

Callus Induction and Plant Regeneration from Mature Embryos of Glutinous Rice (*Oryza sativa L.*) Cultivar TDK1

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ABSTRACT

The present study was conducted to determine the optimum *in vitro* conditions for callus induction and plant regeneration from mature embryos (seeds) of glutinous rice cultivar TDK 1. It was revealed that embryos cultured on Murashige and Skoog (MS) agar medium supplemented with 2 mg/l 2,4-D and 500 mg/l L-proline under light condition produced the highest percentage of callus formation (96.91 %). The average size of callus produced was large (6.02 mm). The calli dehydrated by placing in petridishes with covers for 7 days under light condition before transferring onto regeneration medium generated higher frequency of shoot regeneration than the calli cultured on regeneration medium without dehydration. The most suitable medium for plant regeneration from dehydrated calli was MS agar medium supplemented with 1 mg/l IAA, 4 mg/l BA and 800 mg/l casein hydrolysate which induced the highest percentage of calli forming shoots (45.00 %) and each callus produced the largest number of shoots (average 15 shoots). The shoots were rooted at the highest rate (100 %) when transferred onto MS agar medium supplemented with 1 mg/l NAA. The complete plantlets were thereafter transplanted to grow under greenhouse condition. They were morphologically normal and fertile.

Key words: callus, regeneration, embryo, glutinous rice

INTRODUCTION

Laos is the largest producer and consumer of glutinous rice in the Asian. Approximately 85 % of annual rice production in Laos is glutinous rice. TDK 1 has proven to be the most popular variety of glutinous rice characterized by good tillering ability, highly nitrogen response and resistance to brown planthopper. However, TDK1 gives moderate yield with susceptibility to green leafhopper and poor in grain milling quality when they are grown under dry season with irrigated condition (Schiller *et al.*, 1999). Therefore, breeding program to develop new varieties that are superior to TDK 1 in both

yield and grain quality is required.

Rice improvement using biotechnology such as somaclonal variation, *in vitro* selection, production of doubled haploid lines from anther culture, and genetic transformation might be an alternative way for achieving desirable rice varieties. However, the basic prerequisite for the potential use of biotechnology in rice improvement is the regeneration ability of cell, tissue and organ of rice plant. Successful regeneration of plant tissue culture mainly depends on genotype, explant type, medium composition, plant growth regulator and culture environment (Khanna and Raina, 1998).

There were numerous reports on callus

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formation and plant regeneration from mature seeds of indica rice especially the non-glutinous type (Vajrabhaya *et al.*, 1986; Raina *et al.*, 1987; Chowdhry *et al.*, 1993; Sripichitt and Chewasestatham, 1994; Burikam *et al.*, 2002). However, information of plant regeneration from tissue and organ culture of glutinous rice is scarcely found.

The objectives of this study were to find a suitable medium and culture condition for callus induction and plant regeneration from mature seeds (embryos) of glutinous rice variety TDK 1. Mature seed was chosen as the explant in this experiment because it was tolerant to surface sterilization and easy to be handled. Moreover, mature seed exhibited high potential of plant regeneration and low rate of albinism (Ogewa *et al.*, 1982).

MATERIALS AND METHODS

Experiment 1. Callus induction

Mature seeds of TDK 1 (Tha Dok Kham 1) were dehusked and surface sterilized by soaking in 70% ethanol for 5 min and in 15% clorox containing a wetting agent "Tween 20" for 20 min followed by three rinses in sterile distilled water. Aseptic seeds were cultured on MS medium (Murashige and Skoog, 1962) supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) at the concentrations of 0, 1, 2, 3, 4 and 5 mg/l, 3% sucrose and 0.7% agar, pH = 5.8. Seed cultures were incubated at 25°C under dark and light condition illuminated by 2,000 lux of cool-white fluorescent light with 16hrs photoperiod for 4 weeks. After 4 weeks of culture, the number of seeds forming callus and the size of callus [(width + length)/2] were recorded to determine the optimum concentration of 2,4-D for callus induction from the seeds.

