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Original Article

How some native upland rice and cultivated lowland rice varieties responded to callus induction and regeneration medium?

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Abstract

The seed collected from ethnic farmers (Pa-gha-ker-yor People), at Pala U village, Hau Hin district, Prachuap Khiri Khan province, Thailand for genetic conservation and investigating feasibility for breeding and improvement. For genetic improvement, information on either some qualification or ability to assist in the breeding process is required, such as the ability to culture seed, explants or other tissues in a sterile laboratory condition. The objective of this study evaluated the effectiveness of callus induction and regeneration upland rice seeds (var. *Nikor*, var. *Raw Bi*, var. *Gi Poo* and var. *Nah San*, var. *Baw Pae Soo* and var. *Pae Taw Gaw Bi*) collected from minority farmers and some lowland cultivated rice varieties (var. *RD51* and var. *Pratumtani1*) in Thailand. The culture medium used in the study were derived from the previously reported formulations that are highly effective in inducing callus (MS1, MS2 and MS8) and regenerating (MS1, MSa and MSb) in rice. The different formulas in medium were from various combinations of plant growth regulator both or either on cytokinin (Benzyl aminopurine; BAP) and/or auxins (Napthalene acetic acid; NAA, 2,4-dichlorophenoxy acetic acid; 2,4-D) for callus induction and regeneration. For statistical analysis, the data have been analyzed using analysis of variance (ANOVA). The means among treatments were compared with the Duncan's new multiple range test (DMRT). The results showed the increasing on callus induction percentage were recorded on MS2 (86%) and MS8 (90.5%) mediums studied on immature and mature seed, respectively. The callus of upland rice varieties induced on a medium which showed higher percentage (90.5% callus induction on MS8 and 0% on MS1) were selected to shoot regeneration experiment contained three media (MS1, MSa, and MSb). However, in the regeneration process, there is no significant difference between medium; the percentage of regenerating callus of these media at 6.25%, and the interaction between media and varieties of rice.

Keywords: Indigenous rice genetic, Plant hormone, Micropropagation, Genetic conservation, Culture medium

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Introduction

Cultivation of upland rice has been reported to relate to cultural heritage of farmers (Zapico et al., 2015), even if most of the upland rice productivity has been reported to be quite low in economic term (Gupta and Toole, 1986; Oonyu, 2011). Nevertheless, in some specific areas, upland rice yield could be increased yield if the farmers adopt new technologies in planting this rice (Wang et al., 2010; Nwinya et al., 2014).

In Thailand, landrace upland rice has a low productivity with an average grain yield at 1.16 t ha^{-1}. This rice has been cultivated mainly in the North of Thailand to maintain genetic diversity (Karladee et al., 2012). Most of the upland rice varieties are light sensitive and have been planted in the highland area in which rice cultivation depends on the rain, as the only source of water during rainy season (Gupta and Toole, 1986; Karladee et al., 2012). Upland rice is one of the crops that are tolerant to a very changeable environment, such as water insufficient condition, although the yield is often low under drought condition (Oonyu, 2011; Karladee et al., 2012). In addition, some indigenous rice varieties have been reported to resist to pests (Chanu et al., 2010). Thus, maintaining the genetic diversity of the upland rice will ensure that enough plant material is available for rice genetic improvement.

Although tissue culture has been employed to improve the genetics of lowland rice varieties and for conversation (Lina et al., 2010; Wani et al., 2010). For upland rice, however, most of the studies have been conducted under field and greenhouse conditions (Nwinya et al., 2014; Narenoot et al., 2015). For indica rice, it has been reported that this rice is recalcitrant in *in vitro* culture and its regeneration is quite low compared to japonica rice (Raghvendra et al., 2010). For these reasons, the indica upland rice genetic has been neglected in the laboratory study, resulting in slower genetic improvement and improved productivity.

The objective of this study was to evaluate the effect of media containing different plant growth regulators (PGRs): these PGRs have been reported highly effective in induction callus and regeneration either in upland rice or indica rice (Lina et al., 2010; Shahsavari et al., 2010; Wani et al., 2011) in inducing callus and regenerating shoot in indigenous upland rice varieties (*Oryza sativa* L.) which have been collected from Karen farmers in Prachuap Khiri Khan Province, Thailand. Major of these upland rice varieties has the characteristics; according to seed morphology (Chang, 1985; Sato, 1985; Sato et al., 1990) and molecular analysis by using primers were reported by Lu et al. (2009), tend to be indica rice subspecies.

