# Asian Journal of Plant Sciences

ISSN 1682-3974

science alert

ANSInet
an open access publisher
http://ansinet.com

Asian Journal of Plant Sciences 11 (4): 182-189, 2012
ISSN 1682-3974 / DOI: 10.3923/ajps.2012.182.189


# The Effect of Plant Growth Regulators and Organic Supplements on Callus Induction and Plant Regeneration in Rice (*Oryza sativa* L.)

[1]K. Rattana, [1]P. Theerakulpisut and [2]S. Bunnag
[1]Genomics and Proteomics Research Group for Improvement of Salt-tolerant Rice,
Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[2]Department of Biology, Faculty of Science, Khon Kaen University,
Khon Kaen 40002, Thailand

**Abstract:** Abiotic and biotic stresses are the limiting factors of growth and productivity of rice in many agriculture areas of Thailand. Plant Tissue cultures have been used for breeding purpose, especially in selection for stress tolerance. The aim of this study was to investigate and find out enhancement on callus induction and regeneration in rice cultivars; Khao Dawk Mali 105, Supanburi 1, Chai Nat 1 and Pathum Thani 1. The suitable media for callus induction of KDML 105, Supanburi 1, Chai Nat 1 and Pathum Thani 1 were NN medium supplemented with 2, 1.5, 2.5 and 1 mg $L^{-1}$ 2,4-D, respectively. It was found that addition of 300 mg $L^{-1}$ casein hydrolysate enhanced callus formation in KDML 105. Combination with 300 mg $L^{-1}$ casein hydrolysate and 1,000 mg $L^{-1}$ L-proline can influence for callus induction in Supanburi 1. Addition of 500 mg $L^{-1}$ L-proline can enhance callus induction of Chai Nat 1 and Pathum Thani 1. The MS medium supplemented with 3 mg $L^{-1}$ BA and 300 mg $L^{-1}$ casein hydrolysate was suitable for regeneration of KDML 105. The most suitable medium for regeneration in Supanburi 1 was MS medium combination with 3 mg $L^{-1}$ BA, 0.5 mg $L^{-1}$ NAA and 500 mg $L^{-1}$ L-proline. Addition of 5 mg $L^{-1}$ BA, 0.5 mg $L^{-1}$ NAA and combination with 300 mg $L^{-1}$ casein hydrolysate in MS medium were the optimal for regeneration in Chai Nat 1. Combination with 5 mg $L^{-1}$ BA and 300 mg $L^{-1}$ casein hydrolysate can influence for regeneration in Pathum Thani 1.

**Key words:** Callus induction, organic supplement, plant regeneration, rice, abiotic stress biotic stress, casein hydrolysate

## INTRODUCTION

Rice (*Oryza sativa* L.) is a staple food for Thai population. However, rice yield are affected by disease and insect pest, as well as by environmental stress such as drought, chilling temperature, acidified soil and saline soil. Plant breeder attempts to improve rice by using biotechnology. Therefore, plant tissue culture has also become an important tool for breeding improvement in rice (Ge *et al*., 2006).

Plant tissue culture is a technique for culture of cells, tissue and organs under aseptic conditions. The advantage of tissue culture is applied in cell behavior, plant modification and improvement, pathogen free plants and germplasm storage, clonal propagation and product formation (Thorpe, 2007). The repeated subculture of plant affected on variation in morphological, agronomical, physiological and biochemical characters. This variation in tissue culture, regenerate plants and their progenies is described as somaclonal variation (Collin and Edwards, 1998). Consequently, somaclonal variation can be applied for selection of admirable characterization in plant breeding program (Thadavong *et al*., 2002). The application of tissue culture for rice improvement depends on genotypes, explants, plant growth regulators, basal salts of culture medium, organic supplements and culture conditions. High efficiency of production in callus and plant regeneration is very important for rice improvement. Callus induction has been developed though utilization of basal components in cultured media. The MS (Murashige and Skoog, 1962) and N6 (Chu *et al*., 1975) media have been widely used in the rice tissue culture (Ge *et al*., 2006). Auxin such as 2,4-D is most commonly used to induce the different parts of rice for callus growth (Yang *et al*., 1999; Thadavong *et al*., 2002; Saharan *et al*., 2004; Htwe *et al*., 2011). In addition, combination with auxin and cytokinin is widely used to enhance callus induction and maintenance. Other reports showed that the addition of NAA or IAA can enhance the quality of initiated callus (Ge *et al*., 2006). There are many reports that investigation of organic supplement on callus induction such as addition of casein hydrolysate and proline (Khaleda and Al-Forkan, 2006). Casein hydrolysate is a source of calcium, several micronutrients,

**Corresponding Author:** Sumontip Bunnag, Department of Biology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand Tel: 66 43 342908/3207, 0818074700

vitamins and amino acid which used for callus induction in rice (Htwe *et al*., 2011). Proline is a kind of amino acid provided plant cells as a source of nitrogen. It can be uptake more than an organic component (Khaleda and Al-Forkan, 2006).

In addition, there are several reports are studied on increasing of regeneration in rice under *in vitro* culture. Plant growth regulators are an important factor for initiation and regeneration of plant (Wani *et al*., 2010). The successful of regeneration in rice also depends on genotypes and the developmental stage of the explants (Hoque and Mansfield, 2004). Application of various types and concentration of plant growth regulators is widely used to increase of plant regeneration. Cytokinin was used for stimulating cell division, as well as for formation and growth of axially and adventitious shoots. Addition of 6-benzylaminopuirine (BA) and 6-furfurylaminopurine (kinetin) in regeneration medium are widely applied for shoot regenerations in rice (Chuenboonngarm *et al*., 2001). Combination of cytokinin and auxin is known to promote plant regeneration frequency in some rice cultivar (Rueb *et al*., 1994). There are several reports on high efficiency on plant regeneration of rice. The regeneration medium supplemented with casein hydrolysate, proline or other organic supplement also enhanced in shoot regenerations. In addition, the desiccation treatments have been reported on the beneficial for embryogenesis and regeneration in rice (Wagiran *et al*., 2008).