Experiment 2. Enhancing callus formation

Mature seeds were cultured on MS medium

supplemented with 2,4-D at the optimum concentration (result from experiment 1) in combination with 0.3 and 0.5 mg/l kinetin (K), 50 and 100 mg/l L-tryptophan (Tryp), 300 and 500 mg/l casein hydrolysate (CH), 300 and 500 mg/l L-proline (P), 10 and 15% (v/v) coconut water (CW). Three percent of sucrose and 0.7% of agar were also added to the medium. Seed cultures were incubated at 25°C under dark and light condition (as previously mentioned in experiment 1) for 4 weeks. Four weeks after culture, the number of seed forming callus (%) and the size of callus [(width + length)/2] were recorded to investigate the kind and concentration of supplements to 2,4-D which can enhance the frequency of callus formation.

Experiment 3. Plant regeneration

Four-week-old calli derived from seeds cultured on suitable callus induction medium (result from experiment 2) were divided into 3 groups. The first group, calli were cut into 0.5 mm pieces and cultured immediately on regeneration medium. The second group, calli were dehydrated by placing on the single layer of filter paper in petridishes and sealed by plastic film (parafilm). The petridishes were kept at 25°C under dark condition for 7 days. The third group, calli were dehydrated by the method previously described and kept at 25°C under light condition for 7 days.

Non-dehydrated and dehydrated calli under dark and light conditions were cultured on MS medium supplemented with 1 mg/l indoleacetic acid (IAA) in combination with benzyladenine (BA) or kinetin at the concentrations of 1, 2, 3 and 4 mg/l, 3% sucrose and 0.7% agar. The cultures were incubated at 25°C under light condition. Four week after culture, the number of calli forming shoots and the number of shoots per callus were counted to determine the optimum combination of IAA and BA or kinetin for plant regeneration from seed-derived calli.

Experiment 4. Enhancing plant regeneration capacity

Dehydrated calli under light condition were cultured on MS medium added with the optimum combination of IAA and BA or kinetin (result from experiment 4) and supplemented with 20 and 40 mg/l adenine sulfate (AS), 50 and 100 mg/l L-tryptophan, 500 and 800 mg/l casein hydrolysate, 1 and 2 g/l yeast extract (YE) and 10 and 20 % coconut water. Three percent of sucrose and 0.7 % of agar were also added to the medium. The cultures were incubated at 25°C under light condition. Four weeks after culture, the number of calli forming shoots and the number of shoots per callus were counted to verify the kind and concentration of supplements which could enhance the frequency of plant regeneration.

Experiment 5. Root induction

Regenerated shoots were rooted by culturing on MS medium without growth regulators or supplemented with α -naphthaleneacetic acid (NAA) at the concentration of 0.5, 1, and 2 mg/1,3 % sucrose and 0.7 % agar. Two weeks after culture, the number of shoot forming roots and the number of roots per shoot were recorded to investigate the suitable medium for root induction.

RESULTS AND DISCUSSION

Callus induction

Seeds of glutinous rice variety TDK 1 cultured on MS medium without 2,4-D could not form callus but germinated to be seedlings. Whereas seeds cultured on MS medium supplemented with 2,4-D developed shoots after 2-3 days of culture but later the shoots ceased to grow further. Calli were observed at the base of the shoots about one week of culture. Calli obtained were initiated from dividing cells of scutellum and mesocotyl of the embryos by the induction of 2,4-D (Maeda, 1980). Callus proliferation was continued until the 4th week of culture. Then the number of seeds forming callus

and the size of callus were determined. It was found that the seeds cultured on MS medium supplemented with 2,4-D at every concentration produced callus with relatively high frequencies of 80.00 to 98.43 % under light and dark condition (Table 1). However, seeds cultured under light condition gave higher average frequency of callus formation (92.03 %) and larger average size of callus (3.88 mm) than those cultured under dark condition (87.02 % and 3.44 mm). In addition, calli formed under light condition were mostly embryogenic with creamy, dry and compact appearance, while non-embryogenic calli with white, wet and friable characters were found predominantly under dark condition. It was documented that embryogenic callus displayed higher frequency of plant regeneration than the non-embryogenic one (Nabors *et al.*, 1983; Siriwardana and Nabors, 1983). Janet and Seabrook (1980) reported that callus cultured under light condition showed higher proliferation and plant regeneration because light induces morphogenesis process and green spot formation of callus.