Due to either embryogenic calli produced from dehusked mature seed (Ibandalin et al., 2016) and immature seed (Cantrell and Reeves, 2002) have been reported for efficient gene expression in rice. However, mature seed is easy to collect and keep in storage. Thus, for callus induction in upland rice, both mature and immature seeds were target to study and comparison. Enhancing the efficient tissue culture protocol for callus induction and regeneration would support a breeding program via either involve with mutation or engineering.

Material and Methods

Plant material

Both indigenous upland rice and cultivated lowland rice varieties (*Oryza sativa* L.) were used in this study. The lowland rice (only mature seeds) (var. *RD51* and *Pratumtani1*) and upland rice [only mature seeds (stage R9 of rice reproductive growth stages) (Moldenhauer and Slaton, 2011) including var. *Nikor*, var. *Raw Bi* and var. *Gi Poo* and var. *Nah San*, and both mature and immature seeds (stage R7 of rice reproductive growth stages at ripening phase or hard dough stage) (Moldenhauer and Slaton, 2011) including var. *Baw Pae Soo* and *Pae Taw Gaw Bi*] has presented a difference in rice genetic in the study on callus induction.

Three upland rice varieties (*Nah San*, *Baw Pae Soo* and *Gi Poo*) were used in the following study. These varieties were selected from either the ability of callus induction (var. *Baw Pae Soo* and *Gi Poo*) or popular variety is grown by ethnic minority farmers (var. *Nah San*). Calli derived from the incubated seeds on the callus-inducing medium has been transferred onto a solidified shoot-regenerating medium. The result of this was recorded two weeks after tissue culture (experiment A).

The medium which was effective in inducing the callus (experiment A) was chosen for further studies in inducing callus proliferation and regeneration (experiment B).

Preparing of callus induction and regeneration medium

Callus induction. Three callus-inducing media including MS1, MS2 (Wani et al., 2011) and MS8 (Lina et al., 2010) have been used for this study. The MS (Murashige and Skoog, 1962) was the basal medium for MS1 and MS2 media. MS2 was the MS basal medium which had been supplemented with 560 mg L^{-1} proline. MS1 medium was the MS basal medium which had been added with 2.5 mg L^{-1} Napthalene acetic acid (NAA) and 1 mg L^{-1} Kinetin (Kin). MS2 medium was the MS basal medium which had been added with 2.5 mg L^{-1} 2,4-dichlorophenoxy acetic acid (2,4-D) and 0.5 mg L^{-1}

Kinetin. The MS8 medium was supplemented with 2 mg L^{-1} 2,4-D, 1 mg L^{-1} NAA, 0.5 mg L^{-1} Kin and 2 mg L^{-1} Benzyl aminopurine (BAP).

Preparing of regeneration medium in experiment A. Based on MS base medium, MSr medium was prepared by supplementing the MS medium with 0.5 mg L^{-1} NAA, 0.5 mg L^{-1} Kin and 2 mg L^{-1} BAP (Wani et al., 2011). This medium was chosen for regenerating the plant from inducing callus (experiment A).

Preparing of regeneration medium in experiment B. Three mediums, including MS1, MSa and MSb mediums were used for regenerating shoot of the rice calli. Based on MS medium, both MSa (MSa supplemented with 0.5 mg L^{-1} NAA, 2 mg L^{-1} Kin and 2 mg L^{-1} BAP) and MSb (MSb supplemented with 0.5 mg L^{-1} 2,4-D and 0.5 mg L^{-1} BAP) were prepared (Lina et al., 2010; Shahsavari et al., 2010) These media were used for regenerating plant from calli induced in medium which was selected based on its efficacy in inducing callus (experiment A).

Media for inducing callus (experiment A) and regenerating shoot (experiment B) were supplemented with 30 g L^{-1} sucrose and agar 8 g L^{-1} agar. These media were adjusted to pH 5.8 with 0.1 N NaOH and were subsequently autoclaved at 121°C for 30 mins.

