FACTORS OF CALLUS AND COMBINATION OF PLANT GROWTH REGULATORS EFFECT ON THE PLANT REGENERATION FROM SCUTELLUM OF INDICA RICE (Oryza sativa L. cv. KDML 105)

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ABSTRACT

Factors effect on the plant regeneration of indica rice (*Oryza sativa* L. cv. KDML 105), callus age, desiccation, and combination of plant growth regulator, were investigated in this experiment. Compact and friable calli developed from scutellum cultured on NB basal medium containing 500 mg/l L-proline, 500 mg/l L-glutamine, 300 mg/l casein hydrolysate, 2 mg/l 2,4-D and 30 g/l sucrose. Treatments of four-week-old calli and partial desiccation before transferring clumps of embryogenic calli onto NBR medium showed higher rate of green bud and shoot regeneration than that of eight-week-old calli and non desiccation treatments. The adjustment of BA concentration in combination with 1 mg/l IAA was performed. Maximum shoot bud formation frequency (18.92%) was obtained in NBR medium containing 5 mg/l BA. Regenerated shoots were transferred to normal NB medium and plantets were successfully grown in the green house.

KEYWORDS: Indica rice, KDML 105, regeneration, callus age, partial desiccation, plant growth regulator

1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important crops, which more than 700 million people consume as their main food [1]. Cultivated rice is divided into three subspecies: Indica, Japonica and Javanica. The Indica varieties are widely grown in rice growing regions, especially in developing countries [2]. Khao Dawk Mali 105 (KDML 105), one of the indica's rice, is the most popular aromatic rice variety grown in Thailand [3]. Rice plantation for KDML 105 occupies the area over 2.5 Mha all over the country, 80% of which resides in the Northeastern region. The annual production of KDML 105 approximately 4.5 Mton which is lower than the demand of more than 5 Mton of the world market [4]. Demand for this variety is increasing in both domestic and international markets due to the appreciation of its good quality and high aroma level [5].

Plant tissue culture has played an increasingly important role in rice improvement and rice genetic transformation [6]. Production of callus and its subsequent regeneration from different stages of explants are the primary steps in rice improvement via tissue culture and plant biotechnology. The potential for callus formation and regeneration have been reported to be influence by several factors including the nutrition composition of the culture media, culture conditions and explant origin and genotype [7-10]. Despite of tremendous efforts, the success for reproducible fertile rice regeneration in *in vitro* culture has been limited in indica rice varieties. Significant differences among the genotypes of indica rice may affect the regeneration potential. Therefore, there is need to develop an efficient regeneration protocol for each varieties of indica rice, in order to the useful of rice improvement. The aim of this research was attempt to develop a high regeneration frequency of differential's callus from mature seed of KDML 105.

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2. MATERIALS AND METHODS

2.1 Callus Induction

The plant materials used in this study was jasmine rice (*Oryza sativa* L. ssp. Indica cv. KDML 105). Seeds were dehusked and then surface sterilized by the following steps: immersion in 70 % ethanol for 2 min, soaking in 5 % (v/v) commercial beach (approximately 6 % sodium hypochloride) with 2-3 drops of Tween20® for 30 min, soaking in 30 % commercial beach with 2-3 drops of Tween20® for 30 min and then rinsed 5-6 times with sterile distilled-water. Surface-sterilized seeds were placed in the Petri dishes containing sterile tissue papers. After drying, the seeds were transferred to callus induction medium containing NB salts [11], 500 mg/l L-proline, 500 mg/l L-glutamine, 300 mg/l casein hydrolysate, 2 mg/l 2,4-D, 30 g/l sucrose and 8 g/l agar at pH 5.8. The cultures were incubated under darkness at 25 ± 2 °C for 4 weeks.

