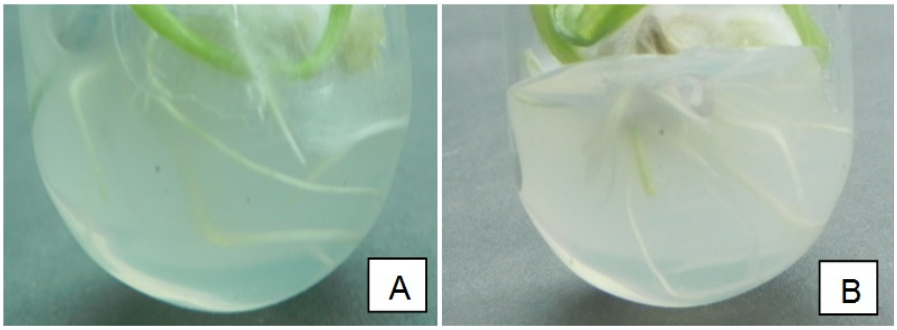

Figure 5: Root induction of BRRI dhan 28 (A) and BRRI dhan 29 (B) on MS media

For regeneration, calli were inoculated on MS and half strength MS media supplemented with different concentration of BA and NAA. Visisble shoot formation was noted within two weeks on the cultivars on BRRI dhan 28 and BRRI dhan 29 variety (Figure 3 and Figure 4). Within 3 weeks, calli were entirely covered with green shoot buds. BRRI dhan 28 showed 87.5±3.365% regeneration on MS media containing 2 mg/l BA + 0.5 mg/l NAA but 100±0% regeneration was observed on half strength MS media containing 1.5 mg/l of BA + 0.5 mg/l of NAA. BRRI dhan 29 showed 62.5±4.059% and 100±0% regeneration on both MS and half strength MS media when 1.5 mg/l of BA + 0.5 mg/l of NAA were used (Figure 8 and Figure 9). Regeneration efficiency of rice plants affected by media composition, explants source and age, and culture environments [10,12,13]. Several reports are available where genotype and nutrient composition of the media was the most important factors for efficient rice plant regeneration [14] and appropriate media composition can increase regeneration efficiency of rice [9,14,15].

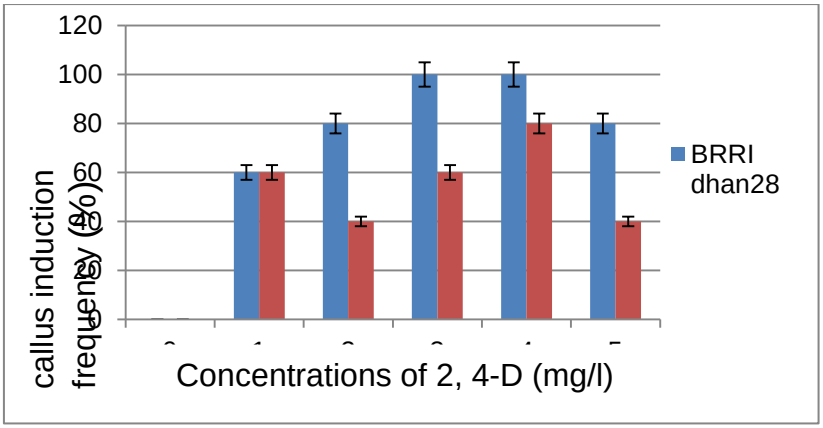

Figure 6: Effect of different concentration of 2, 4-D on MS media for callus formation

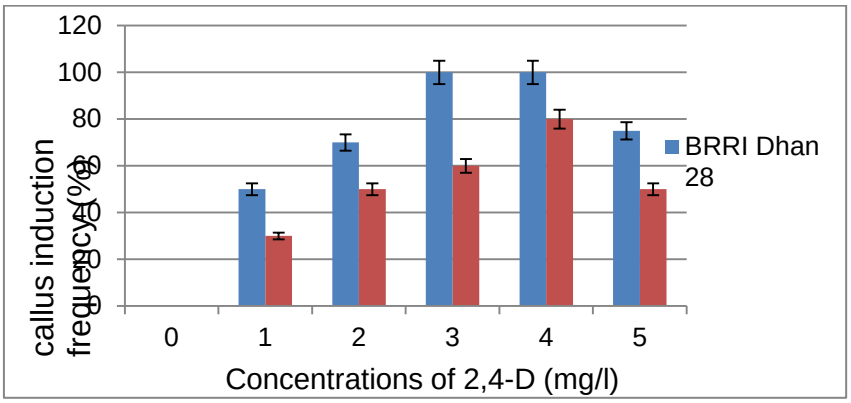

Figure 7: Effect of different concentration of 2, 4-D on half strength MS media for callus formation