Callus induction both from immature and mature embryo of rice

The rice seeds of both immature and mature seeds have been dehusked. These dehusked seeds have then been soaked in 15% and 10% mercuric chloride solution for 15 and 10 mins, respectively. After that they have been rinsed three times with distilled sterile water and cultured at 25±2 °C in dark condition for three weeks on the callus inducing media (both experiment A and B). Both experiments have been conducted in the callus inducing medium in a 100 mm diameter Petri dishes.

Plant regeneration from rice calli

After three weeks of incubation, all calli were transferred onto MSr regenerating medium and cultured for two weeks in experiment A. In experiment B, the calli were transferred onto three regenerating media and cultured for eight weeks. Calli were placed on the cabinet at 25±2 °C under 16 hrs. -photoperiod with 2,735 lux illumination. Both experiments were conducted for regenerating shoot in the 4 oz. bottles.

Data calculation

The percentage of callus induction at three weeks after culturing in the callus inducing media (in experiment A and B) and plant regenerating media (at two and eight weeks in experiment A and B, respectively) have been measured using the following formulas (Zaidi et al., 2006):

Callus induction (%) = (No. calli/No. incubated seeds) x 100

Plant regeneration (%) = (No. regenerated calli/ No. incubated calli) x 100

Other characteristics such as size, fresh weight and color (data was not shown) of the calli in the callus inducing media (experiment A) have also been recorded.

Experimental design and statistical analysis

The 3x9 and 3x3 factorials in completely randomized design (CRD) have been conducted with four replicated in experiments A and B, respectively. In callus induction and continuous for shoot regeneration in experiment A, two factors, including three callus inducing media (MS1, MS2, MS8) and nine rice varieties have been studied. Each replication had five Petri dishes contained 25 seeds in each Petri dish.

For shoot regeneration in the experiment B, two factors including three regenerating media (MS1, MSa and MSb) and three rice varieties have been studied. Each replication contained four of 4 oz. bottles, in which each bottle contained one callus. The shoot regeneration from callus were determined 30 days after cultured on shoot induction medium. The characteristics included the differentiated callus as morphological changes (callus proliferation and shoot regeneration) were observed. However, shoot regeneration percentage was only recorded.

For statistical analysis, the means among treatments were compared with the Duncan's new multiple range test (DMRT). The data have been analyzed using analysis of variance (ANOVA) by using R program version 3.3.1 (R Core Team, 2016). The data which have been analyzed with *Shapiro-Wilk* Test was subsequently analyzed by the non-parametic using *Kruskal-Wallis* Test. Treatments were compared using *Mann-Whitney* Test with the *Gnu PSPP* software (Free Software Foundation Inc., http://www.gnu.org/software/pspp /get.html).

This study has been carried out from January to May in 2015 at Tissue Culture Laboratory of Animal Sciences and Agricultural Technology, Silpakorn University, IT Campus, Cha-Am, Phetchaburi, Thailand.

Results

For callus induction in experiment A, the percentages of callus induction on different media (MS1, MS2 and MS8) have been shown 21 days after culturing in Table 1. Highly significant different ($P<0.01$) is observed on the percentage of callus induction in two factors

including different of media and rice varieties. Types of media and rice varieties were also interacted, affecting the callus induction (Table 1).

The results also show that MS8 medium (90.5%) has induced callus formation higher than MS2 medium (86.0%). However, callus formation did not occur in MS1 medium. *Pae Taw Gaw Bi* both induced from immature (65.7%) and mature (66.2%) has a higher percentage of callus formation with non-significant difference with *RD51* (66.0%), *Pratumtani1* (66.5%) and *Gi Poo* have (65.4%) (Table 1).

Seeds culturing on MS8 medium have a higher callus formation than those in other media. Nevertheless, there is no difference in callus formation (comparing MS2 with MS8) in some rice varieties such as *Pae Taw Gaw Bi* [both immature (97.2% and 100%) and mature (98.7% and 99.8%) seeds], *Pratumtani 1* (100% and 99.4%), *Baw Pae Soo* (94.3% and 98.4%), *Ni Kor* (18.4% and 18%) and *Gi Poo* (96.6% and 99.5%) (Table 1). Both diameter and fresh weight of the calli has been observed 21 days after incubation on MS2 and MS8. The diameter of the callus was significantly affected by media, rice varieties and the interaction between these two factors (Table 2).

