

Research Article

Development of a Technique for Callus Induction and Plant Regeneration in *Oryza sativa* L. var. MRQ74 and MR269

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Abstract: The objective of the present study is to develop callus induction and plant regeneration; an experiment was conducted to investigate the effect of growth regulators on callus induction in rice mature embryo culture. In this experiment, callus was initiated from the mature seed scutellum of two Malaysian indica rice, MRQ74 and MR269. Callus was induced using different concentrations of 2, 4-D and BAP on MS medium under dark condition or under 16/8 h photoperiod conditions. For complete plant regeneration, the calli of both varieties were planted for shoot regeneration on MS media supplemented with different concentrations of BAP and NAA, for root regeneration on IBA. The results revealed that the maximum callus inductions in MRQ74 and MR269 were observed in the MS medium containing 3 mg/L 2, 4-D with 0.1 mg/L BAP under dark conditions. Furthermore, our protocol uses mature seeds as the explants in both rice varieties in terms of callus induction. Additionally, green plant production of plants was also observed in all of the media tested for shoot regeneration and MS media supplemented with 3 mg/L of BAP and 0.1 mg/L of NAA was found to be responsible for the recovery of more highly regenerated shoots. Effective root induction (100%) was achieved in a medium containing 1mg/LIBA. The described protocol can be useful in the establishment of an *in vitro* system for rice that can be a major technique for a genetic transformation system and crop improvement.

Keywords: Callus induction, growth regulators, illumination, plant regeneration

INTRODUCTION

Rice (*Oryza sativa* L.) is the paramount food crop globally and feeds over half of the world's population (International Rice Genome Sequencing Project, 2005; Amarawathi *et al.*, 2008). It plays an important role as a staple food crop and is utilized in feeding more than three billion people resulting in a daily caloric intake of between 50 to 80% (Khush, 2005). The need for rice is correlated with population that is expected to rise more than 38% within 30 years, according to the United Nations (Satyanarayana, 2005). Rice is a widely consumed food crop and is grown on 160 million hectares worldwide (FAO, 2007). Asian-grown rice is an important cereal crop for world food security and nutrition, particularly for developing countries (Vaughan *et al.*, 2008). The cultivable area under rice needs to be increased to improve the production

demands. MRQ74 is a special rice variety officially released by Malaysian Agricultural Research and Development Institute (MARDI) in February 2005 (Suhaimi *et al.*, 2009). Indica rice variety MRQ74 is an aromatic variety selected from a cross involving Q34, KhawDawk Mali and Kasturi. Early generation selections were of slender grain type with aroma, a short, erect plant type with good panicle characteristics and it has slender and long grain shape, moderate alkali spreading value, moderately soft gel consistency and high amylose content. These characteristics produce flaky and non-sticky cooked rice with aroma. It is different from the imported aromatic rice which gives very sticky and soft cooked rice. MRQ74 has been planted under aerobic and organic culture and gives a good yield. It is recommended that MRQ74 be licensed to any interested party with marketing a good level (Ramli *et al.*, 2008). MARDI in 2012 once again

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managed to develop new rice varieties namely MR269. This new breed of rice produced is usually one of hybrid varieties that have better resistance to pests and diseases and it is of high yields (MIATE, 2012).

The progress of a highly effective plant regeneration system *in vitro*, such as breeding using genetic transformation and somaclonal variation, is a prerequisite for the development of crops through many modern biotechnological means (Zhao *et al.*, 2009). Tissue culture of rice from mature embryos has several advantages, including a supply of plant material without the restriction of the geographical environment and season, fewer infections by microorganisms and easy operation. Consequently, this technology is used widely by many experts of rice biotechnology (Li-na *et al.*, 2010). Callus cultures are major tools in biotechnology. Manipulation of the plant growth regulators such as cytokinin to auxin ratio in the plant tissue culture media can lead to the development of somatic embryos, roots or shoots (Kadhimi *et al.*, 2014b). The whole plants can subsequently be produced (Tariq *et al.*, 2008). The solicitation of biotechnology in combination with conventional breeding techniques can properly enhance food production. Effective plant regeneration by micropropagation *in vitro* is important for the effective use of biotechnology techniques in rice crop development (Hoque *et al.*, 2007).

MATERIALS AND METHODS

Seed material and explant preparation: Two rice (*Oryzasativa* L.) varieties (MRQ74 and MR269) were used for this study. For *in vitro* establishment, rice seeds were dehusked and surface sterilized in a laminar air flow cabinet and the seeds were completely washed with sterilized distilled water 3 times. The seeds were then washed with 70% ethanol for 2-3 min. Then, seeds were treated with 0.1% (w/v) mercuric chloride (HgCl₂), with the addition of a few drops of Tween-20 for inner surface sterilization for 4-6 min. Finally, the seeds were washed several times with sterilized, distilled water to remove all of the chemical sterilizing agents (Zinnah *et al.*, 2013).

