J. Bio-Sci. 32(1): 83-94, 2024 **ISSN 1023-8654**
http://www.banglajol.info/index.php/JBS/index
DOI: doi.org/10.3329/jbs.v32i1.74990

INFLUENCE OF PLANT GROWTH REGULATORS ON EFFICIENT CALLUS INDUCTION AND PLANT REGENERATION USING FIVE RICE GENOTYPES IN BANGLADESH

Toyfiquz Zaman and S M Shahinul Islam*
Plant Biotechnology and Genetic Engineering Lab., Institute of Biological Sciences, University of Rajshahi, Rajshahi-6205, Bangladesh

Abstract

An efficient and reproducible *in vitro* regeneration process is required for the production of abiotic stress tolerance in rice through biotechnological approaches. In this study, optimization of culture media, callus induction, and plant regeneration of five Aman rice genotypes *viz*. BRRI dhan34, BRRI dhan51, BRRI dhan71, BRRI dhan79, and BRRI dhan95 were considered and mature seeds of those genotypes were collected from BRRI Regional Station, Rajshahi. To investigate the effects of different concentrations of 2,4-D and other plant growth regulators on callus induction and regeneration some experiments were conducted and evaluated. Two basal media *viz*. MS and N6 were considered for the experiments out of four (MS, B5, N6 and SK-3) studied previously under this research works. It was observed that all five rice genotypes showed higher callus induction on MS medium supplemented with 3.0 mg/l 2,4-D than other concentrations. Based on callus induction data were recorded and BRRI dhan34, BRRI dhan51, BRRI dhan71, BRRI dhan79 and BRRI dhan95 gave 63.4%, 50.4%, 40.4%, 58% and 53% embryogenic calli, induction respectively. In combination with BAP (2.0 mg/l), Kin (2.0 mg/l) and NAA (0.5 mg/l) added to MS medium data showed better performance on regeneration. They can be used as a baseline for demand-driven *in vitro* rice propagation, providing useful information that can be combined with other agronomic features in rice development to improve the abiotic stress-tolerant cultivars through biotechnological methods in future.

Key words: Callus induction, Plant regeneration, Rice, Plant growth regulators.

Introduction

Rice is one of the most important cereal crops in the world including Bangladesh. Almost 50% of the world's population relies primarily on rice as their source of energy, making it one of the essential staple food crops. By 2050, the global population will have risen to 9.5, necessitating a 70% increase in food production (Sandhu and Kumar 2017). As a result, it is necessary to boost rice productivity by overcoming the lower crop output brought on by numerous biotic and abiotic challenges. The Government of Bangladesh currently emphasized to boost domestic rice production to meet the needs of the nation's expanding population. Bangladesh plans to boost its level of food security to 80% by 2030, which will increase demand for food. But rice production is threatened by various abiotic and biotic stress. Salinity is the main problem in coastal areas in Bangladesh which affects rice cultivation. In these areas Aman is the main crop which is affected by the salinity problem. To address these obstacles, new rice varieties could be developed using a combination of traditional breeding, genetic engineering and *in vitro* tissue culture methods (Khatun et al. 2010). Earlier

*Author for correspondence: shahin_ibsc@ru.ac.bd

studies revealed that the indica rice genotypes resistance to diverse *in vitro* tissue culture conditions was mostly due to its inferior callusing and regeneration capacities (Silva 2010) compared to the japonica subspecies (Kalhori et al. 2017). Somatic embryogenesis is an important method for plant propagation that can be used in a variety of projects aimed at agricultural improvement (Karthikeyan et al. 2009, Talapatra et al. 2014, Haque et al. 2015 & 2019). The position of embryonic cells, which reveals the mechanisms driving cell growth as well as the ability for regeneration that is used in plant biotechnology, highlights the earliest stage of secondary embryogenesis induction (Zimmerman 1993, Morshed et al. 2014 & 2016, Wójcikowska and Gaj 2017). Efficient callus induction and plant regeneration are the main procedures in this process before executing any genetic improvement program (Datta 2004, Rahman et al. 2010, Haque et al. 2015, Dievart et al. 2016). Somatic embryogenesis has also been related to successful regeneration, necessitating the development of embryogenic calli (Haque et al. 2015, Islam and Haque 2016). For the formation of calli and ensuing regeneration, exogenously administered plant growth regulators (PGRs) of the right type and concentration are required (Nic-Can and Loyola-Vargas 2016, Seldimirova et al. 2016, Wójcikowska and Gaj 2017). As PGRs 2,4-D alone or in combination with 1-naphthalene acetic acid (NAA) have been used to successfully induce rice callus was reported by Hiei and Komari (2008) and Zuraida et al. (2011).