The size of the callus was biggest on MS2 (0.314 cm), followed by MS8 (0.22 cm). However, the varieties of rice also influence the size of the callus. *Baw Pae Soo* had the biggest callus [in immature seed (0.349 cm) and mature seed (0.326 cm)]. Which *Raw Bi* had the smallest callus [mature seed (0.157 cm)].

Callus fresh weight was highly affected by media, rice varieties and the interaction between these two factors (Table 2). MS8 induced callus fresh weight increased (0.0228 g) more than MS2 did (0.0182 g). Callus fresh weight of the immature seed of *Baw Pae Soo* was the highest (0.0336 g), followed by mature seed of *Gi Poo* (0.0307 g). The lowest callus fresh weight was found in the mature seed of *Nikor* (0.0065 g).

The effect of the interaction between rice varieties and different media on increasing the callus fresh weight have been detected in the mature seeds of *RD51*, *Pae Taw Gaw Bi*, *Nikor* and *Gi Poo* on MS2. In contrast, *Baw Pae Soo*, *Pae Taw Gaw Bi*, *Pratumtani1* and *Raw Bi* had an increased callus fresh weight on MS8 (Table 2).

MSr media had no effect on regenerating callus 14 days after incubation. All calli died, based on the observed syndrome such as the color change in callus from yellow to pale yellow, brownish yellow and tissue necrosis on the regenerating medium (data were not shown).

In the experiment B, induced calli on MS8 were transferred to three regenerating media, including as MS1, MSa and MSb. The result show that rice varieties and the regenerating media did not affect the primary establishment of shoot on callus and there was also no interaction between these two factors (Table.3). All regenerating media did show the same effect based on the percentages of regenerating calli with an average of three rice varieties (*Nah San*, *Baw Pae Soo* and *Gi poo*) at 6.25%. However, the varieties showed significant difference on inducing calli to regenerating shoot by non-parametric test. *Baw Pae Soo* had the percentage of regenerating calli at 12.50%, followed by *Nah San* at 6.25% and *Gi Poo* at zero%.

Table 1. Percent of callus induction (Mean ± SE) of some indigenous upland rice and lowland rice varieties on different mediums at 21 days after culturing.

Medium	Immature seeds		Mature seeds							Means ± SE (medium)
	Varieties		Varieties							
	Baw Pae Soo	*Pae Taw Gaw Bi*	*RD51*	*Pratum-tani1*	*Baw Pae Soo*	*Pae Taw Gaw Bi*	*Nikor*	*Raw Bi*	*Gi Poo*	
MS1	0.0f	0.0f	0.0f	0.0f	0.0f	0.0f	0.0f	0.0f	0.0f	0.0z
MS2	89.2 ± 3.9c	97.2 ± 1.5ab	98.0 ± 0.7b	100.0 ± 0.0a	94.3 ± 3.0b	98.7 ± 0.8ab	18.4 ± 0.2a	48.1 ± 0.1d	96.6 ± 1.8ab	86.0 ± 12.7y
MS8	99.8± 0.2a	100a	100a	99.4 ± 0.2a	98.4 ± 0.6ab	99.8 ± 0.2a	18.0 ± 1.9a	100a	99.5 ± 0.5a	90.5 ± 13.0x
Means ± SE (varieties)	63.0 ± 23.4mn	65.7 ± 24.3m	66.0 ± 24.4m	66.5 ± 24.5m	61.5 ± 24.4n	66.2 ± 24.4m	11.6 ± 4.7p	49.6 ± 23.6o	65.4 ± 24.2m	
F-test	Medium = **, Varieties = **, Media x Varieties = **, CV(%) = 3.87									

Note: ** Significant different at 99 percent of confident (P<0.01).

a,…,f different letter means significant different at 95 percent of confident (P<0.05).

x,y,z different letter in same column means significant different at 95 percent of confident (P<0.05).

m,..,p different letter in same row means significant different at 95 percent of confident (P<0.05).

Table 2. Diameter (cm) and fresh weight (g) (in parenthesis) of induced calli (Mean ± SE) of some indigenous upland rice and lowland rice varieties on different induction mediums at 21 days after culturing.