An *in vitro* technique is the effective biotechnological technique to create the variation by the means of somaclonal variation. To optimize this method, there is a strong need to understand the factors affecting the numerous aspects of callus induction for repeatable success in rice tissue culture (Shanthi *et al.* 2010). Response of rice seeds to callus induction is highly reliant on by the culture media and the genotypes (Kadhimi *et al.* 2014a; Htwe *et al.* 2011). The main problem of the plant tissue culture media of indica varieties are the low rate of somatic embryogenesis, callus induction, and plant regeneration system (Chu and Croughan, 1990). Some of the rice *Oryza sativa* L. varieties showed low rate of callus induction without plant regeneration (Gueye and Ndir, 2010).

The objective of this study was to develop an effective *in vitro* technique using growth regulators for callus induction employing mature seeds of Malaysian rice MRQ74 and MR269 varieties with respect to their callus induction and plant regeneration. An effective improvement of callus induction and plant regeneration will be used for a variety of transformation techniques and will pave the way to introducing agriculturally useful traits into MRQ74 and MR269, providing the best chance for improved quality of the crop.

Callus induction: Explants seeds prepared were cultured under aseptic conditions on gelrite solidified callus induction medium. The endosperm of the seed was within the medium and the embryo is exposed on the surface. Five sterilized seeds were cultured per petri dish callus induction medium and one-hundred seeds of each treatment were cultured with ten replicates, each replicate with ten seeds. Callus induction medium, containing semi-solid MS medium (Murashige and Skoog, 1962) added with vitamins supplemented with growth regulators 2,4-D (2, 3, 4 mg/L) and BAP (0, 0.1, 0.5 mg/L), with 3% sucrose and 3 g/L gelrite were added per litre of the medium for callus induction, before autoclaving, pH (5.7±0.1) of the medium was adjusted to the cultures that were incubated under dark conditions or 16/8 hours photoperiod condition, at 25±2°C for four weeks with sub culturing every two weeks. The data were expressed as the percentage of callus induction (%). After four weeks of incubation, data were recorded on the performance of total callus induction frequency, only embryogenic calli were selected and non-embryogenic calli were discarded. The calli were classified as embryogenic or non-embryogenic using visual characters according to Nabors *et al.* (1983) and Peterson and Smith (1991). Embryogenic rice callus is compact, smooth with knobby in appearance and white in colour, while necrotic and non-embryogenic calli (rough to crystalline in appearance, yellow to translucent and wet) was discarded; callus presenting a heterogeneous appearance was considered as embryogenic. Callus induction frequency (%) was calculated as follows:

$$\text{Frequency of callus induction (\%)} = \frac{\text{Number of explants producing callus}}{\text{Number of explants planted}} \times 100$$

The callus growth rate was taken on a per day basis of callus graded and induction through comparison in four categories: very poor (+), poor (++), good (+++) and very good (++++). The effect of the callus morphology, such as colour, pigmentation, texture, appearance, etc., was observed as per plant growth regulators treatments.

Shoot regeneration from callus: Eight-week-old embryogenic calli were selected and high-quality embryogenic calli from (3 mg/L 2, 4-D with 0.1 mg/L BAP under dark conditions) in the previous experiment were cultured on MS media supplemented with BAP (2,3,4 mg/L) and NAA (0.1, 0.5 mg/L), 3% sucrose and 3 g/L gelrite, to examine the influence of shoot induction media on subsequent regeneration frequencies. Regeneration cultures during 4 weeks were kept at (25±2)°C under a 16/8 h (light/dark) photoperiod. The frequencies of regeneration were determined as the percentage of calli producing fully regenerated plants. Percentage of shoot induction, number of shoot callus and length of shoot (cm) were recorded to investigate the suitable medium for shoot induction. The regeneration response of each genotype was calculated as follows:

$$(\%) \text{ of shoot induction} = \frac{\text{Number of calli producing plant}}{\text{Total number of calli on the regeneration media}} \times 100$$

Root induction: When shoots grew to approximately 4-6 cm in height, they were separated aseptically from each other and transferred to freshly prepared rooting medium. Regenerated shoots from highest of shoot induction (3 mg/L BAP with 0.1 mg/L NAA), were rooted by culturing on MS medium supplemented with different concentrations of (1,2,3 mg/L) IBA, 3%

sucrose and 3 g/L gelrite and were kept at (25±2)°C under a 16/8 h (light/dark) photoperiod. Six weeks after culturing, the number of shoot-forming roots and the % root induction, number of root and root length (cm) per shoot were recorded to investigate a suitable medium for root induction.