For somatic embryogenesis MS medium is most widely used as a basal medium for indica and japonica rice varieties. Highest callus induction was reported from basmati rice cv. 370 in MS medium supplemented with 2.0 mg/l 2,4-D by Hiei and Komari (2008). Khatun et al. (2010) achieved somatic embryos in N6 and MS medium where they used 2.0 mg/l 2,4-D and kinetin as PGRs, respectively. There are some reports using N6, LS and SK1 media which gave a better response on *in vitro* tissue culture in rice than others (Islam et al. 2013, Khatun et al. 2010 & 2012). In addition to the composition of culture media, the concentrations of plant growth regulators also influence the process of callus induction and regeneration in rice (Mohiuddin et al. 2006). Khatun et al. (2010) tested two basal media e.g. MS and N6 for callus induction in five indica rice genotypes. For *in vitro* micropropagation and advance biotechnological research a suitable protocol is very essential, particularly for callus induction, multiplication and plantlet regeneration of rice in Bangladesh. As far as it is known, till now there is not enough report on efficient regeneration system for Bangladeshi indica rice genotypes. Therefore, this study was undertaken with the following objectives- i) to screen the suitable indica rice cultivars for *in vitro* culture systems as well as for callus induction and plant regeneration for advanced biotechnological research, and ii) to investigate the effect of various media and PGRs for efficient callus induction and plant regeneration with stress pre-treatments factors.

Materials and Methods

Plant materials

Till now the Bangladesh Rice Research Institute-BRRI (Bangladesh Rice Knowledge Bank 2024; http://knowledgebank-brri.org/brri-rice-varieties) introduced a number of 103 rice released varieties (Boro = 41, Aus = 21 and Aman = 41). Rice is cultivated as Aus, Aman or Boro throughout the year in Bangladesh. To improve callus induction and plant regeneration, five Aman rice genotypes namely BRRI dhan34, BRRI dhan51, BRRI dhan71, BRRI dhan79, and BRRI dhan95 have been selected for this experiment. Mature seeds of those rice varieties were collected from BRRI Regional Station, Shaympur in Rajshahi.

Seed sterilization and culture media

The dehusked seeds were surface sterilized by dipping in 3% sodium hypochlorite for five minutes, then in 95% ethanol for twenty seconds (from dipping to washing with distilled water), and finally washed 5-6 times in sterile distilled water (Abuhena et al. 2022). The seeds were then shaked on an orbital shaker at 120 rpm for 30 minutes. The sterilized seeds were rinsed 3 times with sterile double-distilled water and then blot dried on sterilized filter paper before inoculation. Then the surface sterilized seeds were carefully inoculated into MS (Murashige and Skoog 1962 and N6 (Chu et al. 1975) media. Different modes of incubation were assessed based on the germination percentage following the formula mentioned below-

$$\text{Germination (\%)} = \frac{\text{Number of germinated seeds}}{\text{Total number of inoculated seeds}} \times 100$$

Effect of media and plant growth regulators on callus induction

To find out the effect of different doses (1-3 mg/l) of 2,4-D two basal media like MS and N6 were considered in this case. Before autoclaving, the pH of the media was adjusted to 5.7 to 5.8. Culture medium was sterilized for 20 minutes at 121°C and 15 psi (103.4 kPa) and the sterilized medium was dispensed to the Petri dishes having 20-25 ml each. In order to maintain sterility, 20-25 ml of medium was then added to Petri dishes. In aseptic conditions, sterilized seeds were horizontally cultivated on the medium and incubated them at 25°C in the dark. After 3 weeks of culture, the frequency of callus induction was measured. The petri dishes were sealed with parafilm and incubated them at 27±2°C in the dark for three to four weeks. Then the vessels were kept under 36 Watt white fluorescent lamps with light intensity of 5000 lux at 16/8 hours cycle of light/dark. The temperature of growth chamber was maintained at 25±1°C.