Medium	Immature seeds		Mature seeds							Means ± SE (medium)
	Varieties		Varieties							
	Baw Pae Soo	*Pae Taw Gaw Bi*	*RD51*	*Pratumtani1*	*Baw Pae Soo*	*Pae Taw Gaw Bi*	*Nikor*	*Raw Bi*	*Gi Poo*	
MS2	0.389 ± 0.005b (0.0293 ± $2.0x10^{-4}$ C)	0.394 ± 0.002ab (0.0118 ± $1.0x10^{-4}$ G)	0.399 ± 0.003a (0.0204 ± $5.0x10^{-4}$ F)	0.200 ± 0.003h (0.0079 ± $6.0x10^{-4}$ J)	0.386 ± 0.001b (0.0237 ± $2.0x10^{-4}$ F)	0.285 ± 0.001d (0.0189 ± $1.0x10^{-5}$ G)	0.292 ± 0.001d (0.0077 ± $1.0x10^{-4}$ J)	0.188 ± 0.002i (0.0014 ± $1.0x10^{-5}$ L)	0.290 ± 0.003d (0.032 ± $5.0x10^{-4}$ B)	0.314 ± 0.04x (0.0182 ± $5.5x10^{-3}$ Y)
MS8	0.309 ± 0.001c (0.0379 ± $2.0x10^{-4}$ A)	0.216 ± 0.002g (0.0261 ± $3.0x10^{-4}$ E)	0.160 ± 0.001j (0.0178 ± $1.0x10^{-4}$ H)	0.237 ± 0.002f (0.0197 ± $4.0x10^{-4}$ G)	0.266 ± 0.001e (0.0270 ± $1.0x10^{-4}$ D)	0.207 ± 0.003h (0.0124 ± $2.0x10^{-4}$ I)	0.184 ± 0.004i (0.0054 ± $1.0x10^{-4}$ K)	0.127 ± 0.002k (0.0299 ± $1.0x10^{-4}$ C)	0.271 ± 0.003e (0.0294 ± $3.0x10^{-4}$ C)	0.220 ± 0.03y (0.0228 ± $4.7x10^{-3}$ X)
Means ± SE (varieties)	0.349 ± 0.02m (0.0336 ± $2.3x10^{-3}$ M)	0.305 ± 0.05o (0.0225 ± $1.9x10^{-3}$ P)	0.279 ± 0.06p (0.0209 ± $1.6x10^{-3}$ Q)	0.219 ± 0.01s (0.0138 ± $3.1x10^{-3}$ S)	0.326 ± 0.03n (0.0253 ± $8.0x10^{-4}$ O)	0.246 ± 0.02q (0.0157 ± $1.7x10^{-3}$ R)	0.238 ± 0.03r (0.0065 ± $6.0x10^{-4}$ T)	0.157 ± 0.02t (0.0156 ± $7.6x10^{-3}$ R)	0.281 ± 0.006p (0.0307 ± $8.0x10^{-4}$ N)	
F-test	Medium = **, Varieties = **, Media x Varieties = **, CV(%) = 2.17 (in parenthesis; Media = **, Varieties = **, Media x Varieties = **, CV(%) = 2.79)									

Note: ** Significant different at 99 percent of confident (P<0.01).
a,…,k (A,…,L in parenthesis) different letter means significant different at 95 percent of confident (P<0.05).
x,y (X, Y in parenthesis) different letter in same column means significant different at 95 percent of confident (P<0.05).
m,..,t (M,…, T in parenthesis) different letter in same row means significant different at 95 percent of confident (P<0.05).

Table 3. Percent of regenerated calli (Mean), induced callus of some indigenous upland rice and lowland rice varieties on MS8 medium, on different regenerated mediums at 21 days after culturing.

Medium	Varieties§			Mean (Medium) ¶
	Nah San	*Baw Pae Soo*	*Gi Poo*	
MS1	0	18.75	0	6.25
MSa	12.50	6.25	0	6.25
MSb	6.25	12.50	0	6.25
Mean (Varieties) †	6.25 ab	12.50 a	0 c	

Note: §, mature seeds
¶, NS = not significant different at 95 percent of confident (P>0.05).
†, Significant different at 95 percent of confident (P<0.05).
a, b, c different letter means significant different at 95 percent of confident (P<0.05).

Discussion

The callus induction (Table 1) is affected by culture media, concentrations of PGRs and rice varieties. There are various reports in rice culturing in the *in vitro* conditions which indicate that either media of the PGRs (Rueb et al., 1994; Zaidi et al., 2006) or varieties of rice (Shahsavari et al., 2010; Ozawa et al., 2003) affect the establishment of callus.