Considering the effects of 2,4-D at various concentrations, it was shown that 2 mg/l 2,4-D gave the highest percentage of callus formation (98.43 %) and largest size of callus (5.07 mm) when the seeds were cultured under light condition. Whereas under dark condition, seeds cultured on the medium supplemented with 1 mg/l 2,4-D exhibited the highest frequency of callus formation (94.64 %) and largest amount of callus (4.11 mm). It is well known that 2,4-D is the most suitable auxin for callus induction of rice in tissue culture. However, the optimum concentration of 2,4-D varied depending on the explant source and genotype of rice (Raina, 1989). Our result revealed that 2,4-D at the concentrations of 1-2 mg/l were suitable for callus production from rice seed which was in accordance with many previous reports (Vajrabhaya *et al.*, 1986; Raina *et al.*, 1987; Sripichitt and Cheewasestatham, 1993; Burikam *et al.*, 2002).

Table 1 Callus formation of mature embryos cultured on MS medium supplemented with various concentrations of 2,4-D under light and dark condition. Each treatment comprises 70 replications.

2,4-D (mg/l)	Light		Dark	
	% Seed forming callus	Size of callus (mm) ¹	% Seed forming callus	Size of callus (mm) ¹
0	0.00 d ³	0.00 d	0.00 e	0.00 c
1	93.87 b	4.65 ab	94.64 a	4.11 a
2	98.43 a	5.07 a	90.83 b	4.00 a
3	85.50 c	2.96 c	84.61 c	2.61 b
4	87.93 c	2.67 c	85.00 c	2.67 b
5	94.40 b	4.04 b	80.00 d	3.83 a
Average ²	92.03	3.88	87.02	3.44

¹ Size of callus = (width + length)/2

² Control is not included

³ Means within column followed by the same letter are not significantly different at 95 % level of confidence by DMRT

Enhancing callus formation

Seeds were cultured on MS medium added with 2 mg/l 2,4-D in combination with various kinds and concentrations of organic substances under light and dark conditions to promote callus formation. It was observed that under light condition organic supplements enhanced callus induction and size of callus from 88.14 % and 4.34 mm (with no supplement) to 89.78 % and 4.79 mm (Table 2). However, organic supplements could not promote callus production under dark condition. It is clearly shown that light condition had stimulatory effect on callus proliferation from embryos of this glutinous rice variety.

Comparing the effects of various kinds and concentrations of organic supplements on enhancing callus formation, it was found that proline supplemented to the medium at the concentration of 500 mg/l under light condition promoted the highest percentage of seeds forming callus (96.91 %) and large size of callus (6.02 mm) (Figure 1A). However, the other organic supplements including 0.5 mg/l kinetin, 100 mg/l tryptophan and 10 % coconut

water could enhance callus formation as well. Although 2,4-D alone induced callus formation from rice embryos (Vajrabhaya *et al.*, 1986; Raina *et al.*, 1987; Sripichitt and Cheewasestatham, 1994), some organic substances such as kinetin, tryptophan, casein hydrolysate, proline and coconut water added to the callus induction medium containing 2,4-D could enhance the efficiency of callus formation (Siriwardana and Nabors, 1983; Vajrabhaya *et al.*, 1986; Sripichitt and Cheewasestatham, 1994; Rueb *et al.*, 1994). Especially, proline was found to be the most effective supplement for callus promotion in this experimenter. This is because proline is a kind of amino acid and is used as a precursor in the process of metabolism and cell division (Santos *et al.*, 1996). Moreover, calli of TDK 1 derived from seeds cultured on the medium supplemented with proline, tryptophan and kinetin were mostly embryogenic calli. This is in agreement with the previous works showing many organic substances added to the callus induction medium including proline, tryptophan, casein hydrolysate, coconut water and kinetin increased the frequency of

Table 2 Callus formation of mature embryos cultured on MS medium supplemented with 2 mg/l 2,4-D and various kinds and concentrations of organic substances under light and dark conditions. Each treatment comprises 70 replications.