Plant regeneration and acclimatization

Four to five weeks old calli derived from five Aman rice cultivars were used for regeneration, which were then placed on plant regeneration medium (RM) containing MS and N6 basal media supplemented with BAP (1.0 - 2.0 mg/l), Kin (1.0 - 2.0 mg/l) and NAA (0.5 mg/l). As gelling agent 4.0 g/l of phytagel (Sigma-Aldrich) were used before autoclaving. Four different concentration of PGRs e.g. (i) RM1 = 2 mg/l BAP + 0.5 mg/l NAA, (ii) RM2 = 2 mg/l BAP + 0.5 mg/l NAA + 1 mg/l Kin, (iii) RM3 = 2 mg/l BAP + 2 mg/l Kin + 0.5 mg/l NAA, and (iv) RM4 = 1 mg/l BAP + 0.5 mg/l NAA + 1 mg/l Kin. Three replicates of each treatment were conducted with similar number and amount of calli were inoculated in each time. Thereafter, all cultures were incubated at ± 2°C with a 16:8 (light/dark) photoperiod. For shoot elongation, the *in vitro* regenerated plantlets were moved into culture tubes containing the same basal media. Every week, the *in vitro* regeneration response based on the commencement of various kinds of green spots was noticed. The plantlets were moved to culture tubes containing the same basal medium for shoot elongation after being initiated. After transplanting, the quantity of regenerated shoots was counted in every two weeks. The regenerated *in vitro* plantlets were moved to a half-strength basal medium for 7 days without any PGRs for root proliferation. To avoid fungal assaults, the plantlet was then carefully rinsed with sterile water to thoroughly remove the adhering media. After that, the

in vitro plantlets with good roots were placed in pots of soil to acclimate. The regeneration percentage were measured following the formula mentioned below:

$$\text{Regeneration (\%)} = \frac{\text{Number of green spots}}{\text{Total number of calli}} \times 100$$

Furthermore, the calli and regenerated plantlets were recorded. After replanting the plants into pots with the best regeneration media at 2 weeks old, the survival percentage of *in vitro* grown plantlets under natural environmental circumstances was measured. A fully randomized block design was used to set up the studies.

Statistical analysis

Three replicates of the callus induction frequency and plantlet regeneration were observed. The data analysis was carried out in R 2021, and Tukey's test was used to assess mean differences. At the $p<0.05$ level, the differences were considered statistically significant. To indicate the significance of the discrepancies, distinct means were labelled with different letters (a, b, c, d, and e).

Results and Discussion

2,4-D and its effect on callus induction

Genetic manipulation to improve a variety must start with a high percentage of re-generable embryogenic calli was reported by Juturu et al. (2016). For more effective plant regeneration, this study attempted to evaluate and enhance the embryogenic potential of calli (Siddique et al. 2014, Ahmad et al. 2016, Abiri et al. 2017, Mostafiz and Wagiran 2018). *In vitro* seed derived calli of five indica rice genotypes were examined and the developmental stages are shown in Fig. 1 & 2. There were no discernible changes in the CIF- callus induction frequency (%) across different media ($p<0.001$). For all cases in addition of 3.0 mg/l 2,4-D showed high yielding calli in a range of 40 - 60% (Fig. 1 & 2). The callus percentage gradually increased with an increase in 2,4-D up to 3 mg/l. Fig.1 shows the effect of 2,4-D added to MS medium containing of 3 mg/l for BRRI dhan34 (59-63%), BRRI dhan79 (54-58%), BRRI dhan85 (54-58%), BRRI dhan51 (47-50%) and BRRI dhan51 (40-42%). Without adding 2,4-D in MS medium used as a control, where there was no sign of callus formation.