Callus induction in MS8 medium has a high percentage (90.5%) of callus formation when this medium is supplemented with AUX (2 mg L^{-1} 2,4-D and 1 mg L^{-1} NAA) and CK (2 mg L^{-1} BAP and 0.5 mg L^{-1} KIN). The number of callus formation is followed by that in MS2 (86%), a medium which has been supplemented with AUX only as 2,4-D (2.5 mg L^{-1}) and KIN (0.5 mg L^{-1}). Both MS8 and MS2 contained 2,4-D and KIN as the supplemented PGRs, a combination of PGRs which is effective in inducing embryogenic callus (Ge et al., 2006). MS1, on the other hand, is not effective in inducing callus formation. This medium was supplemented only with 2.5 mg L^{-1} NAA and 1 mg L^{-1} KIN. This result may indicate the importance of 2,4-D in the medium for inducing callus in rice (Shahsavari et al., 2010; Libin et al., 2012; Poraha et al., 2016). The combination of NAA and KIN supplemented in the medium, however, will be effective for regenerating shoot (Libin et al., 2012).

Regardless of the stages both of maturity and immaturity of the seeds, high percentage of callus may be induced in both *Pae Taw Gaw Bi* and *Baw Pae Soo* (Table 1). Both immature seed (Masuda et al., 1989) and mature seed (Carsono and Yoshida, 2006) were reported could induce

callus. However, non-significant difference in the percentages of callus induction between mature seed and immature seed was observed in this study. This is advantageous because the stored mature seeds can be used for inducing callus, and paving the way in reproducing the progeny of these two rice varieties irrespective of the planting season (Karladee et al., 2012; Poeaim et al., 2016). The callus induction: both in percentage and quality of callus, depends on the interaction between factors including genotype, medium, and explants (Carsono and Yoshida, 2006). Moreover, the success of *in vitro* study depends on the development stages of the mother plants (Rahman et al., 2015). In this study, immature rice seed had been harvested in stage R7 of rice reproductive growth stages (hard dough stage) may have the seed quality is similar with mature seed in stage R9 of rice reproductive growth stages. Seed at stage R7 has a firm of whole grain and it is ready for harvest, although the moisture content inside is higher than seed at stage R9 (Moldenhauer and Slaton, 2011).

Both rice varieties and media influenced the size and fresh weight of the calli (Table 2). Thus, the genetic of rice and both types and concentrations of PGRs play a role in the establishment of callus. The effects of genetic and media for inducing callus have also been investigated in the breeding program in rice (Kuroda et al., 1998; Sankepally et al., 2016). There was an interaction between rice varieties and media, which has affected the size and weight of calli in this study. This confirms the importance of these two factors in inducing callus in *in vitro* culture.

Both immature and mature seeds of *Baw Pae Soo* had high values of diameter and fresh weight of the induced calli (Table 2). The genetic has been reported to influence the proliferation and growth of the calli in rice in either normal or stressful conditions. (Kuroda et al., 1998; Gomez and Kalamani, 2002). The values of heritability for the number of active young shoots on the callus and emerging roots had been reported as 79.5% and 74.60%, respectively (Gomez and Kalamani, 2002). These high values of heritability indicate that genetic influences the emergence of shoot and root more than the inducing media, which have the respective values of heritability for the diameter and weight of calli at 18.5% and 35.6% (Gomez and Kalamani, 2002).

Moreover, the results of this study show that cultural media and the interaction between rice varieties and media may affect the callus growth. For this reason, both callus diameter and callus weight have been used in a cooperated study with other characteristics for an effective plant genetic improvement and a genetic study under *in vitro* selection (Johnson et al., 1955; Gomez and Kalamani, 2002).

As yellow or cream colored callus, embryogenic callus is usually also denser callus (Tam and Lang, 2003). The result shows a contrast between the media with higher values on callus diameter (MS2) and callus fresh weight (MS8). An exception has been found in *Bae Pae Soo* and *Gi Poo* which had higher callus diameter and weight (Table 2).