Acclimatization of tissue-cultured plants: The regenerated plantlets with well-developed roots were washed under running tap water to clear off the residual gelrite medium to check the chances of contamination in soil. The regenerated plantlets were transferred to small (8 cm) diameter polystyrene tea cups containing an autoclaved mixture of red soil, sand and vermiculite sterilized in an autoclave in growth chamber, were covered with polythene bags to prevent excessive evapotranspiration and the covers were removed after week of hardening in the plant growth chamber. Selection of the hardened plants that recovered well after three weeks were successfully established in pots and the plantlets were transferred to the glasshouse where they developed into mature plants (Plate 1).

Statistical analysis: The experiment was designed as a factorial experiment based on a CRD (completely randomized design). The factors included; for callus induction 2, 4-D (3 levels: 2; 3; 4 mg/L) and BAP (3 levels: 0; 0.1; 0.5 mg/L) under Dark or 16/8 hours

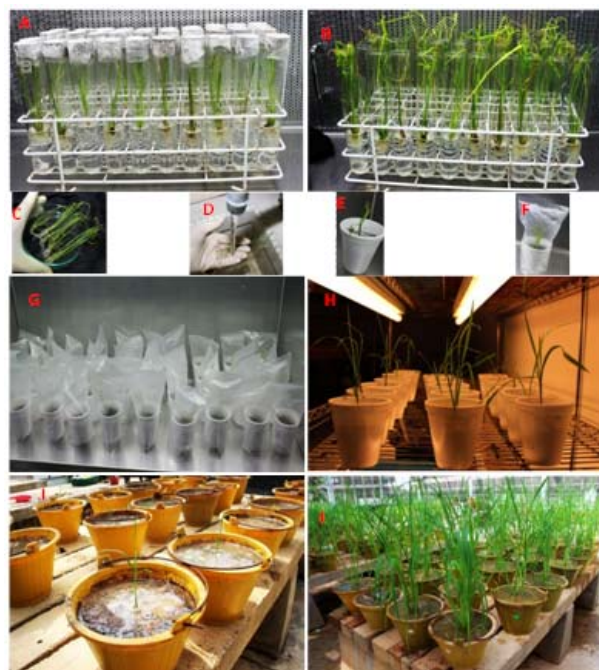


Plate 1: Various stages of in vitro propagation and acclimatization of MRQ74 and MR269: (A): Complete in vitro plantlet having healthy shoots and roots from embryogenic callus, (B) a well-rooted shoot in media, (C) and (D) well-developed roots were washed under running tap water, (E) Regenerated plants transplanted in the soil (mixture of red soil, sand and vermiculite), (F) Covered with polythene bags for one week, (G) Acclimatization of plantlet under growth chamber, (H) A plant 3 weeks after acclimatization under culture room conditions, (I) Acclimatized and hardened plantlets in soil glasshouse conditions, (J) Tissue culture rice plant established in the glasshouse

photoperiod conditions. For shoot induction NAA (2 levels: 0.1; 0.5 mg/L) and BAP (3 levels: 2; 3; 4 mg/L). For root induction one factors included IBA (3 levels: 1; 2; 3 mg/L). In this study ten replications with three samples per each replication were applied. The data were subjected to the normality test prior to analysis of variance using SAS programme (Release 9.1 for Windows, Version 6.1.7600, Software SAS 9.1.3 Service Pack 4 XP_PRO platform, SAS Institute Inc., Cary, NC, USA) software. Significant differences among the mean values of treatments were determined using the Duncan's Multiple Range Test (DMRT) and the Least Significant Difference was calculated at $\alpha = 0.05$ level.

RESULTS AND ANALYSIS

Callus induction under dark conditions: The experiments of this study for callus induction and plant regeneration in rice were conducted in two varieties, (MRQ74 and MR269). Callus formation was developed from the scutellar region of the seeds. The callus was induced by using the mature embryo as explant and the medium used was MS (Murashige and Skoog, 1962) media containing different concentrations (2, 3 and 4 mg/L) of 2,4-D and (0,0.1 and 0.5 mg/L) BAP under dark conditions or under 16/8 hours photoperiod. The overall frequency (%) of callus induction under dark

conditions was found to be better than that under 16/8 hours photoperiod. The results in Table 1 showed that by using 3 mg/L 2,4-D and 0.1 mg/L BAP under dark conditions, the callus induction frequency was the highest for MR268 (97.20%) and lowest for MRQ74(78.50%).