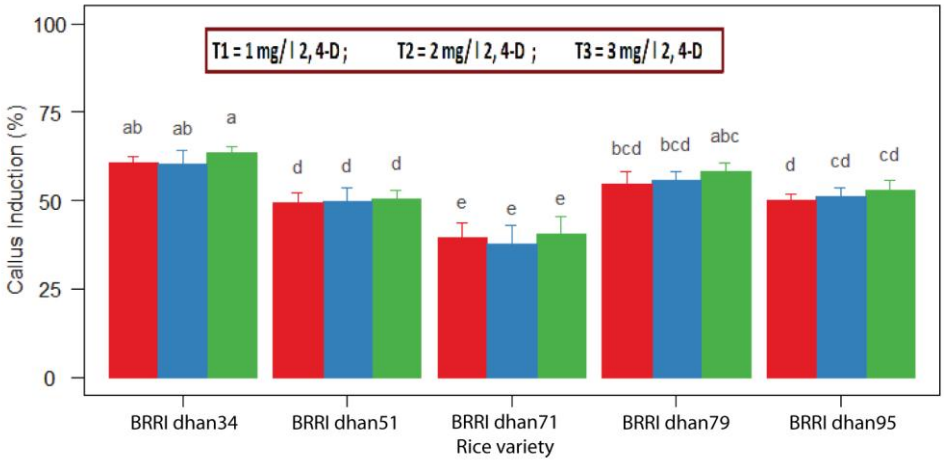

Fig. 1: Calli induction frequency in five rice genotypes supplemented with different concentration of 2,4-D in **MS** medium. Mean values of calli induction frequency marked with the same letters do not differ significantly ($p \leq 0.05$) in Tukey's test.

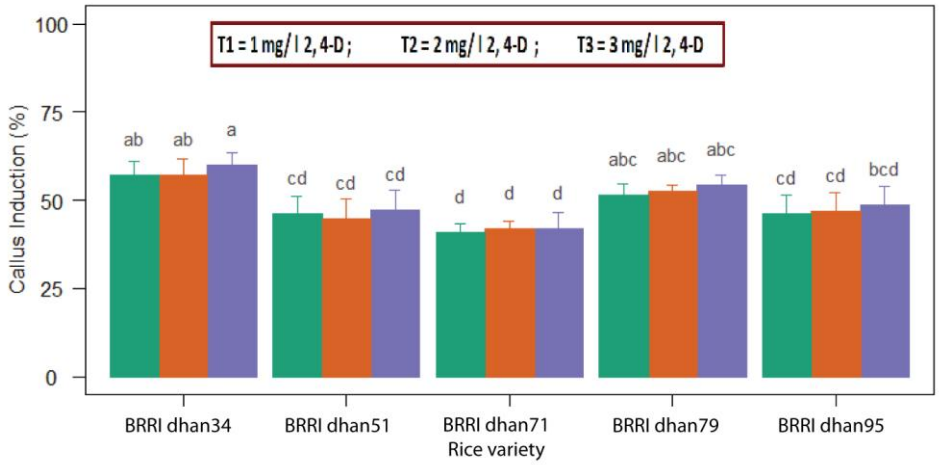

Fig. 2: Calli induction frequency in five indica rice genotypes (BRRI dhan34, BRRI dhan51, BRRI dhan71, BRRI dhan79 and BRRI dhan95) supplemented with different concentration of 2,4-D on **N6** basal medium. Mean values of calli induction frequency marked with the same letters do not differ significantly ($p \leq 0.05$) in Tukey's test.

For somatic embryogenesis, a number of PGRs are essential for cell division and differentiation was reported by Haque et al. (2015), and Lee & Huang (2013). Auxin is in charge of the creation of shoots inside various PGRs was reported by Meneses et al. (2005). Previous studies showed that callogenesis is mostly

influenced by the type and concentration of auxin as well as its external supply (Naik et al. 2017). As a result, in this investigation, the control treatment (0.0 mg/l 2,4-D) did not induce any calluses. Additionally, this study discovered that higher doses of 2,4-D reduced callus induction (Table 1).

Table 1: Effects of NS and MS medium on callus induction of five rice genotypes in Bangladesh.

Season	Genotypes	Basal media	
		N6 (% ± SD)	MS (% ± SD)
T. Aman	BRRI dhan95	48.8 ± 5.07^{bc}	53 ± 2.74^{bc}
	BRRI dhan79	54.2 ± 2.77^{ab}	58 ± 2.55^{ab}
	BRRI dhan71	42 ± 4.53^{c}	40.4 ± 5.13^{d}
	BRRI dhan51	47.2 ± 5.81^{bc}	50.4 ± 2.41^{c}
	BRRI dhan34	59.8 ± 3.70^{a}	63.4 ± 1.95^{a}
Analysis of variance			
Df		4	4
Sum Square		1493.4	930.8
Mean Square		373.3	232.70
F. value		37.41	11.49
Significance		***	***

Within column, mean ± SD values. Significance symbol ($p<0.05$), according to Tukey multiple comparisons of means test (n = 5). $^{ns}p \geq 0.05$, $^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$. Bars without shared letters indicate significant differences.