A difference of callus diameter and fresh weight has been found in different media such as MS2 and MS8. One reason for this difference of callus which formed these media could be explained by their origins. Indeed, they come from different types and concentration of PGRs. Both of BAP and NAA as PGRs were added only in MS8 medium. However, distinctions on morphogenic expression of callus of various plant species (Maciel et al., 2010) and rice (Mannan et al., 2013) have been found depending on the species, genotypes, explants, nutritive medium and PGRs plus the interaction between the factors during culturing processes and the cell properties. Although, MSr medium has been reported for being an effective callus inducing medium for indica rice (var. *PAU 201*) (Wani et al., 2011). It was not effective to induce callus in rice in this study (data were not shown). Although, MSr medium supplemented with many PGRs such as NAA and BAP has been reported to be effective in regenerating the plants in indica rice (Kumar and Ajinder, 2013; Poraha et al., 2016) and KIN (Sankepally et al., 2016). This medium was not effective in regenerating plants from callus in upland rice [both two lowland rice varieties (var. *RD51* and *Pratumtani1*) and five upland rice varieties (var. *Nikor, Raw Bi*, *Gi Poo*, *Baw Pae Soo* and *Pae Taw Gaw Bi*)] in this study (data were not shown). Although auxin (NAA) and cytokinin (BAP) were found to be important for undifferentiated tissue for shoot regeneration (Din et al., 2016). Balancing between cytokinins and auxins must be carefully considered under the study in different varieties. The proportion between auxins and cytokinins can result in the initiation or inhibition for regeneration in induced calli (Lee and Huang, 2014). Moreover, non-dehydrated calli was practiced before regeneration in this study. So that, this may be a reason this study found non-effectiveness in shoot regeneration because the partial desiccation has been reported to promote the plant regeneration in rice (Saharan et al., 2004). Especially in indica rice varieties which are considerd recalcitrant to regeneration (Khanna and Raina, 1997), increased plantlets after desiccation was reported (Saharan et al., 2004). In addition, number of gene products was reported to regulate shoot regeneration under *in vitro* culture (Xue et al., 2017). There were studies which had reported that the successful shoot regeneration in rice was dependent on the concentration of PGRs which had been used to

culture the calli (Sikder et al., 2006).

MS8 containing higher weight of callus in experiment A, was selected as inducing callus medium for the study on plants regeneration in experiment B. Two of three varieties (*Nah San* and *Baw Pae Soo*), except in *Gi Poo*, have been found to produce a regenerating shoot on calli at 21 days after incubation (Table 3). Difference in rice genetic may also severely affect the calli's regeneration in the media, although all three varieties (*Nah San*, *Baw Pae Soo* and *Gi Poo*) were collected from the fields in the same area. For *in vitro* culture, different genotypes is important factor effect to the variation of responding with *in vitro* culture, especially in indica subspecies of rice (Rahman et al., 2010). In this study, percent of regeneration in all upland rice varieties is low, which many methods were reported increasing plant regeneration in rice including dehydration callus before the regeneration (Haq et al., 2009). Transferred callus to hormone-free MS medium for regeneration (Benlioglu et al., 2015) and supplement combination between sucrose and sorbitol in regeneration medium (Al-Khayri et al., 1996)

The induced calli has a statistically significant difference among rice varieties with non-parametic test, but the type of the media was not different in inducing the callus. *Baw Pae Soo* has produced regenerating plant on calli in all mediums (Table 3). MSa and MSb have induced regenerating plants for these two varieties (Table 3).

Conclusion

The types and concentrations of PGR and the interaction between these factors have affected the callus establishment of both upland rice (both immature and mature seeds) and lowland rice seeds in tissue culture. MS8 medium is most effective in inducing callus formation, based on the percentage of induced callus and fresh weight. 2,4-D (2,4-dichlorophenoxy acetic acid) is the most effective of supplement in MS2 and MS8 media, that induces callus formation at 86% and 90.5%, respectively. Callus formation in the inducing media is highly affected by the varieties of rice (except *Nikor* and *Raw Bi*). *Baw Pae Soo*, an upland rice variety, has the most percentage increases of callus formation; either in immature (63%) or mature seeds (61.5%), and callus fresh weight; either in immature (0.0336 g) or mature seeds (0.0253 g). Regenerating shoot is not successful on MSr medium in the experiment A, but is accomplished on MS1, MSa and MSb media in the experiment B. Rice genetics, types of medium and the combination of these two factors play a role in determining the success of callus formation and plant regeneration.

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