Therefore, the objectives of this research were to study the effects of plant growth regulators and organic supplements on callus induction and regeneration in rice cv. Khao Dawk Mali 105, Supanburi 1, Chai Nat 1 and Patum Thani 1. Application of this study should facilitate the generation of somaclonal variation, *in vitro* selection, crop improvement and other biotechnological objectives.

## MATERIALS AND METHODS

**Measurement of callus induction in rice:** Mature seeds of rice cv. Khao Dawk Mali 105 (KDML105), Supanburi 1, Chai Nat 1 and Pathum Thani 1 were surface sterilized with 70% (v/v) ethanol for 5 min and 30% (v/v) Clorox® with 2-3 drops of Tween-20 as a wetting agent for 30 min. After three washes in sterile water, these seed were cultured on NN medium (Nitsch and Nitsch, 1969) supplemented with 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mg $L^{-1}$ 2, 4 dichlorophenoxy acetic acid (2,4-D), 20 % (w/v) sucrose and 4 g $L^{-1}$ (w/v) gelrite (Phytagel; Sigma), pH = 5.7. The cultures were maintained under condition of 16 h photoperiod (light intensity 40 μmol $m^{-2}$ $sec^{-1}$) at 25±2°C. After 4 weeks of culture, the frequency of callus induction, the average size, fresh weight and dry weight of calli were recorded.

**Estimation of the effects of organic supplements on enhancement of callus formation in rice:** Sterilized seeds were cultured on NN medium supplemented with the optimal concentration of 2, 4-D in each cultivar and combination with organic supplements as shown in Table 1. The medium were adjusted pH to 5.7 before adding 4 g $L^{-1}$ (w/v) gelrite (Phytagel; Sigma). Sterile seed cultures were incubated at 25±2°C under light condition for 4 weeks. The average size, fresh weight and dry weight of calli were recorded.

**Measurement of plant regeneration in rice:** Four-week old calli were cultured on MS medium supplemented with 0 and 0.5 mg $L^{-1}$ NAA combination with 0, 1, 2, 3, 4 and 5 mg $L^{-1}$ BA, 3% (w/v) sucrose and 4 g $L^{-1}$ (w/v) gelrite (Phytagel; Sigma). The cultures were incubated at 25±2°C under light condition. Four weeks after culture, the number of calli forming shoots, the average number of shoots per callus was determined.

**Estimating the effect of organic supplements on enhancement of plant regeneration in rice:** Four-week old calli were cultured on MS medium added with the optimal combination of NAA and BA in each cultivar and supplemented with 500, 1,000 mg $L^{-1}$ L-proline, 300, 600 mg $L^{-1}$ casein hydrolysate, 20, 40 g $L^{-1}$ sorbitol and 10, 20% coconut water. The cultures were incubated at 25±2°C under light condition. Four weeks after culture, the percentages of plant regeneration, the number of shoots per callus was recorded to determine the suitable supplements for enhancement the frequency of plant regeneration.

**Statistical analysis:** Statistical analysis of all parameters was analyzed as CRD using SPSS 11.5 for Windows (SPSS, Inc., Chicago, IL). All of data were analyzed by the Analysis of Variation (ANOVA). Significant difference at $p<0.05$ between treatments were evaluated by Duncan's Multiple Range Test (DMRT).

## RESULTS AND DISCUSSION

**The effect of 2,4-D on callus induction in rice:** The effect of 2,4-D on callus induction was observed at 3-4 days after cultured on NN medium containing 2,4-D at the concentrations of 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mg $L^{-1}$ under light condition. The result showed that, rice seeds cultured on NN medium without 2,4-D can not produce calli, but seed cultivated on NN medium supplemented with 2,4-D found that calli were initiated from scutellar region. The color of calli was yellow and their appearance of calli was nodular in all four rice cultivars (Fig. 1). There

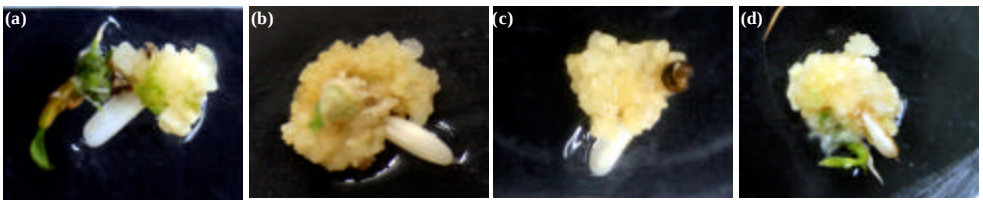


Fig. 1(a-d): Characterizations of calli in rice cultivars; (a) KDML105, (b) Supanburi 1, (c) Chai Nat 1 and (d) Pathum Thani 1 after cultured on NN medium supplemented with 2,4-D. Bar = 5 mm

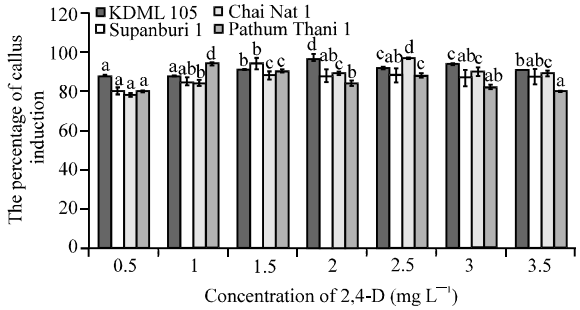


Fig. 2: The percentages of callus induction in four rice cultivars cultured on NN medium supplemented with 2,4-D; the different letters show the significant difference at $p<0.05$

Table 1: MS medium supplemented with organic supplement on enhancement for callus induction

| Treatment | Various kinds and concentration of organic supplements |
|---|---|
| 1 | NN+2,4-D |
| 2 | NN+2,4-D+50 mg $L^{-1}$ tryptophan |
| 3 | NN+2,4-D+100 mg $L^{-1}$ tryptophan |
| 4 | NN+2,4-D+500 mg $L^{-1}$ L-proline |
| 5 | NN+2,4-D+1,000 mg $L^{-1}$ L-proline |
| 6 | NN+2,4-D+300 mg $L^{-1}$ casein hydrolysate |
| 7 | NN+2,4-D+300 mg $L^{-1}$ casein hydrolysate+50 mg $L^{-1}$ tryptophan |
| 8 | NN+2,4-D+300 mg $L^{-1}$ casein hydrolysate+100 mg $L^{-1}$ tryptophan |
| 9 | NN+2,4-D+300 mg $L^{-1}$ casein hydrolysate+500 mg $L^{-1}$ L-proline |
| 10 | NN+2,4-D+300 mg $L^{-1}$ casein hydrolysate+1,000 mg $L^{-1}$ L-proline |