The calli were also compared for the mean rate of callus growth under dark conditions and under 16/8 hours photoperiod containing different concentrations of 2, 4-D and BAP (Table 2). Two varieties showed the rate of callus growth was very good on media containing 3 mg/L 2, 4-D and 0.1 mg/LBAP under dark conditions. It was observed that for both of the rice varieties (MRQ74 and MR269), MS medium supplemented with 3 mg/L2, 4-D and 0.1 mg/LBAP proved to be the best combination in terms of callus growth. It is important that under dark conditions, the embryogenicity of the callus produced is important for plant regeneration (Plate 2 and 3(A, C and E).

Callus induction under 16/8 hour photoperiod: The callus was induced from mature embryo as an explant and the medium used was MS media containing different levels of 2,4-D and BAP under 16/8 h photoperiod. The data pertaining to callus induction of rice varieties (MRQ74 and MR269) are given in Table 1. It was observed that for both varieties, MS medium supplemented with 2, 4-D and BAP effected callus

Table 1: Effect of different concentrations of 2, 4-D, BAP and illuminations (dark or 16/8 hours photoperiod) on the percentage of Rice var. MRQ74 and MR269 callus induction (%)

2,4-D (mg/L) Concentration	BAP (mg/L) Concentration	Callus induction (%) var.MRQ74		Callus induction (%) var.MR269	
		Dark	16/8 hours Photoperiod	Dark	16/8 hours Photoperiod
2	0.0	55.70e	59.70d	73.70f	54.80f
	0.1	64.30cd	61.00d	92.80b	61.90e
	0.5	63.20d	62.10d	81.70d	66.00d
3	0.0	72.40b	68.70bc	80.10de	75.10c
	0.1	78.50a	72.00ab	97.20a	86.00a
	0.5	71.00b	73.90a	92.40b	80.00b
4	0.0	58.20e	68.10c	77.60e	75.80c
	0.1	67.20c	71.20abc	91.70bc	84.10a
	0.5	64.90cd	70.00bc	89.70c	77.60bc

Different letters in each column show significant difference at the 5% level by DMRT

Table 2: Effect of different concentrations of 2, 4-D, BAP and illuminations (16/8 hours photoperiod or dark) on the response of callus growth rice var. MRQ74 and MR269

2,4-D (mg/L) Concentration	BAP (mg/L) Concentration	Callus growth var.MRQ74		Callus growth var.MR269	
		Dark	16/8 hours Photoperiod	Dark	16/8 hours Photoperiod
2	0.0	+	+++	+++	+
	0.1	++	++	+++	+++
	0.5	++	++	++	+++
3	0.0	+++	++++	+++	++++
	0.1	++++	+++	++++	+++
	0.5	+++	++	+++	+++
4	0.0	+	++	+++	+++
	0.1	+++	++	+++	++
	0.5	++	+	++	++

Very poor (+), poor (++) , good (+++) and very good (++++)

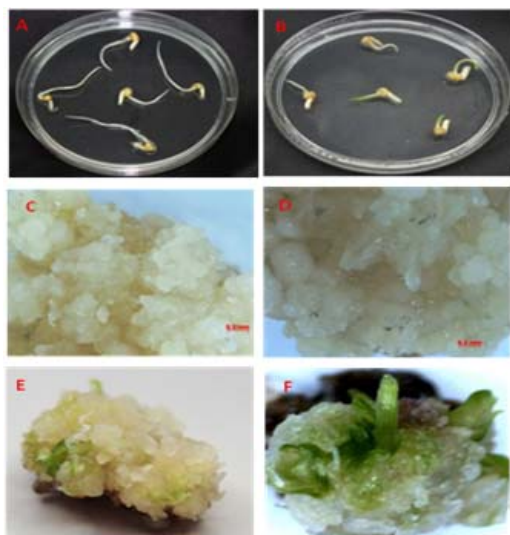


Plate 2: Plant regeneration from mature embryo-derived embryogenic callus indica rice MRQ74: (A) Callus induction from mature seed embryos on callus induction medium with 3 mg/L 2,4-D and 0.1 mg/L BAP after 2 weeks of culture under dark condition, (B) Callus induction from mature seed embryos on callus induction medium with 3 mg/L 2,4-D and 0.1 mg/L BAP after 2 weeks of culture under 16/8 hours photoperiod condition, (C) Photographs were taken under electron microscope for callus induced under dark condition, (D) Photographs were taken under electron microscope for callus induced under 16/8 h photoperiod condition, (E) Plant regenerated from embryogenic callus of culture on medium with 3 mg/L BAP and 0.1 mg/L NAA, (F) Photographs were taken under electron microscope for somatic embryo initiation, turned to green