In comparison to MS for BRRI dhan71, the CIF on N6 was higher. For BRRI dhan95, BRRI dhan79, BRRI dhan34, and BRRI dhan51, the CIF on MS was higher than the CIF on N6. However, there was no significant difference between MS and N6 (Table 1). The callus percentage of the BRRI dhan71 was practically identical to that of the cultures on MS and N6 media. In a previous Rahman et al. (2015) demonstrated that the majority of rice calluses were induced from MS medium but with varied auxin treatments. According to the current study, auxin affected the frequency of callus induction but varied between types, which is consistent with a prior study (Shahsavari et al. 2010). The results of this study also demonstrated that auxin had an impact on the responses of several rice types, which were distinct. According to Bevitori et al. (2014), rice somatic embryogenesis is influenced by a number of variables, including explant age, donor plant genotype, nutritional media, auxin concentration, and ambient circumstances (Islam et al. 2013, Islam and Haque 2016).

Plant regeneration

It was decided to test the potential of high-quality embryogenic calli for plant regeneration by transferring them to regeneration medium (RM). We employed calli from the most responsive callus induction media e.g. MS and N6 with 3.0 mg/l 2,4-D to evaluate their efficiency on regeneration. Here, Table 2 displays the frequency of plantlet regeneration derived from calli of five rice genotypes under this study. Between the regeneration media and various concentration of plant growth regulators plants regeneration (%) of five studied rice genotypes were differed (Table 2). In the majority of the types, RM3 and RM2 showed the highest plantlet regeneration. Whereas, BRRI dhan79 showed the highest (28-32%) and BRRI dhan95 in RM_1 showed the lowest (18%) regeneration (Table 2).

Table 2: The effect of different concentration (mg/l) of BAP + Kin + 0.5 NAA in MS and N6 media on plantlet regeneration (%) from the calli of five different rice genotypes.

Genotypes	Treatments	Shoot regeneration (% ± SE)	
		MS	N6
BRRI dhan95	RM_1	21.25±1.25	18.75±1.25
	RM_2	23.75±1.25	21.25±2.39
	RM_3	25.00±2.04	22.50±1.44
	RM_4	23.75±1.25	20.00±2.04
BRRI dhan79	RM_1	28.75±1.25	28.75±1.25
	RM_2	32.50±1.44	23.75±1.25
	RM_3	32.75±1.25	32.75±1.25
	RM_4	30.00±2.04	22.50±1.44
BRRI dhan71	RM_1	22.50±1.44	21.25±1.25
	RM_2	28.75±1.25	23.75±1.25
	RM_3	28.00±0.00	26.75±1.25
	RM_4	26.25±1.25	25.00±2.04

Contd. Table 2

BRRI dhan51	RM_1	27.50±1.44	22.50±1.44
	RM_2	22.50±1.44	21.25±1.25
	RM_3	27.75±1.25	26.25±2.39
	RM_4	18.75±1.25	22.50±1.44
BRRI dhan34	RM_1	26.25±1.25	23.75±1.25
	RM_2	21.25±1.25	26.25±1.25
	RM_3	26.50±1.44	24.50±1.44
	RM_4	23.75±2.39	22.50±1.44

RM_1 = 2 mg/l BAP + 0.5 mg/l NAA, RM_2 = 2 mg/l BAP + 0.5 mg/l NAA + 1 mg/l Kin, RM_3 = 2 mg/l Kin + 0.5 mg/l NAA, RM_4 = 1 mg/l BAP + 0.5 mg/l NAA + 1 mg/l Kin.

After two weeks of transferring calli to regeneration medium it shows becoming greenish of the calli on the RM. After three weeks, the green patches continued to develop into numerous shoot primordia, *in vitro* plantlets, and finally, healthy plants in the pot (Fig. 3 C-G). In the current investigation, it was discovered that for all five kinds of rice varieties, the percentage of plantlet regeneration were increased significantly with rising of cytokinin (RM3) concentrations in addition to the medium (Table 2). Rahman et al. (2015) analysis different culture media and found that NAA showed better performance than others. They also found that without NAA, regenerated plantlets were weaker. Higher concentrations of BAP and Kin may enhance the first round of cell division and be crucial for somatic embryogenesis and plantlet regeneration was reported by Rueb et al. (1994), Haque and Islam (2015 & 2019) and Khatun et al. (2010 & 2012). According to the results all five rice genotypes had the greatest impact on plantlet regeneration where 2.0 mg/l BAP + 2.0 mg/l Kin + 0.5 mg/l NAA were added in the culture medium. By regulating mitosis, cytokinesis, total protein synthesis, lignin biosynthesis, vascular differentiation, and the differentiation of mature chloroplasts from protoplastids, kinetin has been shown in a previous study to aid improve somatic embryogenesis (Abe and Futsuhara 1986, Morshed et al. 2014 & 2016). On regeneration media, greenish of the callus was noticed after 21 days of culture initiation, and all five kinds of rice thereafter showed signs of regenerated shoot and plantlet regeneration. Numerous plantlets produced from somatic embryos were successfully acclimated to *ex-vitro* settings. The plant's roots were cleaned before being planted in soil for 10 days (Fig. 3G). The BRRI dhan79 had the highest rate of plant survival, followed by BRRI dhan 95, BRRI dhan 34, BRRI dhan 71, and BRRI dhan 51. Plants were exposed to their natural surroundings after 10 days. It was noted that plants had impressive development after 30 days (Fig. 3G). After two weeks in the natural environment, the plants that had been restored on RM3 medium survived perfectly.