2.2 Callus age on plant regeneration

In this experiment, source of calli were received from time difference of calli initiation. The first group was four-week-old calli and the second group was eight-week-old calli. Eight-week-old calli were produced from six-week-old calli and then subcultured onto fresh medium for 2 weeks as described by Imjongjirak (2000). The both groups of calli were transferred to regeneration medium containing NB salts, 500 mg/l L-proline, 1 g/l yeast extract, 300 mg/l casein hydrolysate, 1 mg/l IAA, 4 mg/l BA, 30 g/l sucrose and 8 g/l agar at pH 5.8 (NBR medium). Calli were cultured under 16 hr/day photoperiod at 25 ± 2 °C and subcultured every 2 weeks to fresh NBR medium. All green spot and shoot bud formation were scored after transfer to NBR medium within 2-6 weeks.

2.3 Effect of partial desiccation of calli

Four-week-old calli were used for each treatment of desiccation experiment. The first group of calli was cultured directly onto NBR medium as refer to non desiccated calli. The second group of calli was transfer to the Petri dishes containing sterile Whatman-1 filter papers, sealed with parafilm and kept at 25 ± 2 °C in dark condition for 7 days. After desiccation treatment, these calli were transferred to NBR medium. Both of calli were cultured in the same regenerative condition as described above. After 2-6 weeks, green spot and shoot bud formation were observed. New shoot buds were subculture to NB medium

2.4 Effect of BA on plant regeneration

Four-week-old calli were used as explants. Desiccated calli were cultured on NBR medium with the adjustment of 6-bensyl adenine (BA) to 3, 4, 5 and 6 mg/l. The cultures were then maintained under condition of plant regeneration. These calli were subcultured every 2 weeks to fresh NBR medium containing the same concentration of BA. An entire of green spot and shoot bud formation on the calli were examined.

3. RESULTS AND DISCUSSION

3.1 Callus Induction

Several reports have been shown the various factors affect callus formation and plant regeneration frequency in the rice [12-14]. In this experiment, callus formation was developed from the scutellar region of the seeds within 4 weeks on callus induction medium that contains 2 mg/l 2,4-D. Yellowish friable and some compact calli were obtained and used for the regeneration of subsequent experiment. The pretreatment of rice seedling with 2,4-D has been report that reduced the time required for shoot regeneration and increased the number of shoot regeneration [6]. This positive effect may due to the fact that the stimulated auxin may have changed the balance of endogenous growth regulator inside the plant cell.

3.2 Factors effect on plant regeneration

Table 1 shows the effect of initial callus age on the plant regeneration. After 2-6 weeks of culture on NBR medium, four-week-old calli were covered with green spots and shoot buds higher percentage than eight-week-old calli (11.2 % and 2.94 %, respectively). This evidence indicated that four-week-old calli are more suitable for rice regeneration than eight-week-old.

Development stage of callus has been reported to be effective for the regeneration [15-16]. This result is the same as previous report that four-week-old callus is suitable for green spots and shoot buds production of indica rice [15, 17-18]. Due to number of living cell and age of callus may affect rice regeneration.

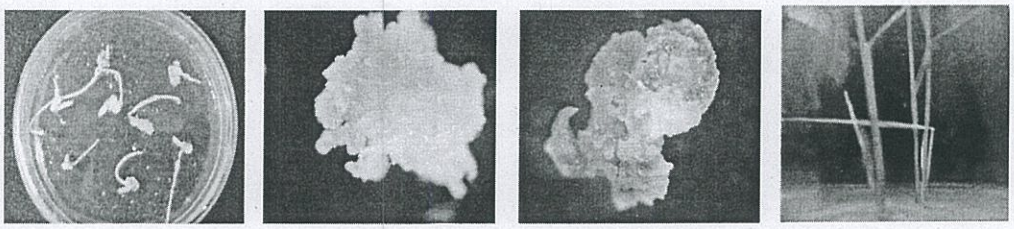
After desiccation of callus in the dark condition for 7 days and then transfer to regeneration medium, the desiccated calli treatment shows the increase of shoot buds formation comparing with the non desiccated calli approximately 4 fold (12.12 % shoot buds formation, Table 2).