Table 2: The analysis of variances for the percentage of callus induction in four rice cultivars cultured on NN media supplemented with various concentration of 2,4-D

| Source of variation | df | Mean square | F value |
|---|---|---|---|
| Media | 6 | 215.187 | 12.484* |
| Cultivars | 3 | 225.591 | 13.088* |
| Media x Cultivars | 18 | 79.381 | 4.605* |
| Error | 112 | 17.237 | |

Level of significance are represented by (*) at $p<0.05$

are also reports that rice mature seed is the best parts used in tissue culture because it can induce embryogenic callus for complete shoot regeneration (Raina, 1989). After cultured 4 weeks, it was found that seeds of all rice cultivars cultured on NN medium containing 2,4-D in every concentration produced calli. The percentage of seed forming calli in each cultivar was recorded. The optimal concentration of 2,4-D for KDML105, Supanburi 1, Chai Nat 1 and Pathum Thani 1 were 2, 1.5, 2.5 and 1 mg $L^{-1}$, respectively. The percentages of callus induction in all rice cultivars were 96.89, 94, 97 and 94, respectively (Fig. 2). The analysis of variances for the percentage of callus induction in those cultivar was differently significant ($p<0.05$) as show in Table 2. The average size of calli obtained from mature embryos of KDML105, Supanburi 1, Chai Nat 1 and Pathum Thani 1 were 0.97, 0.89, 1.01 and 1.06 cm, respectively. The average fresh weights of calli were 0.25, 0.38, 0.27 and 0.35 g, respectively. The average dry weights of four rice cultivars were 0.07, 0.09, 0.04 and 0.07 g, respectively (Fig. 3).

Auxin is most commonly used to induce callus growth and callus formation (Yang *et al*., 1999; Thadavong *et al*., 2002; Saharan *et al*., 2004; Htwe *et al*., 2011). In addition other auxin such as IAA, NAA 2,4,5-T (2,4,5-Trichlorophenoxyacetic acid) can be also used for callus induction (Bhaskaran and Smith, 1990). However, the optimal concentration of 2,4-D depend on the explants and genotypes of rice (Raina, 1989). Michiba *et al*. (2001) reported that addition of high concentration of 2,4-D can inhibit growth of callus. Khan *et al*. (1999) reported that addition of higher concentration of 2,4-D may effected to higher frequency of albino shoot regeneration in rice. In this study found that 2,4-D at the concentration of 1-2.5 mg $L^{-1}$ were suitable for callus induction. It was agreed with Wen *et al*. (1991), Datta *et al*. (1992), Yin *et al*. (1993), Rashid *et al*. (2001), Hoque *et al*. (2007) and Jubair *et al*. (2008). They reported that addition of 2,4-D at the concentration between 1 to 2.5 mg $L^{-1}$ was suitable for callus induction.

**The effect of organic supplements on enhancement of callus formation in rice:** Mature seeds of KDML105, Supanburi 1, Chai Nat 1 and Pathum Thani 1 were cultured on NN medium supplemented with various concentrations of 2,4-D and supplemented with various concentrations of organic supplements (Table 1) were studied. It was found that no different significance ($p<0.05$) between organic supplements and the percentage of callus formation in four rice cultivars. However, supplements with organic

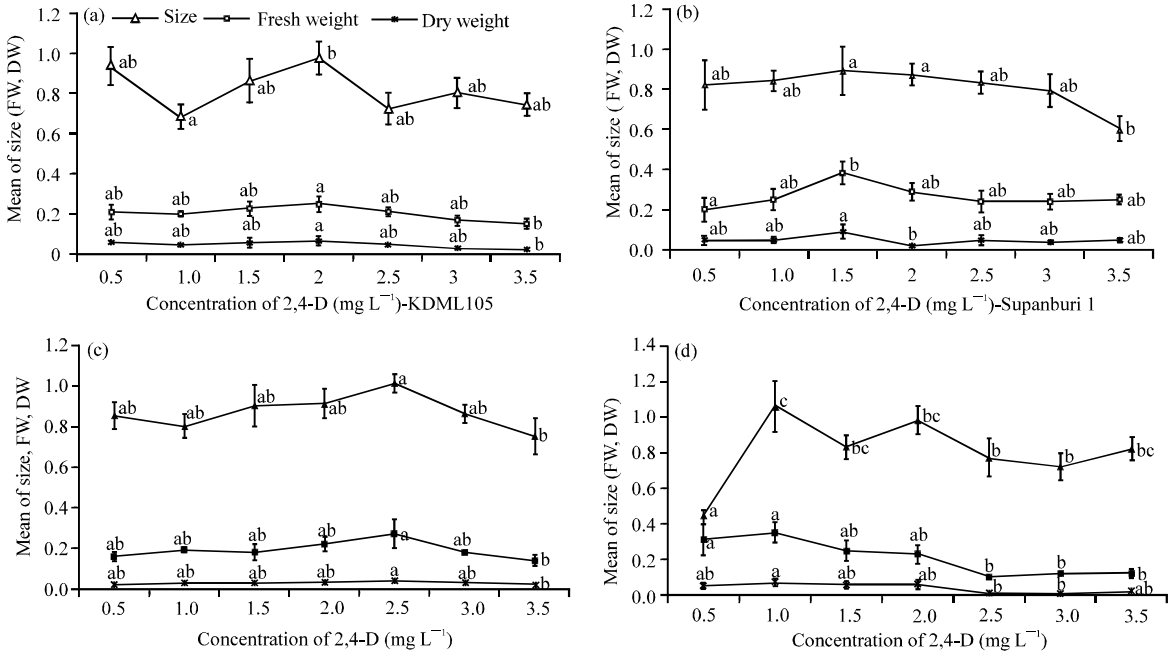


Fig. 3(a-d): The average size (mm), fresh weight (g) and dry weight (g) in calli of rice cultivars, (a) KDML105, (b) Supanburi 1, (c) Chai Nat 1 and (d) Pathum Thani 1 after culture on NN medium supplemented with various concentrations of 2,4-D, the different letters show the significant difference at p = 0.05