Supplement to 2 mg/l 2,4-D	Light		Dark	
	% Seed forming callus	Size of callus (mm) ¹	% Seed forming callus	Size of callus (mm) ¹
No supplement	88.14 a ³	4.34 bc	95.90 a	3.86 a
K 0.3 mg/l	88.70 a	4.29 bc	92.77 a	4.05 a
K 0.5 mg/l	93.56 a	5.83 a	81.54 a	3.69 a
Trypt 50mg/l	89.27 a	4.25 bc	87.65 a	3.48 a
Trypt 100mg/l	96.34 a	4.97 ab	91.43 a	3.55 a
CH 300 mg/l	78.68 b	3.34 c	90.13 a	3.66 a
CH 500mg/l	87.97 a	4.37 bc	83.55 a	2.98 a
P 300mg/l	89.29 a	3.63 c	89.47 a	3.64 a
P 500mg/l	96.91 a	6.02 a	88.94 a	3.58 a
CW 10 %	90.59 a	6.05 a	86.78 a	3.14 a
CW 15 %	86.49 a	5.21 ab	82.07 a	3.68 a
Average ²	89.78	4.79	87.43	3.54

¹ Size of callus = (width + length)/2

² No supplement is not included

³ Means within column followed by the same letter are not significantly different at 95 % level of confidence by DMRT

embryogenic callus formation (Siriwardana and Nabors, 1983; Armenia and Futsuhara, 1992; Chowdhry *et al.*, 1993; Rueb *et al.*, 1994).

Callus proliferation was carried out to be used in the other experiments by culturing the embryos on MS medium supplemented with 2 mg/l 2,4-D and 500 mg/l proline under light condition.

Plant regeneration

After the calli were transferred onto regeneration medium for 1 week, green spots and roots were induced from the calli. Thereafter the green spots were developed to be shoots or plantlets on the 2nd week of culture. Plant regeneration of calli observed in this experiment was the result of organogenesis process because shoots and roots were not originated simultaneously from the same cells. Shoots were developed firstly followed by

root formation at the base of some shoots. The process of plant regeneration from rice calli were classified into organogenesis (Abe and Futsuhara, 1989) and embryogenesis (Ling *et al.*, 1983) of which shoots and roots were developed simultaneously from the same originated cell.

Non-dehydrated calli cultured on MS medium without growth regulator or supplemented with 1 mg/l IAA and various concentration of either kinetin or BA formed green spots with varying frequencies from 6.86 to 37.47 % (Table 3). However, low frequencies of calli (0-5.0 %) of which green spots gave rise to shoots were observed. Among them calli cultured on MS medium supplemented with 1 mg/l IAA and 1 mg/l BA gave the highest percentage of shoot regeneration (5 %) and largest number of shoots per callus (1.66 shoots/callus).

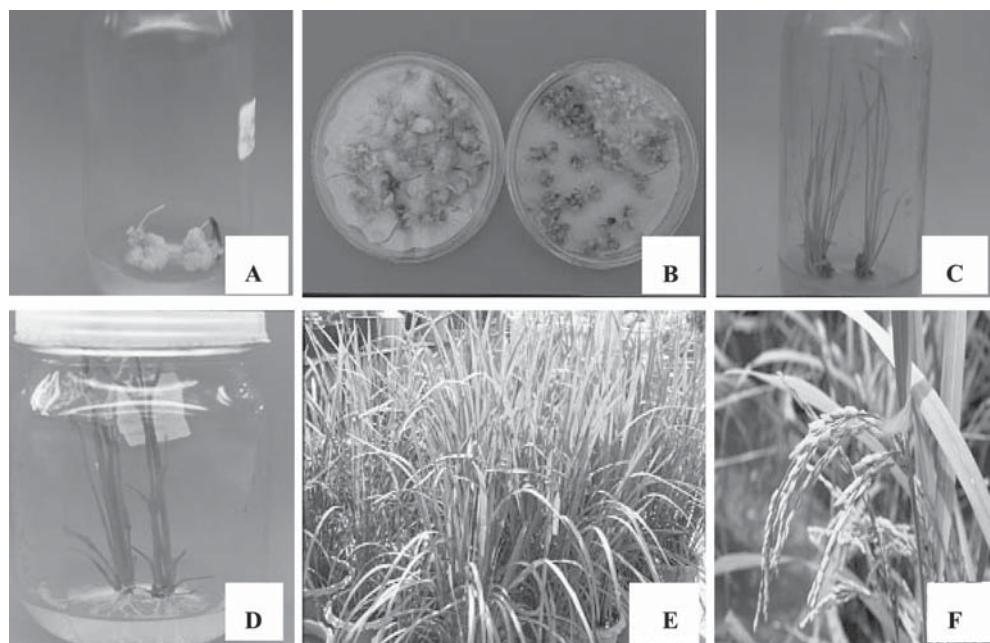


Figure 1 Callus formation and plant regeneration from mature embryos of TDK 1.