Partial desiccation has been found to promote the rice regeneration [10, 19-21, 22-23]. Desiccated callus has water-deficit from relative humidity outside cell lower than inside cell. Thus this callus had more efficiently absorbing nutrient from medium when it was transferred in regeneration medium [24].

According to high frequency of regeneration from desiccated calli, these calli were used for increase efficiency of this experiment. NBR medium containing 1 mg/l IAA with different concentrations of BA (3-6 mg/l) were tried and found that the combination of 1 mg/l IAA and 5 mg/l BA gave rise to the highest number of green spots covering calli and also shoot bud formation. The regeneration frequency in this treatment was 18.92 % (Table 3). This compares to 12.12, 5.88 and 2.78 % when 4, 3 and 6 mg/l BA were used in combination with 1 mg/l IAA, respectively.

Plant regulators have an important role in callus cultures and plant regeneration. Medium containing a combination of auxin and cytokinin is used for plant regeneration [25]. Of all the cytokinins, BA is most used for plant tissue culture [26].

Somatic embryo formation has been reported in cereals when cultures were transferred from a medium with a high to a low concentration of auxin [27]. Also optimal concentration of low auxin (IAA) combination with high cytokinin (BA) affects the increase of somatic embryo formation. In plant regeneration experiment, we found that the suitable of BA concentration of KDML 105 is slightly higher that of other indica rice varieties [3, 10, 15, 17, 20, 28-29]. Plantlets also formed roots on NB medium without supplement, and they were well established when transferred to the soil.



(A) (B) (C) (D)

Figure 1. Regeneration of rice plants varieties Khao Dawk Mali 105:
(A) Callus induction from scutellum of mature seed for 21 days
(B) Characteristic of green spots on the surface of callus in regeneration medium (NBR medium) for 21 days
(C) Shoot regeneration of callus on callus in regeneration medium (NBR medium) for 28 days
(D) Plantlet of rice from callus in NB medium for 14 days

Table 1. Green spot and shoot bud formation from four- and eight-week-old callus of Indica rice (*Oryza sativa* L. cv. KDML 105). Evaluation within 6 weeks after transfer of callus to the regeneration medium.

Treatment	Number of callus	Development of callus			
		Produced green spot (no.)	% Green spots formation	Produced shoot bud (no.)	% Shoot buds formation
Four-week-old	34	4	11.2	1	2.94
Eight-week-old	33	8	24.2	4	12.12

Table 2. Green spot and shoot bud formation from partial desiccation and non desiccation callus of Indica rice (*Oryza sativa* L. cv. KDML 105). Evaluation within 6 weeks after transfer of callus to the regeneration medium.

Treatment	Number of callus	Development of callus			
		Produced green spot (no.)	% Green spots formation	Produced shoot bud (no.)	% Shoot buds formation
Non desiccation	34	4	11.2	1	2.94
desiccation	33	8	24.2	4	12.12

Table 3. Green spot and shoot bud formation received from four-week-old callus with partial desiccation of Indica rice (*Oryza sativa* L. cv. KDML 105) on NBR medium containing 3, 4, 5 and 6 mg/l BA combination with 1 mg/l IAA. Evaluation within 6 weeks after transfer of callus to the regeneration medium.

Treatment	Number of callus	Development of callus			
		Produced green spot (no.)	% Green spots formation	Produced shoot bud (no.)	% Shoot buds formation
3 mg/l BA	34	7	20.6	2	5.88
4 mg/l BA	33	8	24.2	4	12.12
5 mg/l BA	37	15	40.5	7	18.92
6 mg/l BA	36	5	13.9	1	2.78

4. CONCLUSIONS

In conclusion, increase of plant regeneration from callus derived from scutellum of indica rice (*Oryza sativa* L. cv. KDML 105) was achieved by using proper age of callus and by partial desiccation of callus. Moreover the suitable concentration of auxin and cytokinin combination on shoot bud regeneration of callus should be variable depend on indica rice varieties.

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