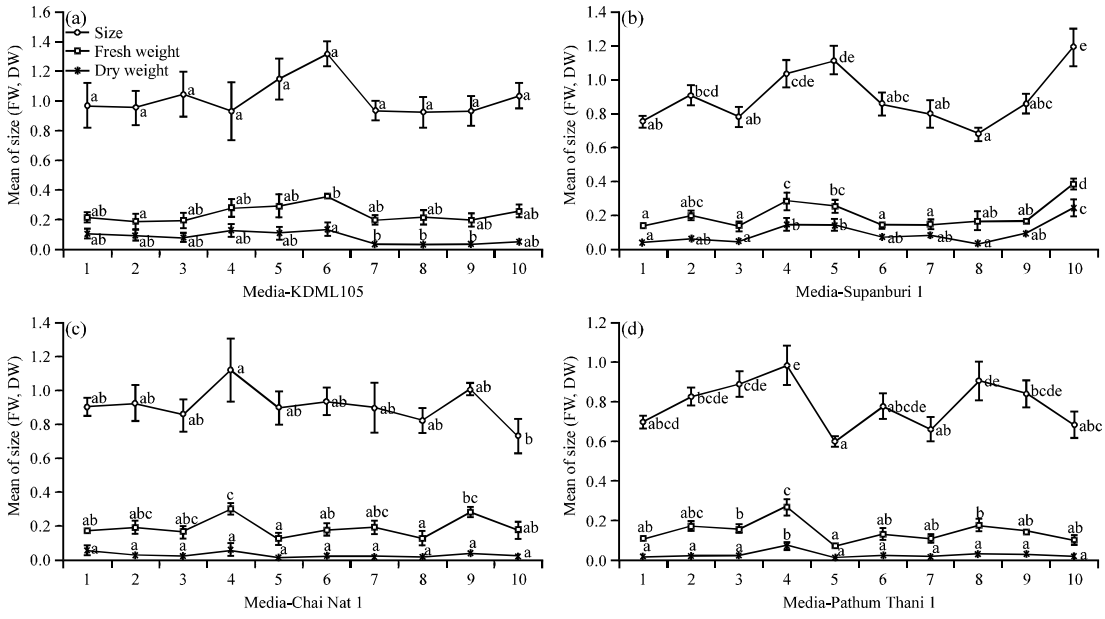


Fig. 4(a-d): The average size (mm), fresh and dry weight (g) in calli of rice cultivars, (a) KDML105, (b) Supanburi 1, (c) Chai Nat 1 and (d) Patum Thani 1 after culture on NN medium supplemented with various concentrations of organic supplements, the different letters show the significant difference at p = 0.05

supplements in callus cultured medium can enhance in size, fresh weight and dry weight. Addition of 300 mg L$^{-1}$ casein hydrolysate enhanced callus formation in KDML105; the average size of calli, fresh weight and dry weight were 1.31 cm, 0.36 g and 0.14 g, respectively (Fig. 4a). Rice cv. Supanburi 1 was cultured on NN medium supplemented with 300 mg L$^{-1}$ casein hydrolysate combination with 1,000 mg L$^{-1}$ L-proline influenced the

highest average size of callus (1.19 cm), fresh weight (0.38 g) and dry weight (0.24 g) (Fig. 4b). Additions of 500 mg $L^{-1}$ L-proline can enhance in callus induction of Chai Nat 1 and Patum Thani 1. The average size of callus of Chai Nat 1 was 1.12 cm and the average fresh weight and dry weight were 0.31 g and 0.06 g (Fig. 4c), respectively. The average size, fresh weight and dry weight of calli of Pathum Thani 1 were 1.12 cm, 0.35 g and 0.08 g (Fig. 4d), respectively.

Addition of 2,4-D in culture medium can induce callus formation from rice seed (Thadavong *et al*., 2002; Saharan *et al*., 2004; Sikder *et al*., 2006). Several reports revealed that some organic supplements such as casein hydrolysate, tryptophan and L-proline added to the medium could enhance efficiency of callus induction (Rueb *et al*., 1994; Thadavong *et al*., 2002). While, supplemented with 0.4 g $L^{-1}$ of casein hydrolysate was found useful for callus induction in rice (Htwe *et al*., 2011). Pravin *et al*. (2011) reported that addition of casein hydrolysate in MS medium increased production of embryogenesis calli. In this study, the NN medium containing 300 mg $L^{-1}$ casein hydrolysate was highly initiated callus induction in KDML105. However, Pongtongkam *et al*. (2004) reported that addition of 20 μM L-lysine can promote callus formation in rice cv. KDML105.

In this study, it was found that addition of 500 mg $L^{-1}$ L-proline can enhance callus induction in Chi Nat 1 and Patum Thani 1. The additions of the amino acid such as L-proline and tryptophan have benefited for culturing of embryogenic calli. Thadavong *et al*. (2002) revealed that seeds of rice cv. TDK1 were cultured on MS medium supplemented with 500 mg $L^{-1}$ L-proline enhanced the percentage of callus induction form 88.14 to 96.91. Three varieties of japonica rice show increasing of fresh weight in cell mass after 2 days incubation in media supplemented with 500 mg $L^{-1}$ L-proline (Wagiran *et al*., 2008). The use of glutamine, arginine and L-proline was found improvement the conversion of somatic embryos to plantlets in *Cryptomeria japonica* (Igasaki *et al*., 2006). While, the combination of 300 mg $L^{-1}$ casein hydrolysate with 1,000 mg $L^{-1}$ L-proline was suitable for enhancement of callus induction in rice cv. Supanburi 1. Saharan *et al*. (2004) reported that addition of 300 mg $L^{-1}$ casein hydrolysate combination with 500 mg $L^{-1}$ L-proline can influent callus induction in rice.