- (A) Callus formation of mature embryos cultured on MS medium containing 2 mg/l 2,4-D and 500 mg/l proline.
- (B) Non-dehydrated calli (left) and dehydrated calli under light condition (right).
- (C) Shoot regeneration from dehydrated calli (under light condition) cultured on MS medium containing 1 mg/l IAA, 4 mg/l BA and 800 mg/l casein hydrolysate.
- (D) Root formation of regenerated shoots cultured on MS medium containing 1 mg/l NAA.
- (E) Regenerated plants transplanted in the greenhouse showing normal morphological characters and well seed setting (F).

Calli dehydrated for 7 days under dark condition when culturing on every kind of regeneration medium including control could develop green spots with varying degree from 10.17 to 52.00 % (Table 4). Nevertheless, relatively low percentages of calli (0-11.26 %) developed shoots from green spots were recovered. Calli cultured on MS medium added with 1 mg/l IAA and 4 mg/l BA exhibited the highest rate of shoot regeneration (11.26 %) and largest number of shoots per callus (3.75 shoots/callus) comparing with the calli cultured on other kinds of regeneration medium.

Calli dehydrated for 7 days under light condition (Figure 1 B) formed green spots with the frequencies from 25.00 to 50.00 % when culturing

on regeneration medium (Table 5). Relatively high frequencies of calli forming shoots (0-37.00 %) were achieved. Calli cultured on MS medium supplemented with 1 mg/l IAA and 4 mg/l BA showed the highest regeneration ability (37.00 % calli forming shoots and 12.30 shoots/callus) among the calli cultured on all kinds of regeneration medium.

Comparing the non-dehydrated calli, dehydrated calli for 7 days under dark and light condition, it was revealed that calli dehydrated under light condition showed the highest average percentage of calli forming green spots (38.07 %) and shoots (12.80 %) and largest average number of shoots per callus (4.26 shoots/callus) (Table 5).

Table 3 Plant regeneration from non-dehydrated calli cultured on MS medium supplemented with 1mg/l IAA and various concentrations of either K or BA.

Growth regulator (mg/l)	No. of calli cultured	No. of calli forming			No. of shoots/callus
		Green spot (%)	Roots (%)	Shoots (%)	
0 (control)	19	37.47 a ¹	68.00 d	0.00 d	0.00 e
IAA : K					
1:1	35	13.83 d	54.83 e	3.84 b	1.28 c
1:2	29	33.88 a	57.69 e	0.00 d	0.00 e
1:3	36	22.48 c	54.76 ef	0.00 d	0.00 e
1:4	22	25.02 b	50.00 f	4.54 a	1.51 b
IAA:BA					
1:1	39	10.54 e	96.42 a	5.00 a	1.66 a
1:2	33	10.78 e	73.68 c	0.00 d	0.00 e
1:3	40	12.83 de	76.66 c	2.50 c	0.08 d
1:4	32	6.86 f	84.61 b	0.00 d	0.00 e
Average		19.29	68.51	1.76	0.50

¹ Means within column followed by the same letter are not significantly different at 95 % level of confidence by DMRT

Table 4 Plant regeneration from dehydrated calli (under dark condition) cultured on MS medium supplemented with 1mg/l IAA and various concentrations of either K or BA.

Growth regulator (mg/l)	No. of calli cultured	No. of calli forming			No. of shoots/callus
		Green spot (%)	Roots (%)	Shoots (%)	
0 (control)	38	34.52 d ¹	40.00 e	0.00 d	0.00 c
IAA : K					
1:1	64	40.62 c	35.40 g	0.00 d	0.00 c
1:2	58	43.98 b	37.50 fg	5.29 c	1.76 b
1:3	62	43.34 b	63.80 b	0.00 d	0.00 c
1:4	64	50.12 a	70.00 a	9.79 b	3.26 a
IAA:BA					
1:1	66	35.89 d	32.50 h	6.06 c	2.02 b
1:2	58	10.27 e	48.00 c	0.00 d	0.00 c
1:3	60	10.17 e	39.50 ef	0.00 d	0.00 c
1:4	70	52.00 a	45.70 d	11.26 a	3.75 a
Average		35.65	45.82	3.60	1.19

¹ Means within column followed by the same letter are not significantly different at 95 % level of confidence by DMRT

Table 5 Plant regeneration from dehydrated calli (under light condition) cultured on MS medium supplemented with 1mg/l IAA and various concentrations of either K or BA.