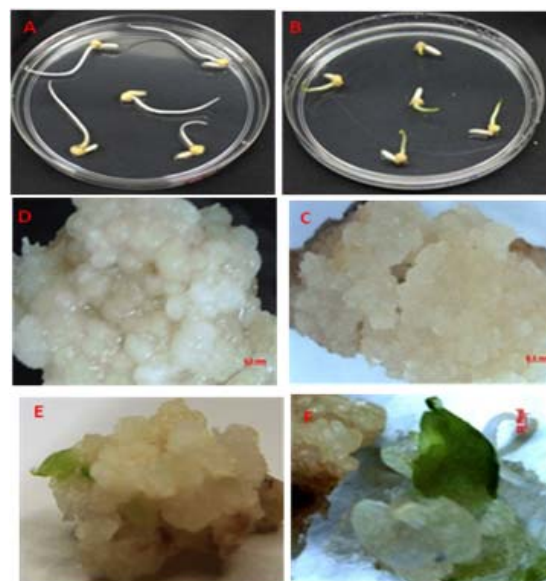


Plate 3: Plant regeneration from mature embryo-derived embryogenic callus indica rice MR269: (A) Callus induction from mature seed embryos on callus induction medium with 3 mg/L 2,4-D and 0.1 mg/L BAP after 2 weeks of culture under dark condition, (B) Callus induction from mature seed embryos on callus induction medium with 3 mg/L 2,4-D and 0.1 mg/L BAP after 2 weeks of culture under 16/8 hours photoperiod condition, (C) Photographs were taken under electron microscope for callus induced under dark condition, (D) Photographs were taken under electron microscope for callus induced under 16/8 h photoperiod condition, (E) Plant regenerated from embryogenic callus of culture on medium with 3 mg/L BAP and 0.1 mg/L NAA, (F) Photographs were taken under electron microscope for somatic embryo initiation, turned to green

induction. However, callus induction percentages were highest 72.00% and 73.90% at 2,4-D 3 mg/L + BAP 0.1 mg/L; and 2,4-D 3 mg/L + BAP 0.5 mg/L respectively under a 16/8 h photoperiod for MRQ74, but the percentages of callus induction were 86.00, 84.10% for 2,4-D 3 mg/L+ BAP 0.1 mg/L and 2,4-D 4 mg/L+ BAP 0.1 mg/L for MR269. Both the varieties effect and interaction between varieties and plant growth regulators were statistically significant. The maximum and minimum responses to callus induction were clearly shown by MR269 (86.00%) and MRQ74 (73.90%) (Table 1, Plate 2 and 3(B, D and F).

Significant differences in the rate of callus growth were observed among the varieties as well as among the 2,4-D and BAP concentrations tested under 16/8 h photoperiod (Table 2); however, different concentrations of 2, 4-D and BAP were used to determine their effect on the rate of callus growth. Significant differences were also observed in growth between treatments. The data show that 3 mg/L of 2, 4-D alone increased the both of the rice varieties (MRQ74 and MR269) and gave the highest callus growth. This callus, when sub-cultured, showed vigorous growth 2,4-D has been shown to be useful for producing calli.

The effect of media composition on shoot regeneration:

The different plant growth regulators are widely used for shoot induction and multiplication in tissue culture studies. The experimental results of the effect of BAP and NAA on the percentage of shoot induction, number of shoot callus and length of shoot (cm) in the rice varieties (MRQ74 and MR269) was studied and the results are presented on Fig. 1 and 2(A to C). It was observed that the plant regeneration ability of plated calli depends on the variety and growth regulators. The highest percentage of shoot induction of MRQ74 was 61% (Plate 4A) and MR269 was 73% (Plate 5A) obtained on media with 3 mg/L BAP and 0.1 mg/L NAA. The highest production of multiple shoots from callus on MR269 was obtained when treated with 3 mg/L BAP and 0.1 mg/L NAA resulting in 20 multiple shoots, combined with MR269, the highest significant effect were obtained on 3 mg/L BAP with (0.1 or 0.5) mg/L NAA. On the other hand, MS media containing 2 mg/L BAP in combination with 0.1 mg/L, NAA produced the lowest percentage of shoot induction and number of shoot callus in both varieties.