**The effect of BA and NAA on plant regeneration in rice:** Three-week old calli were transferred to MS medium supplemented with various concentrations of BA and NAA. After cultured for four weeks, the number of callus forming shoots and the number of shoots per callus were examined. It was found that green spots were appeared and developed to shoot after cultured for 2 week. Sahrawat and Chand (2001) reported that shoot bud started from nodular green spots after transferred to regeneration medium. In this study found that the percentages of shoot regeneration were differently significant ($p<0.05$) in all rice cultivars. The MS medium containing 3% sucrose, 0.4% gelrite supplemented with 3 and 5 mg $L^{-1}$ BA was suitable for regeneration of KDML105 and Patum Thani 1, respectively. Addition of 3 mg $L^{-1}$ BA combination with 0.5 mg $L^{-1}$ NAA and 5 mg $L^{-1}$ BA combination with 0.5 mg $L^{-1}$ NAA was suitable for regeneration of Supanburi 1 and Chai Nat 1, respectively. The highest regeneration percentages of KDML105, Supanburi 1, Chai Nat 1 and Pathum Thani 1 were 66.7, 44.4, 33.3 and 32, respectively. The number of shoots per callus was 6, 8, 3 and 3 shoots, respectively as shown in Table 3.

Cytokinin such as BA, Kinetin usually use for plant regeneration from embryonic callus in some rice variety (Rueb *et al*., 1994). In this study showed that addition of BA can induce callus growth of KDML105 and Pathum Thani 1 similarly to reported of Yang *et al*. (1999). They reported that the regeneration medium supplemented with 55.48 μM BA affected to the adventitious buds and the number of shoots per callus in rice. In the other hand, combination of auxin and cytokinin usually use for plant regeneration from embryonic callus in some rice variety (Rueb *et al*., 1994). In this study showed that combination of BA and NAA can induce callus growth of Supanburi 1 and Chai Nat 1. Sikder *et al*. (2006) reported that MS medium supplemented with 5 mg $L^{-1}$ BA combination with 0.05 mg $L^{-1}$ NAA is suitable for plant regeneration in rice cv. Chiniguri. Tariq *et al*. (2008) also found that N6 media added with 1 mg $L^{-1}$ NAA and 2.5 mg $L^{-1}$ BAP was optimal for shoot regeneration in rice. In the other hand, Lee *et al*. (2002) reported that Kinetin was found more effective for shoot regeneration in rice compared with BA. Jubair *et al*. (2008) also reported that combination of 3 mg $L^{-1}$ BA with 0.5 mg $L^{-1}$ NAA and 0.5 mg $L^{-1}$ Kinetin was the suitable media for regeneration in rice variety Topa. Moreover, Islam *et al*. (2004) reported that influence of shoot regeneration was obtained from calli cultured on media supplemented with 1 mg $L^{-1}$ 2,4-D, 2 mg $L^{-1}$ α-NAA and 1 mg $L^{-1}$ kinetin.

**The effect of organic supplements on enhancement of plant regeneration in rice:** Desiccated calli were cultured on suitable regeneration medium for each rice cultivar. In attempt to enhance of shoot regeneration; 500 and 1,000 mg $L^{-1}$ L-proline, 300 and 600 mg $L^{-1}$ casein

Table 3: The percentages of shoot regeneration and the average number of shoots/callus cultured on MS medium supplemented with various concentrations of BA and NAA in four rice cultivars

| | KDML105 | | Supanburi 1 | | Chai Nat 1 | | Pathum Thani 1 | |
|---|---|---|---|---|---|---|---|---|
| BA/NAA (mg $L^{-1}$) | RF (%) | Shoots/callus | %RF | Shoots/callus | %RF | Shoots/callus | %RF | Shoots/callus |
| 0 | 13.3$^{b}$ | 1.0$^{ab}$ | 20.0$^{b}$ | 1.0$^{a}$ | 4.0$^{a}$ | 1.0$^{a}$ | 0$^{a}$ | 0.00$^{a}$ |
| 1/0 | 26.6$^{c}$ | 2.8$^{d}$ | 26.6$^{c}$ | 3.0$^{cd}$ | 29.6$^{f}$ | 1.6$^{abc}$ | 8$^{c}$ | 2.00$^{bcd}$ |
| 2/0 | 60.0$^{f}$ | 3.0$^{d}$ | 26.6$^{c}$ | 2.5$^{bc}$ | 28.0$^{f}$ | 1.6$^{abc}$ | 12$^{d}$ | 1.34$^{bc}$ |
| 3/0 | 66.7$^{g}$ | 6.0$^{f}$ | 6.6$^{a}$ | 2.0$^{b}$ | 10.0$^{bc}$ | 2.6$^{cb}$ | 16$^{e}$ | 1.00$^{ab}$ |
| 4/0 | 60.0$^{f}$ | 3.8$^{de}$ | 26.6$^{c}$ | 5.7$^{f}$ | 17.9$^{d}$ | 1.4$^{ab}$ | 16$^{e}$ | 1.5$^{bcd}$ |
| 5/0 | 26.6$^{c}$ | 2.5$^{cd}$ | 33.3$^{d}$ | 2.4$^{bc}$ | 11.5$^{c}$ | 2.0$^{bc}$ | 32$^{h}$ | 3.00$^{d}$ |
| 0/0.5 | 0.0$^{a}$ | 0.0$^{a}$ | 6.6$^{a}$ | 2.0$^{b}$ | 11.5$^{c}$ | 2.0$^{bc}$ | 0$^{a}$ | 0.00$^{a}$ |
| 1/0.5 | 33.3$^{cd}$ | 1.4$^{bc}$ | 26.6$^{c}$ | 2.5$^{bc}$ | 8.3$^{b}$ | 1.6$^{abc}$ | 16$^{e}$ | 1.50$^{bcd}$ |
| 2/0.5 | 33.3$^{cd}$ | 2.8$^{d}$ | 33.3$^{d}$ | 3.4$^{de}$ | 5.6$^{c}$ | 2.0$^{bc}$ | 24$^{g}$ | 2.30$^{cd}$ |
| 3/0.5 | 46.6$^{e}$ | 4.8$^{ef}$ | 44.4$^{e}$ | 8.0$^{g}$ | 20.0$^{de}$ | 2.4$^{bcd}$ | 20$^{f}$ | 2.00$^{bcd}$ |
| 4/0.5 | 33.4$^{cd}$ | 2.6$^{cd}$ | 20.0$^{b}$ | 4.0$^{e}$ | 20.8$^{e}$ | 2.2$^{bcd}$ | 5b | 1.00$^{ab}$ |
| 5/0.5 | 40.0$^{d}$ | 3.5$^{d}$ | 20.0$^{b}$ | 1.0$^{a}$ | 33.3$^{g}$ | 3.0$^{d}$ | 0$^{a}$ | 0.00$^{a}$ |
| Significance$^{\delta}$ | * | * | * | * | * | * | * | * |