Growth regulator (mg/l)	No. of calli cultured	No. of calli forming			No. of shoots/callus
		Green spot (%)	Roots (%)	Shoots (%)	
0 (control)	32	37.50 c ¹	80.00 c	0.00 e	0.00 d
IAA : K					
1:1	40	40.00 c	56.25 de	0.00 e	0.00 d
1:2	40	40.00 c	55.55 ef	0.00 e	0.00 d
1:3	40	45.00 b	55.55 ef	7.69 d	2.56 c
1:4	46	29.63 d	52.63 f	11.11 c	3.70 c
IAA:BA					
1:1	32	30.55 d	84.61 b	0.00 e	0.00 d
1:2	40	25.00 e	90.00 a	29.40 b	9.80 b
1:3	42	45.00 b	58.82 d	30.00 b	10.00 b
1:4	42	50.00 a	41.17 g	37.00 a	12.30 a
Average		38.07	63.84	12.80	4.26

¹ Means within column followed by the same letter are not significantly different at 95 % level of confidence by DMRT

followed by calli dehydrated under dark condition giving an average of 35.65 % and 3.60 % calli forming green spots and shoots and an average of 1.19 shoots/callus (Table 4) and non-dehydrated calli exhibiting an average of 19.29 % and 1.76 % calli forming green spots and shoots and an average of 0.50 shoots/callus (Table 3), respectively. Interestingly, calli dehydrated under light condition performed the highest plant regeneration ability (50.00 % and 37.00 % calli forming green spots and shoots and 12.30 shoots/callus) when they were cultured on MS medium containing 1 mg/l IAA and 4 mg/l BA (Table 5).

Dehydration of calli for 7 day under dark and light condition before transferring to regeneration medium promoted plant regeneration capacity comparing to the non-dehydrated calli. A similar stimulatory effect of dehydration on plant regeneration of rice callus has been manifested in several previous studies (Tsukahara and Hirosawa, 1992; Sripichitt and Cheewasestatham, 1994; Jain

et al., 1996; Burikam *et al.*, 2002). Gray (1987) suggested that callus has lost water from the cells to become at quiescent stage during dehydration and reabsorbed water and nutrients when transferring to regeneration medium which resulted in higher capacity of plant regeneration. Whereas, Rueb *et al.* (1994) demonstrated that non-dehydrated callus released the excess water from the cells to the surface of callus which obstructed the regeneration process of the embryogenic callus. The result showed that dehydration of callus under light condition increased the frequency of plant regeneration when compared to dehydration under dark condition. Armenia and Futsuhara (1992) suggested that regeneration process of callus depended not only on genotype and suitable regeneration medium but also on the light.

The suitable medium for plant regeneration from dehydrated calli under dark and light condition in this experiment was MS medium supplemented with 1 mg/l IAA and 4 mg/l BA. It is well recognized

that regeneration of callus to become shoots (plantlets) or roots depends mainly on the proportion of auxin and cytokinin in the medium. Shoot regeneration is achieved in the medium supplemented with low ratio of auxin to cytokinin while root formation preferred the medium with high ratio of auxin to cytokinin. Thus, increasing the concentration of BA in the medium reduced the ratio of auxin to cytokinin and consequently enhanced the frequency of shoot regeneration. Although kinetin is a kind of cytokinin, it induced lower rate of shoot regeneration than BA in this study.