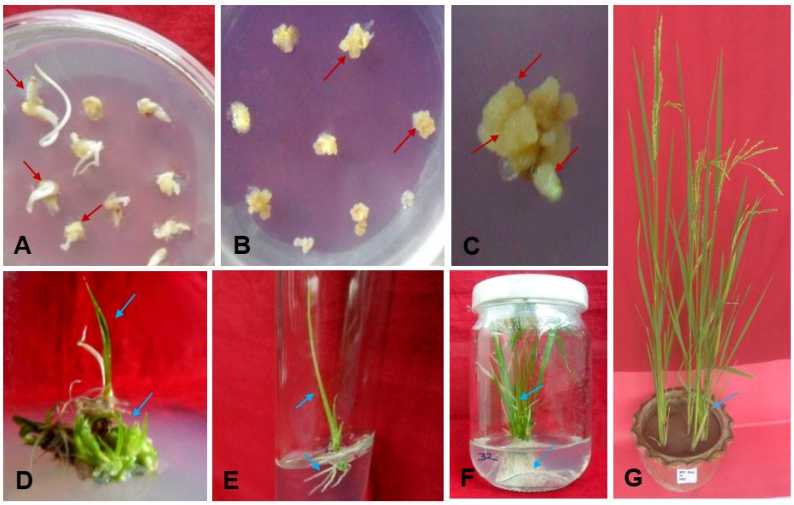

Fig. 3(A-G): Inoculated of mature rice seeds and several steps for development of calli and its subsequent regeneration. A) 4-5 days of inoculated seeds started calli. B) 2-3 weeks old calli separated from rice seeds, C) 2-3 weeks old calli were transferred to regeneration medium which showed becoming greenish, D) Seed derived calli started to regeneration, E) Green plantlets with good root and shoots, F) Plants were grown in pots, G) Plants were transferred to pots after successful acclimatization.

Conclusion

In this work, five distinct indica rice cultivars were used to examine *in vitro* callus induction and its subsequent regeneration. In this case five rice genotypes, two basal media and various plant growth regulators (2,4-D, NAA, Kin, NAA) were examined to improve callus induction and plant regeneration frequency. Here, all rice genotypes showed a greater callus induction frequency with 3 mg/l 2,4-D. The frequency of callus induction did not significantly differ when 2,4-D and NAA were used in combination. Compared to N6 a higher frequency of callus induction was found in MS. The RM3 (modified MS), the regeneration was higher than others three combinations. This study demonstrated the potential for the PGRs in combination with 2 mg/l BAP, 2 mg/l Kin, and 0.5 mg/l of NAA enhanced regeneration efficiency under the studied rice genotypes. Hope those genotypes can be used for further advance research in biotechnology for rice improvement in Bangladesh.

Conflict of interest

The authors declare no conflicts of interest.

Acknowledgements

The authors are grateful to Institute of Biological Sciences, University of Rajshahi, Bangladesh for providing fellowships, lab and related other facilities for this study.

Conflict of interest: The authors hereby declare no conflict of interest regarding the publication of this article.

References

Abe T and Futsuhara Y (1986). Genotypic variability for callus formation and plant regeneration in rice (*Oryza sativa* L.). Theoretical and Applied Genetics 72: 3-10.

Abiri R, Maziah M, Shaharuddin NA, Yusof ZAB, Atabaki N, Hanafi MM, Sahebi M, Azizi P, Kalhori N and Valdiani A (2017). Enhancing somatic embryogenesis of Malaysian rice cultivar MR219 using adjuvant materials in a high-efficiency protocol. International Journal of Environmental Science and Technology 14: 1091-1108.