*level of significance are represented at $p<0.05$, Means followed by the same letter within each column are not significantly different using Duncan's multiple range test at 95%, RF: Regeneration frequency

Table 4: The percentage of shoot regeneration and average number of shoots/callus cultured on MS medium supplemented with various concentrations of organic supplements in four rice cultivars

| | KDML105 | | Supanburi 1 | | Chai Nat 1 | | Patum Thani 1 | |
|---|---|---|---|---|---|---|---|---|
| Media | RF (%) | Shoots/callus | RF (%) | Shoots/callus | RF (%) | Shoots/callus | RF (%) | Shoots/callus |
| Control | 66.5$^{cd}$ | 5.31$^{b}$ | 33.0$^{a}$ | 7.7$^{bc}$ | 33.8$^{ab}$ | 3.0$^{ab}$ | 33.3$^{a}$ | 3.0$^{ab}$ |
| 500 mg $L^{-1}$ L-proline | 51.1$^{abc}$ | 5.00$^{ab}$ | 66.0$^{d}$ | 8.0$^{c}$ | 37.5$^{abc}$ | 3.8$^{bc}$ | 30.0$^{a}$ | 2.6$^{a}$ |
| 1,000 mg $L^{-1}$ L-proline | 43.9$^{ab}$ | 4.78$^{ab}$ | 50.0$^{c}$ | 6.7$^{bc}$ | 31.3$^{a}$ | 2.8$^{a}$ | 33.3$^{a}$ | 2.8$^{a}$ |
| 300 mg $L^{-1}$ casein hydrolysate | 75.0$^{d}$ | 7.00$^{c}$ | 55.5$^{cd}$ | 7.0$^{bc}$ | 62.6$^{f}$ | 7.0$^{e}$ | 60.0$^{c}$ | 6.0$^{d}$ |
| 600 mg $L^{-1}$ casein hydrolysate | 43.3$^{ab}$ | 4.11$^{ab}$ | 47.2$^{bc}$ | 6.4$^{ab}$ | 43.8$^{bcd}$ | 3.6$^{abc}$ | 44.0$^{b}$ | 4.2$^{bc}$ |
| 20 g $L^{-1}$ sorbitol | 50.6$^{abc}$ | 5.00$^{ab}$ | 33.3$^{a}$ | 5.0$^{a}$ | 43.8$^{bcd}$ | 3.8$^{bc}$ | 53.5$^{c}$ | 4.8$^{cd}$ |
| 40 g $L^{-1}$ sorbitol | 58.3$^{bc}$ | 4.67$^{ab}$ | 34.2$^{ab}$ | 5.0$^{a}$ | 56.3$^{e}$ | 5.0$^{d}$ | 33.3$^{a}$ | 4.8$^{cd}$ |
| 10% coconut water | 41.1$^{a}$ | 3.22$^{a}$ | 55.5$^{cd}$ | 6.4$^{ab}$ | 46.8$^{ede}$ | 4.0$^{c}$ | 33.3$^{a}$ | 3.8$^{abc}$ |
| 20% coconut water | 43.3$^{ab}$ | 4.00$^{ab}$ | 55.5$^{cd}$ | 6.3$^{ab}$ | 50.0$^{de}$ | 5.6$^{d}$ | 33.3$^{a}$ | 3.4$^{abc}$ |
| Significance$^{\delta}$ | * | * | * | * | * | * | * | * |

*Level of significance are represented at $p<0.05$, Means followed by the same letter within each column are not significantly different using Duncan's multiple range test at 95%, RF: Regeneration frequency

hydrolysate, 20 and 40 g $L^{-1}$ sorbitol and 10 and 20% coconut water were added to the media. It was found that the percentage of shoot regeneration and the average number of shoots/callus were differently significant ($p<0.05$). In this study, it was found that the addition of 300 mg $L^{-1}$ casein hydrolysate in the medium can enhance the percentage of shoot and number of shoots per callus in KDML105, Chai Nat 1 and Patum Thani 1. The highest percentages of shoot were 75, 62.6 and 60 and the averages shoots per callus were 7, 7 and 6 shoots, respectively (Table 4). The most suitable organic supplement for enhancement of callus induction in Supanburi 1 was 500 mg $L^{-1}$ L-proline. The highest percentage of shoots was 66 and the average shoot per callus was 8 shoots as shown in Table 4. In this study found that regeneration frequency increased to 1-1.8 folds after cultured rice calli on media supplemented with organic supplements.

Thadavong *et al.* (2002) reported that addition of 800 mg $L^{-1}$ casein hydrolysate can enhance callus forming with green spots and shoot regeneration of rice cv. TDK1. L-proline are also used for shoot regeneration. Khaleda and Al-Forkan, 2006) demonstrated that plant regeneration media supplemented with L-proline stimulated plant regeneration in rice. Different reports have shown that many factors affect on plant regeneration in rice such as genotypes and type of media (Gioi and Tuan, 2002; Khatun *et al.*, 2003; Hoque and Mansfield, 2004; Shahnewaz *et al.*, 2004; Htwe *et al.*, 2011). Desiccated calli also advantaged for plant regeneration. Dehydration of calli for 24 h before transfer to regeneration medium was found greatly promotes plant regeneration for japonica rice (Tang *et al.*, 2000). Saharan *et al.* (2004) reported that shoot regeneration frequency increased from 1.2 to 5.6 fold after 48 h of rice callus desiccations. However, in this study desiccations of calli for 7 day produced shoot regeneration in all rice cultivars. Addition of 20 g $L^{-1}$ sorbitol also enhanced rice regeneration (Geng *et al.*, 2008; Wagiran *et al.*, 2008). Shahsavari (2010) reported that the highest regeneration of rice (78.7%) was observed in MS medium supplemented with 10 g $L^{-1}$ sorbitol. Naqvi *et al.* (2005) reported that sorbitol and mannitol increased green spots in callus and the percentages of shoot regeneration in indica rice.