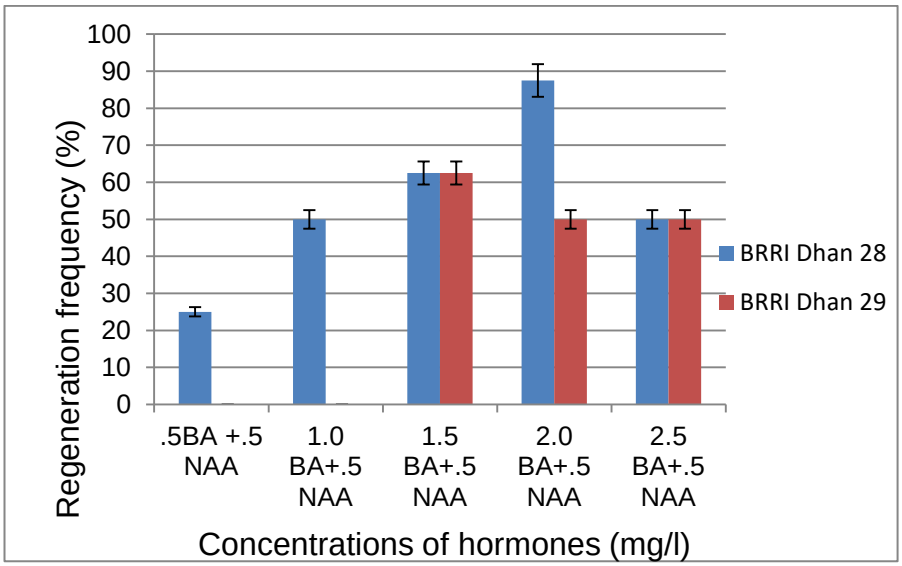

Figure 8: Effect of different concentrations of BA and NAA on shoot formation on MS media.

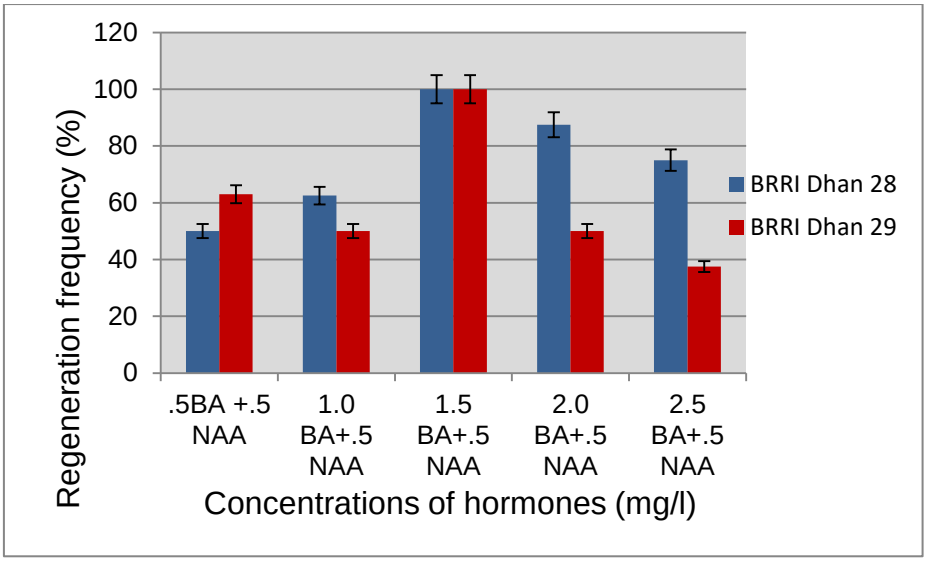

Figure 9: Effect of different concentrations of BA and NAA on shoot formation on MS half strength media.

According to [16] high concentration of cytokinin and low level of auxin promoted plantlet regeneration. Similar reports have shown that some combinations of auxin and cytokinin along with the effect of basal salts played an important role for callus formation and subsequent plant regeneration of rice [9,17]. MS media and half strength MS media showed similar response in case of callus induction for both varieties. On the other hand, half strength MS media showed better response than MS media in case of regeneration of both rice varieties.

Small shoots were shifted to rooting media containing different concentration of IBA, NAA alone and in combination. Maximum rooting was recorded when MS media supplemented with IBA 2 mg/l for both rice cultivar (Figure 5). The rooted shoots were removed from the culture tubes, washed with tap water and transferred to plastic pots with the mixture of field soil. The regenerated plantlets are gradually exposed to the stress of lower relative humidity (30-60%), higher light intensity and flexible tempature to adopt the natural enviornment.

4. Conclusion

This research was conducted for establishment of callus initiation and regeneration system of two important rice cultivars BRRI dhan 28 and BRRI dhan 29 on MS and half strength MS media. Using mature seed explants, callus formation and regeneration of complete plantlets were attained. According to this study, there was no significant difference between MS media and half strength MS media for callus induction rate in both varieties but they exhibit noteworthy differences in shoot induction capability. Shortly, MS half strength media was the best for in vitro plantlet regeneration of these two varieties. Thus, this study will be helpful for further improvement of BRRI dhan 28 and BRRI dhan 29.

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