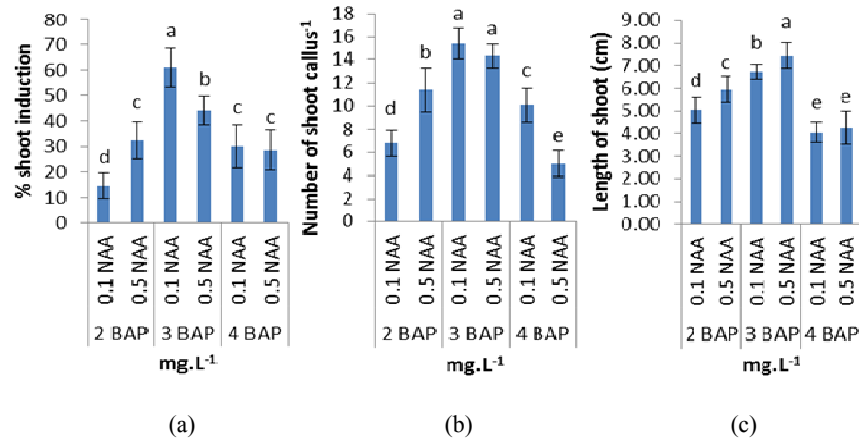


Fig. 1: (A, B, C) Effect of different concentrations of BAP and NAA on % of shoot induction, number of shoot callus and length of shoot (cm) of Rice var. MRQ74. Similar letters show non-significant differences at the 0.05 level by DMRT

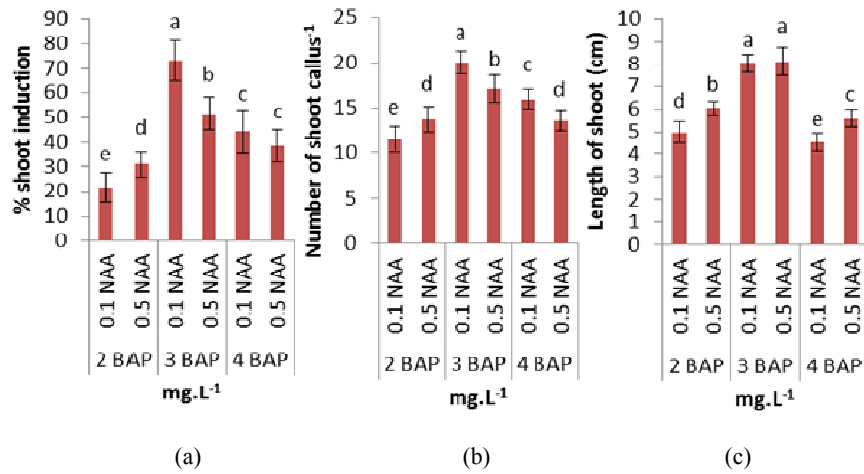


Fig. 2: (A, B, C) Effect of different concentrations of BAP and NAA on % of shoot induction, number of shoot callus and length of shoot (cm) of Rice var. MR269. Similar letters show non-significant differences at the 0.05 level by DMRT

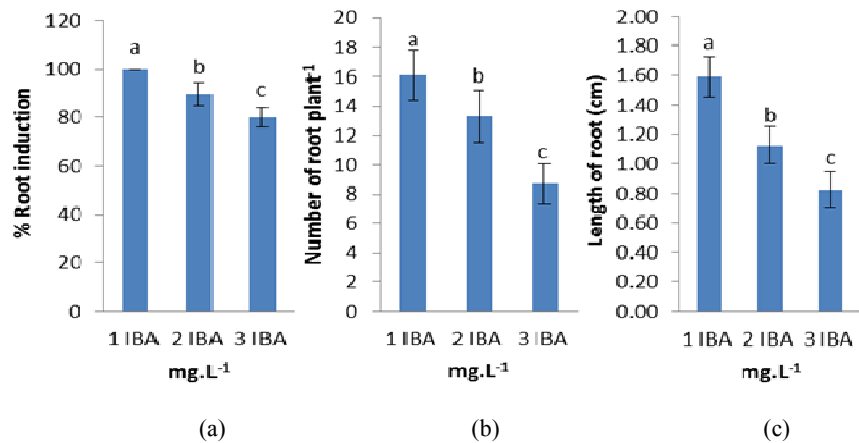


Fig. 3: (A, B, C) Effect of different concentrations of IBA on % root induction, number of root/plant, and length of root (cm) of Rice var. MRQ74; Similar letters show non-significant differences at the 0.05 level by DMRT

In general, the length of shoot in MR269 was higher compared to MRQ74 and it was observed that the significant length of the shoot was obtained on media containing 3 mg/LBAP and 0.1 or 0.5 mg/L