Rooting of the regenerated plants from selected calli was also observed. After regenerated shoots in all rice cultivars were transferred to MS medium without of plant growth regulators. It was found that shoot can produce in normal root in all rice cultivars.

## CONCLUSION

In conclusion, it is confirmed that plant growth regulators and organic supplements were successfully enhanced on callus induction and shoot regeneration in rice cultivars; KDML105, Supanburi 1, Chai Nat 1 and Pathum Thani 1. The suitable medium for callus induction in KDML105 was NN medium supplemented with 2 mg $L^{-1}$ 2,4-D and 300 mg $L^{-1}$ casein hydrolysate. The optimal medium for callus induction in Supanburi 1 was NN medium supplemented with 1.5 mg $L^{-1}$ 2,4-D, 300 mg $L^{-1}$ casein hydrolysate and 1,000 mg $L^{-1}$ L-proline. Additions of 2.5 mg $L^{-1}$ 2,4-D and 500 mg $L^{-1}$ L-proline in NN medium were suitable callus cultured in Chai Nat 1. The optimal medium for callus cultured in Pathum Thani 1 was NN medium supplemented with 1 mg $L^{-1}$ 2,4-D and 500 mg $L^{-1}$ L-proline. The suitable medium for shoot regeneration in KDML105 was MS medium supplemented with 3 mg $L^{-1}$ BA and 300 mg $L^{-1}$ casein hydrolysate. Addition with 3 mg $L^{-1}$ BA, 0.5 mg $L^{-1}$ NAA and 500 mg $L^{-1}$ L-proline in MS medium was the most suitable for plant regeneration in Supanburi 1. The most suitable shoot regeneration medium in Chai Nat 1 was MS medium supplemented with 5 mg $L^{-1}$ BA, 0.5 mg $L^{-1}$ NAA and 300 mg $L^{-1}$ casein hydrolysate. Addition of 5 mg $L^{-1}$ BA and 300 mg $L^{-1}$ hydrolysate in MS medium was the most suitable for plant regeneration in Pathum Thani 1. Improvement of callus induction and shoot regeneration can be used and applied to *in vitro* selection for improvement in rice for salt tolerance in the future.

## ACKNOWLEDGMENTS

This work was financially supported by a Ph.D. scholarship from the Office of the Higher Education Commission, Ministry of Education, Thailand and the Genomics and Proteomics Research Group for Improvement of Salt-tolerant.

## REFERENCES

Bhaskaran, S. and R.H. Smith, 1990. Regeneration in cereal tissue culture: A review. Crop Sci., 30: 1328-1337.

Chu, C.C., C.C. Wang, C.S. Sun, K.C. Hsu, K.C. Yin, C.Y. Chu and F.Y. Bi, 1975. Establishment of an efficient medium for anther culture of rice through comparative experiments on the nitrogen sources. Sci. Sinica, 18: 659-668.

Chuenboonngarm, N., S. Charoonsote and S. Bhamarapravati, 2001. Effect of BA and 2iP on shoot proliferation and somaclonal variation of *Gardenia jasminoides* ellis *in vitro* culture. ScienceAsia, 27: 137-141.

Collin, H.A. and S. Edwards, 1998. Selection for Somaclonal Varisation. In: Plant Cell Culture, Collin, H.A. and S. Edwards (Eds.). Bios Scientific Publishers, UK, pp: 91-101.

Datta, K., I. Potrykus and S.K. Datta, 1992. Efficient fertile plant regeneration from protoplasts of the Indica rice breeding line IR72 (*Oryza sativa* L.). Plant Cell Rep., 11: 229-233.

Ge, X., Z. Chu, Y. Lin and S. Wang, 2006. A tissue culture system for different germplasms of indica rice. Plant Cell Rep., 25: 392-402.

Geng, P., H. La, H. Wang and E.J.C. Stevens, 2008. Effect of sorbitol concentration on regeneration of embryogenic calli in upland rice varieties (*Oryza sativa* L.). Plant Cell Tissue Organ Cult., 92: 303-313.

Gioi, T.D. and V.D. Tuan, 2002. Effect of different media and genotypes on anther culture efficiency of $F_1$ plants derived from crosses between IR64 and new plant type rice cultivars. Omonrice, 10: 107-109.

Hoque, E.H. and J.W. Mansfield, 2004. Effect of genotype and explant age on callus induction and subsequent plant regeneration from root-derived callus of Indica rice genotypes. Plant Cell Tissue Organ Cult., 78: 217-223.

Hoque, M.N., L. Rahman and L. Hassan, 2007. Effect of culture media on seed dormancy and callus induction ability of some wild and cultivated rice genotypes. Biotechnology, 6: 61-63.

Htwe, N.N., M. Maziah, H.C. Ling, F.Q. Zaman and A.M. Zain, 2011. Responses of some selected Malaysian rice genotypes to callus induction under *In vitro* salt stress. Afr. J. Biotechnol., 11: 350-362.

Igasaki, T., N. Akashi, T. Ujino-Ihara, Y. Matsubayashi, Y. Sakagami and K. Shinohara, 2006. Phytosulfokine stimulates somatic embryogenesis in *Cryptomeria japonica*. Plant Cell Physiol., 44: 1412-1416.

Islam, M.M., S.K. Adhikary, P. Gain, M.M. Rahman and N.A. Siddique, 2004. Effect of plant growth regulators on callus induction and plant regeneration in anther culture of rice. Pak. J. Biol. Sci., 7: 331-334.