Enhancing plant regeneration capacity

Dehydrated calli (under light condition) were cultured on MS medium containing 1 mg/l IAA and 4 mg/l BA (result of the previous experiment) in conjunction with various kinds and concentrations of organic substances to increase the frequency of

plant regeneration. It was found that organic supplements including tryptophan, casein hydrolysate, yeast extract and coconut water induced higher percentage of calli forming green spot and shoots and number of shoots per callus than those of the control (no supplement) (Table 6). Especially, casein hydrolysae at the concentration of 800 mg/l gave the highest frequency of calli forming green spots (80 %) and shoots (45 %) and largest number of shoots per callus (15 shoots/callus) (Figure 1 C) followed by 500 mg/l casein hydrolysate exhibiting 75 % and 40 % calli forming green spots and shoots and 13.3 shoots/callus and 2,000 mg/l yeast extract showing 57.14 % and 35.71 % calli forming green spots and shoots and 11.90 shoots/callus, respectively. There were several reports demonstrating that casein hydrolysate, yeast extract, tryptophan and proline could promote the efficiency of plant regeneration from rice callus (Ling *et al.*, 1983; Raina *et al.*, 1987; Sahrawat and Chand,

Table 6 Plant regeneration from dehydrated calli (under light condition) cultured on MS medium supplemented with 1mg/l IAA, 4mg/l BA and various kinds and concentrations of organic substances.

Supplement to 1mg/l IAA+4mg/lBA	No. of calli cultured	No. of calli forming			No. of shoots/ callus
		Green spot (%)	Roots (%)	Shoots (%)	
No supplement	57	20.24 j ¹	30.62 a	4.73 g	1.57 f
AS 20 mg/l	31	29.00 i	16.12 d	0.00 h	0.00 f
AS 40 mg/l	33	72.72 c	30.30 a	0.00 h	0.00 f
Tryp 50 mg/l	32	62.50 d	25.00 b	0.00 h	0.00 f
Tryp 100 mg/l	20	50.00 f	30.00 a	10.00 f	3.30 e
CH 500 mg/l	20	75.00 b	10.00 e	40.00 b	13.30 b
CH 800 mg/l	20	80.00 a	8.00 f	45.00 a	15.00 a
YE 1,000 mg/l	24	37.50 h	8.33 f	29.16 d	9.72 c
YE 2,000 mg/l	28	57.14 e	3.57 g	35.71 c	11.90 b
CW 10 %	30	36.66 h	23.33 c	16.00 e	5.30 d
CW 20 %	20	40.00 g	5.00 g	15.00 e	5.00 d
Average		50.97	17.29	17.78	5.91

¹ Means within column followed by the same letter are not significantly different at 95 % level of confidence by DMRT

Table 7 Root formation of regenerated shoots cultured on MS medium supplemented with various concentrations of NAA.

NAA (mg/l)	No. of shoots cultured	No. of shoots forming roots	% Root formation
0	39	36	92.30
0.5	63	62	98.41
1.0	56	56	100.00
2.0	53	53	100.00

1997). However, the beneficial effect of organic substances on plant regeneration is not clearly known.

Root induction

Regenerated shoots when transferring onto MS medium containing NAA at the concentrations of 0.5-2 mg/l formed roots with the higher frequencies (98-100 %) than those cultured on MS medium without NAA. Especially, NAA at the concentrations of 1-2 mg/l induced the highest percentage of shoots forming roots (100 %) (Figure 1 D). Moreover, shoots produced higher number of roots and longer root length when culturing on rooting medium containing NAA. Auxin such as NAA, IAA and IBA are generally used for regulating cell elongation, cell division, formation of adventitious roots and callus initiation and growth when added to the medium at appropriate concentrations. NAA and IBA have been reported to induce roots from regenerated shoots of rice (Sahrawat and Chand, 1998; Burikam *et al.*, 2002). The complete plantlets obtained were transferred to grow in pots under greenhouse condition until maturity (Figure 1 E). They were morphologically normal and could set seeds well (Figure 1 F).

CONCLUSIONS

1. The appropriate medium for callus induction from mature embryos of glutinous rice cultivar TDK 1 was MS agar medium added with 2

mg/l 2,4-D and 500 mg/l L-proline.

2. Dehydrated calli showed higher capacity of shoot regeneration than non-dehydrated calli.

3. The suitable medium for plant regeneration from dehydrated calli (under light condition) was MS agar medium supplemented with 1 mg/l IAA, 4 mg/l BA and 800 mg/l casein hydrolysate.

4. Root induction was achieved when the regenerated shoots were transferred onto MS agar medium containing 1 mg/l NAA.

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