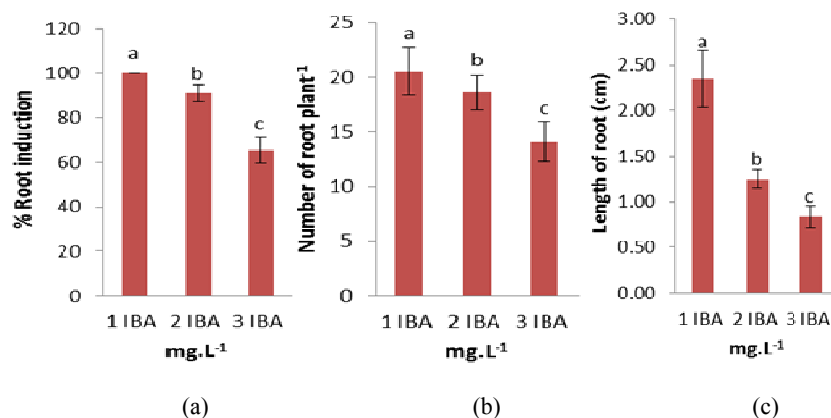


Fig. 4: (A, B, C) Effect of different concentrations of IBA on % root induction, number of root/plant, and length of root (cm) of Rice var. MR269. Similar letters show non-significant differences at the 0.05 level by DMRT

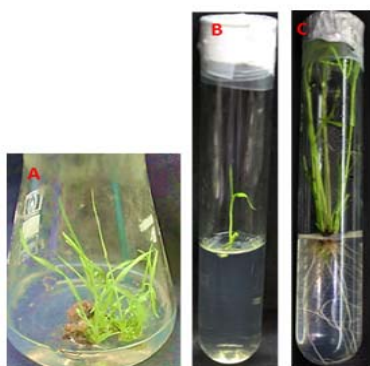


Plate 4: Development of plantlets from mature embryos of MRQ74: (A) Shoot regeneration from embryogenic callus in MS media supplemented with 3 mg/L BAP and 0.1 mg/L NAA, (B) Root formation of regenerated shoots cultured on MS media supplemented with 1 mg/L IBA, (C) Development of root

NAA was higher in MR269 compared with MRQ74 with the highest response to length of shoot obtained at 3 mg/LBAP and 0.5 mg/LNAA.

The Effect of media composition on root induction:

Roots were formed when regenerated shoots were transferred onto MS medium containing IBA at the concentrations of 1,2,3 mg/L. The experimental results of the role of IBA on the % root induction, length (cm) and number of root per plant are shown on Fig. 3 and 4(A to C). All IBA concentrations examined on both of the rice varieties produced an average of % induction of root. On the other hand, 1 mg/L IBA in rooting media of MRQ74 and MR269 produced the highest number of root (16.1 and 20.5 plant⁻¹) and longer roots (1.59 and 2.34 cm), respectively (Plate 4C and 5C), compared to the treatment with 2,3 mg/L IBA.

DISCUSSION

Production of an embryogenic callus with high regeneration ability is a prerequisite for a highly effective method of rice transformation. Several experiments have been performed on selection-appropriate explants in rice to enhance embryogenic calli under suitable culture conditions. In the present study, a strong effect of a combination of 2,4-D with BAP on callus induction was also observed. However, this combination was crucial for callus production.

Plant growth regulators have a record of playing an important role in callus cultures, and the effects of various growth regulators on plant regeneration in rice callus cultures has been intensively investigated (Rueb *et al.*, 1994; Sripichitt and Cheewasestatham, 1994; Mannan *et al.*, 2013). Usually, media for induction and proliferation of rice callus requires a strong auxin, of which 2,4-D is preferred, while for plant regeneration, a growth regulator-free medium or a medium containing

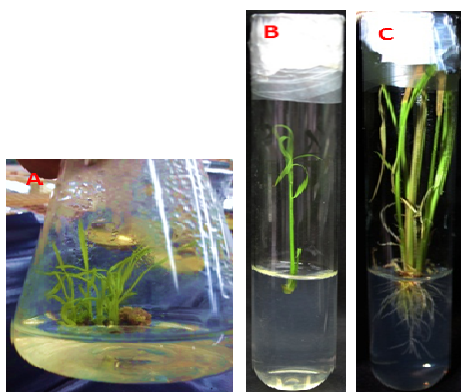


Plate 5: Development of plantlets from mature embryos of MR269: (A) Shoot regeneration from embryogenic callus in MS media supplemented with 3 mg/L BAP and 0.1 mg/L NAA, (B) Root formation of regenerated shoots cultured on MS media supplemented with 1 mg/L IBA, (C) Development of root

a weak auxin in combination with a cytokinin (BAP) is used. Conversely, exposing callus cultures to the high ratios of auxin with cytokinin resulted in root formation (Joyia and Khan, 2012).