Jubair, T.A., U. Salma, N. Haque, F. Aktar, I.J. Mukti, A.K.M.F. Haque and M.R. Ali, 2008. Callus induction and regeneration of local rice (*Oryza sativa* L.) variety topa. Asian J. Plant Sci., 7: 514-517.

Khaleda, L. and M. Al-Forkan, 2006. Stimulatory effects of casein hydrolysate and proline in *in vitro* callus induction and plant regeneration from five deepwater rice (*Oryza sativa* L.). Biotechnology, 5: 379-384.

Khan, Z.I., A. Hussain and M. Sadiq, 1999. Optimization of different media for plant regeneration from callus culture of indica rice (*Oryza sativa*) genotype DM. 25. Pak. J. Biol. Sci., 2: 984-987.

Khatun, M.M., M.H. Ali and N.V. Desamero, 2003. Effect of genotype and culture media on callus formation and plant regeneration from mature seed scutella culture in rice. Plant Tissue Cult., 13: 99-107.

Lee, K., H. Jeon and M. Kim, 2002. Optimization of a mature embryo-based *in vitro* culture system for high-frequency somatic embryogenic callus induction and plant regeneration from *Japonica* rice cultivars. Plant Cell Tissue Organ Cult., 71: 237-244.

Michiba, K., T. Okamoto and M. Mii, 2001. Increasing ploiding level in cell suspension cultures of *Doritaenopsis* by exogenous application of 2,4-dichlorophenoxyacetic acid. Physiol. Planta, 112: 142-148.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant., 15: 473-497.

Naqvi, S.M.S., R. Sultana and H. Rasheed, 2005. Tissue culture studies in *Oryza sativa* L. cvs. Basmati 385 and super basmati. Pak. J. Bot., 37: 823-828.

Nitsch, J.P. and C. Nitsch, 1969. Haploid plants from pollen grains. Science, 163: 85-87.

Pongtongkam, P., S. Peyachoknagul, P. Sripichit, A. Thongpan, K. Klakhaeng, S. Ketsagul and K. Lertsirirungson, 2004. Effects of L-lysine on callus formation, plant regeneration and flowering of Thai Rice c.v. KDML 105. Kasetsart J. (Nat. Sci.), 38: 190-195.

Pravin, V.J., M.S. Dudhare, S. Taranjeet, A.K. Sarawgi, R. Saxena and G. Chandel, 2011. Assessment of critical factors influencing callus induction, *in vitro* regeneration and selection of bombarded *indica* rice genotypes. J. Agric. Biotechnol. Sustainable Dev., 3: 44-59.

Raina, S.K., 1989. Tissue culture in rice improvement: Status and potential. Adv. Agron., 42: 339-398.

Rashid, H., S.Y.A. Bokhari and A. Quraishi, 2001. Callus induction, regeneration and hygromycin selection of rice (Super Basmati). J. Biol. Sci., 1: 1145-1146.

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

Saharan, V., R.C. Yadav, N.R. Yadav and B.P. Chapagain, 2004. High frequency plant regeneration from desiccated calli of *indica* rice (*Oryza sativa* L.). Afr. J. Biotechnol., 3: 256-259.

Sahrawat, A.K. and S. Chand, 2001. High-frequency plant regeneration from coleoptile tissue of indica rice (*Oryza sativa* L.). *In vitro* Cell. Dev. Biol. Plant, 37: 55-61.

Shahnewaz, S., M.A. Bari, N.A. Siddique and M.H. Rahman, 2004. Effects of genotype on induction of callus and plant regeneration potential in vitro anther culture of rice (*Oryza sativa* L.). Pak. J. Biol. Sci., 7: 235-237.

Shahsavari, E., 2010. Evaluation and optimizations of media on the tissue culture system of upland rice. Int. J. Agric. Biol., 12: 537-540.

Sikder, M.B.H., P.K. Sen, M. Abdullah-Al Mamun, M.R. Ali and S.M. Rahman, 2006. *In vitro* regeneration of aromatic rice (*Oryza sativa* L.). Int. J. Agri. Biol., 8: 759-762.

Tang, K., E. Zhao, Q. Hu, J. Yao and A. Wu, 2000. A simple and efficient procedure to improve plant regeneration from protoplasts isolated from long-term cell-suspension cultures of indica rice. *In vitro* Cell. Dev. Biol. Plant, 36: 362-365.

Tariq, M., G. Ali, F. Hadi, S. Ahmad, N. Ali and A.A. Shah, 2008. Callus induction and *in vitro* plant regeneration of rice (*Oryza sativa* L.) under various conditions. Pak. J. Biol. Sci., 11: 255-259.

Thadavong, S., P. Sripichitt, W. Wongyai and P. Jompuk, 2002. Callus induction and plant regeneration from mature embryos of glutinous rice (*Oryza sativa* L.) cultivar TDK1. Kasetsart J. (Nat. Sci.), 36: 334-344.

Thorpe, T.A., 2007. History of plant tissue culture. Mol. Biotechnol., 37: 169-180.

Wagiran, A., I. Ismail, C.R.C.M. Zain and R. Abdullah, 2008. Improvement of plant regeneration from embryogenic suspension cell culture of japonica rice. J. Biol. Sci., 8: 570-576.

Wani, S.H., P.A. Sofi, S.S. Gosal and N.B. Singh, 2010. *In vitro* screening of rice (*Oryza sativa* L) callus for drought tolerance. Commun. Biometry Crop Sci., 5: 108-115.

Wen, F., J. Peng, R.M. Lister and T.K. Hodges, 1991. A procedure for regenerating javanica and indica varieties of rice from protoplasts. Plant Mol. Biol. Rep., 9: 308-321.

Yang, Y.S., Y.D. Zheng, Y.L. Chen and Y.Y. Jian, 1999. Improvement of plant regeneration from long-term cultured calluses of Taipei 309, a model rice variety in *in vitro* studies. Plant Cell Tissue Organ Cult., 57: 199-206.

Yin, Y., S. Li, Y. Chen, H. Guo, W. Tian, Y. Chen and L. Li, 1993. Fertile plants regenerated from suspension culture-derived protoplasts of an indica type rice (*Oryza sativa* L.). Plant Cell, Tissue Organ Cult., 32: 61-68.