Abuhena M, Al-Rashid J, Azim MF, Khan MNM, Kabir MG, Barman NC and Huq MA (2022). Optimization of industrial (3000 L) production of *Bacillus subtilis* CW-S and its novel application for mini-tuber and industrial-grade potato cultivation. Scientific Reports 12(1): 11153.

Ahmad FI, Wagiran A, Abd Samad A, Rahmat Z and Sarmidi MR (2016). Improvement of efficient *in vitro* regeneration potential of mature callus induced from Malaysian upland rice seed (*Oryza sativa* cv. Panderas). Saudi Journal of Biological Sciences 23(1): S69-S77.

Bevitori R, Popielarska-Konieczna M, Dos Santos EM, Grossi-de-Sá MF and Petrofeza S (2014). Morpho-anatomical characterization of mature embryo-derived callus of rice (*Oryza sativa* L.) suitable for transformation. Protoplasma 251: 545-554.

Chu C, Chu WC, San, SC, Chen H, Chu YK and Yin CC (1975). Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Scientia Sinica 18(5): 659-668.

Datta SK (2004). Rice biotechnology: A need for developing countries. AgBioForum 7(1&2): 31-35.

Dievart A, Perin C, Hirsch J, Bettembourg M, Lanau N, Artus F and Guiderdoni E (2016). The phenome analysis of mutant alleles in leucine-rich repeat receptor-like kinase genes in rice reveals new potential targets for stress tolerant cereals. Plant Science 242: 240-249.

Haque M and Islam SMS (2015). Callus age and size of barley (*Hordeum vulgare* L.) improves regeneration efficiency. Notulae Scientia Biologicae 7(2): 188-191, DOI: 10.15835/nsb.7.2.9497.

Haque M and Islam SMS (2019). An efficient regeneration system through *in vitro* somatic embryogenesis of barley (*Hordeum vulgare* L.). Journal of Bio-Science 27: 89-99. doi: https://doi.org/10.3329/jbs.v27i0.44674.

Haque M, Siddique AB and Islam SMS (2015). Effect of silver nitrate and amino acids on high frequency plants regeneration in barley (*Hordeum vulgare* L.). Plant Tissue Culture and Biotechnology 25(1):37-50.

Hiei Y and Komari T (2008). *Agrobacterium*-mediated transformation of rice using immature embryos or calli induced from mature seed. Nature Protocols 3(5): 824-834.

Islam SMS and Haque M (2016). Effect of carbon sources and plant growth regulators on efficient callus induction and regeneration in barley (*Hordeum vulgare* L.). Asia-Pacific Journal of Molecular Biolology and Biotechnology 24(1): 2-11.

Islam SMS, Ara I, Tuteja N and Subramaniam S (2013). Efficient microspore isolation methods for high yield embryoids and regeneration in rice (*Oryza sativa* L.). World Academy of Science, Engineering and Technology International Journal of Biological Science and Engineering 7(12): 891-896.

Juturu VN, Mekala GK, Garladinne M, Reddy PCO and Sekhar AC (2016). Optimization of *in vitro* regeneration protocol for a popular Indica rice (*Oryza sativa* L. cv Swarna). Annals of Plant Sciences 5: 1395-1401.

Kalhori N, Nulit R, Go R, Zulkifly S, Azizi P and Abiri R (2017). Selection, characterizations and somatic embryogenesis of Malaysian salt-tolerant rice (*Oryza sativa* cv. MR219) through callogenesis. International Journal of Agriculture and Biology 19(1): 157-163.

Karthikeyan A, Pandian STK and Ramesh M (2009). High frequency plant regeneration from embryogenic callus of a popular indica rice (*Oryza sativa* L.). Physiology and Molecular Biology of Plants 15: 371-375.

Khatun R, Islam SMS and Miah MAB (2010). Studies on plant regeneration efficiency through *in vitro* micropropagation and anther culture of twenty-five rice cultivars in Bangladesh. Journal of Applied Science and Research 6(11): 1705-1711.

Khatun R, Islam SMS, Ara I, Tuteja N and Bari MA (2012). Effect of cold pre-treatment and different media in improving anther culture response in rice (*Oryza sativa* L.) in Bangladesh. Indian Journal of Biotechnology 11(4): 458-463.