Auxins with cytokinins interact at the concentration of plant growth regulator perception and signal transduction in plants to control many core growth processes (Joyia and Khan, 2012). Our results further revealed that the use of 2,4-D with BAP could be helpful for a high percentage of callus induction and callus growth. Similar observations were also reported in rice (Mannan *et al.*, 2013). Taken together, the findings from this study will be very beneficial for enhancing high a frequency of callus induction that is a prime step for crop development or fast propagation by biotechnological approaches. It also revealed that the genotype of the explant may also be an important factor in callus induction.

In this regard, an attempt was made to increase callus induction percentage and growth of calli through employing different light conditions and concentrations of 2,4-D and BAP. Light is a major of the physical factor for callus induction, cell growth and plant production (Summart *et al.*, 2008). Pan and Staden (1998), observed that the effect of light is a very important factor that affects *in vitro*. It has been proved that it has an effect on growth and organized development tissue cultures. Light conditions for differentiation involve a combination of several requirements, including quality, intensity, and daily light period (Pan and Staden, 1998). On the other hand the degree of responsibility to light is dependent on cell type and rice genotype (Summart *et al.*, 2008). Calli of rice grown under dark conditions had higher cell mass than those grown under light conditions (16/8 h light/dark) (Summart *et al.*, 2008). Dark conditions offer great promise for the induction of higher quality calli of rice and the promotion of cell growth (Summart *et al.*, 2008).

Enhanced callus proliferation in dark and light tends to promote embryogenesis, but calli enhanced and proliferated in dark conditions and gave high regeneration and more plants/seed calli across genotypes (Biswas and Mandal, 2007). The presence of light is not necessary for callus growth and in most reports, callus inductions are conducted in the dark (Seraj *et al.*, 1997). However, there are some reports in which light is used for callus proliferation and maintenance (Kunuvatchaidach *et al.*, 1995). Rice callus grown under dark condition had great promise for the induction of higher quality of callus and the promotion of cell growth than that under (16/8 h light/dark) condition (Summart *et al.*, 2008). 2,4-D generates DNA hypermethylation, which maintains the cells in a highly active mitotic stage and, therefore, in a pro-embryonic phase (Endress, 1994). Meneses *et al.* (2005) reported that under darkness conditions, indirect somatic embryo genesis from mature embryos of rice,

was induced by the auxin 2,4-D at concentrations between (4.52-9.05 μ M). The degree of responsiveness to illumination is dependent on plant species and cell type. Various regeneration media were tested. A combination of specific concentrations of auxin and cytokinin has appeared to play a significant role in enhancing regeneration from MRQ74 and MR269 calli.

Silvarajan *et al.* (2012) showed that the regeneration of Malaysian indica rice MR219 from the exogenous application of auxin with cytokinin has a significant effect on shoot and root. Traiq *et al.* (2008) reported higher frequencies of regeneration of two varieties i.e., Basmati-370 and Basmati-371 were observed on BA 2.5 mg/L and 1 mg/L NAA. Al-Jubair *et al.* (2008) also found that the combination of 3 mg/L BA with 0.5 mg mg/L NAA with 0.5 mg/L Kinetin was a suitable media for regeneration in a rice variety. However, Sripichitt and Cheewasestatham (1994) observed an effect of MS medium supplemented with 4 mg/L BA and 1 mg/L IAA induced the highest percentage of calli-forming shoots. Mannan *et al.* (2013) reported that MS medium supplemented with 0.5 mg mg/L BAP and 0.1 mg/L IBA exhibited the highest range of shoot number. Our study shows that it is now possible to obtain high frequencies of regeneration in MRQ74 and MR269 varieties belonging to the indica group.

CONCLUSION

Optimal media compositions were chosen on the basis that they give a highly significant effect of callus induction. In our investigation, the highest percentage of callus induction in MR269 than MRQ74 could be achieved by culturing the mature seeds on MS media supplemented with 3 mg/L 2,4-D with 0.1 mg/L BAP under dark conditions. However, 2, 4-D with BAP plays a role in the callus induction. Therefore, supplementing with 2, 4-D and BAP in the media can enhance the callusing response of both varieties. Additionally, the protocols presented here were developed for the regeneration of embryogenic callus of rice varieties into plantlets. We have established that a combination of MS media supplemented with 3 mg/L of BAP combination 0.1 mg/L of NAA is suggested for a plantlet regeneration media. The suitable medium for root induction was achieved when the regenerated shoots were transferred onto MS media with 1 mg/L IBA. This method can be incorporated into a genetic engineering programme to introduce desirable agronomic traits in Malaysian rice varieties.

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ABBREVIATIONS

2,4-D : 2, 4-dichlorophenoxy acetic acid
BAP : 6-benzilaminopurina
NAA : α -naphthalene acetic acid
IBA : Indole-3-butyric acid
MS : Murashige and Skoog medium

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