Lee ST and Huang WL (2013). Cytokinin, auxin, and abscisic acid affects sucrose metabolism conduce to de novo shoot organogenesis in rice (*Oryza sativa* L.) callus, Botanical Studies 54: 1-11.

Meneses A, Flores D, Muñoz M, Arrieta G and Espinoza AM (2005). Effect of 2, 4-D, hydric stress and light on indica rice (*Oryza sativa*) somatic embryogenesis. Revista de Biología Tropical 53(3-4): 361-368.

Mohiuddin AKM, Sultana S, Ferdous J and Karim NH (2006). Increased regeneration efficiency in seed derived callus of rice (*Oryza sativa* L.). Plant Tissue Culture and Biotech. 16(1): 45-52.

Morshed S, Abu Baker Siddique AB and Islam SMS (2016). Improvement of embryogenesis and regeneration by air desiccation in maize (*Zea mays* L.). Pakistan Journal of Botany 48(3): 1139-1144.

Morshed S, Siddique AB and Islam SMS (2014). Efficient plant regeneration using mature and immature embryos of maize (*Zea mays* L.). International Journal of Agriculture Innovations and Research 3(3): 2319-1473.

Mostafiz SB and Wagiran A (2018). Efficient callus induction and regeneration in selected indica rice. Agronomy 8(5): 77.

Murashige T and Skoog F (1962). A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiologia Plantarum 15(3): 473-497.

Naik N, Rout P, Umakant N, Verma RL, Katara JL, Sahoo KK, Sing ON and Samantaray S (2017). Development of doubled haploids from an elite indica rice hybrid (BS6444G) using anther culture. Plant Cell, Tissue and Organ Culture 128: 679-689.

Nic-Can GI and Loyola-Vargas VM (2016). The role of the auxins during somatic embryogenesis. Somatic embryogenesis: fundamental aspects and applications 171-182. doi: 10.1007/978-3-319-33705-0_10.

Rahman ZA, Ramli A, Kamaruzaman R, Seman ZA, Othman AN, Zainal Z, Uddain J and Subramaniam S (2015). Efficient plant regeneration of Malaysian aromatic rice (*Oryza sativa* L.) via improved somatic embryogenesis pathway. Emirates journal of Food and Agriculture 857-863.

Rahman ZA, Suri Roowi WZW and Subramaniam S (2010). Regeneration of Malaysian indica rice (*Oryza sativa*) variety MR232 via optimized somatic embryogenesis system. Journal of Phytology 2(3).

Rueb S, Leneman M, Schilperoort RA and Hensgens LAM (1994). Efficient plant regeneration through somatic embryogenesis from callus induced on mature rice embryos (*Oryza sativa* L.). Plant Cell, Tissue and Organ Culture 36: 259-264.

Sandhu N and Kumar A (2017). Bridging the rice yield gaps under drought: QTLs, genes, and their use in breeding programs. Agronomy 7(2): 27.

Shahsavari E, Maheran AA, Akmar ASN and Hanafi MM (2010). The effect of plant growth regulators on optimization of tissue culture system in Malaysian upland rice. African Journal of Biotechnology 9(14): 2089-2094.

Siddique AB, Ara I, Islam SMS and Tuteja N (2014). Effect of air desiccation and salt stress factors on *in vitro* regeneration of rice (*Oryza sativa* L.). Plant Signaling Behavior 9:12 (e977209- 1-10).

Silva TD (2010). Indica rice anther culture can the impasse be surpassed? Plant Cell, Tissue and Organ Culture 100: 1-11.

Talapatra S, Ghoshal N and Raychaudhuri SS (2014). Molecular characterization, modeling and expression analysis of a somatic embryogenesis receptor kinase (SERK) gene in *Momordica charantia* L. during somatic embryogenesis. Plant Cell, Tissue and Organ Culture 116: 271-283.

Wójcikowska B and Gaj MD (2017). Expression profiling of auxin response factor genes during somatic embryogenesis induction in *Arabidopsis*. Plant Cell Reports 36: 843-858.

Zimmerman JL (1993). Somatic embryogenesis: a model for early development in higher plants. The Plant Cell 5(10): 1411.

Zuraida AR, Naziah B, Zamri Z and Sreeramanan S (2011). Efficient plant regeneration of Malaysian indica rice MR 219 and 232 via somatic embryogenesis system. Acta Physiologiae Plantarum 33: 1913-1921.

(Manuscript received on 9th March 2024 and revised on 22nd April 2024)