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Effects of L-lysine on Callus Formation , Plant Regeneration and Flowering of Thai Rice c.v. KDML 105

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ABSTRACT

Seed-cultured of KDML 105 on Murashige and Skoog (MS) medium supplemented with 2 mg/l 2,4–D , 1 g/l L-proline , 1 g/l casein hydrolysate and 20 μM L-lysine produced the highest average percentage of callus formation (95.16%). Regeneration of the calli into plantlets could be best achieved at the average of 63.87 % in the MS medium containing 1 mg/l kinetin, 1 g/l L–proline, 300 mg/l casein hydrolysate and 20 μM L-lysine. Plantlets were cultured in the MS medium containing 3 mg/l BAP, 3 g/l activated charcoal and 20 μM L-lysine before transferring to pots under greenhouse condition. Two plants out of 80 plantlets reached the flowering stage under long photoperiod and produced only 6 seeds altogether. The seeds were subsequently planted for 2 generations and DNA fingerprints of these plants were determined using sequence related amplified polymorphism (SRAP) technique. All 6 clones of the L-lysine-treated plants gave different DNA patterns from that of the original KDML105 suggesting some effects of L-lysine on their genetic variation.

Key words: L-lysine, KDML105, SRAP technique

INTRODUCTION

KDML105 is the most popular variety of rice grown in Thailand due to its special characteristic of soft glutinous texture and pleasant palatable aroma. Rice plantation for KDML 105 occupies the area over 2.5 Mha all over the country, 80% of which resides in the Northeastern part of Thailand (Chotinant, 1999; Department of Agriculture, 2000). The annual production of KDML105 equals to a total of 4.5 Mton which is still below the high demand of more than 5 million ton of the world market (Sithisuang , 1995).

KDML105 is a photo–sensitive variety and could be naturally grown only once a year. Attempts are being made to increase its production by inducing a photo–insensitive variety which can be grown and flower all–year–round. The objective of this study was to find the effect of L–lysine on callus formation, plant regeneration and flowering of KDML105.

MATERIALS AND METHODS

I. Callus formation

Mature seeds of KDML105 were dehusked and surface-sterilized by soaking in 70% ethanol for 3 min and in 15% Chlorox for 20 min followed

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by rinsing 3 times in sterile distilled water. The surface aseptic seeds were cultured on 5 different callus induction media (Murashige and Skoog, 1962) supplemented with 2 mg/l 2,4–D, 1 g/l L–proline, 1 g/l casein hydrolysate and L–lysine at various concentrations of 0, 5, 10, 20 and 40 μM and subjected to a completely randomized design with 4 replications. Each medium formula in the replication contained 150 seeds. Seeds were cultured for 3 weeks under 16 hr/day illumination and callus formation was recorded.

II. Plant regeneration

Calli from seed-cultured were dehydrated by placing on an autoclaved filter paper in a petridish and sealed with a parafilm. The dishes were kept in the dark for 3 days. They were cultured on MS medium supplemented with 1 mg/l kinetin, 1 g/l L–proline, 300 mg/l casein hydrolysate and L–lysine at various concentrations of 0, 5, 10, 20 and 40 μM. The cultures were also incubated under 16 hr/day of light for 3 weeks before changing the medium and allowed to grow for another 6 weeks. The development of callus, percentage of plantlet regeneration, and the number of plantlets per callus were determined.

III. Plantlet multiplication and flowering

Plantlets were transferred to culture on MS medium supplemented with 3 mg/l BAP, 3 g/l activated charcoal, and 5 different concentrations of L–Lysine as previously described. Each medium formula supported the growth of 16 plantlets for 4 weeks. All 80 plants were subsequently transferred to grow in pots under the greenhouse condition for 3 months. Plant height, multiplication rate, and flowering were recorded. The seeds collected from the flowering plants were further cultivated in pots until they are fully mature.

IV. Fingerprint of extracted DNA

The leaves were collected from each flowering plant. DNA was extracted using the modified method of Agrawal *et al*. (1992) and the fingerprintings of these extracted DNA were determined using SRAP (sequence–related amplified polymorphism) technique (Li and Quiros, 2001).

RESULTS AND DISCUSSION

I. Callus formation

After having been cultured in the medium for 3 days, callus began to form at the scutellum of the cultured seed. Most of the forming calli were compact type (Figure 1). They were creamy in color and tightly aggregated showing their embryogenic calli nature. All 5 MS media supplemented with different concentrations of L-lysine gave high percentage of callus formation in the range of 91–95 % (Table 1). Although the medium containing 20 μM L-lysine seemed to be the best to support callus formation, there was no statistical difference among the results in using different amount of L-lysine in the cultured medium or even from L-lysine free medium. The non–significant role of L-lysine on callus formation agreed with the work of Zhongkui (1988) on lotus (*Nelumbo mucifera* Gaerth) which indicated that only low concentration of L-lysine and threonine could promote the formation of callus but higher concentration inhibited the growth of these calli.

II. Plant regeneration

After dehydrating the calli and keeping them in the dark for 3 days, they were transferred onto the regeneration medium. The first appearance of callus growth was observed after one week in the new medium, and green spots on the surface of some calli were distinctly seen 2-3 weeks afterwards (Figure 2). Most of these green spots were eventually developed into complete mature plantlets (Figure 3). However, some of green spots on callus remained the same for some time or turned into black spots. Some calli just developed the rootlets. Although all the cultured calli could

be developed into plantlets, the percentages of plantlet formation varied considerably (Table 2). The medium supplemented with 20 μM L-lysine gave the best plantlet formation (63.87%). Addition of L-lysine to the medium seemed to affect of plantlet regeneration (Figure 4). This finding is in agreement with the work of Vijayan *et al*. (1998) who showed the positive effect of L-lysine on shoot induction of mulberry.

III. Plantlet multiplication and flowering

The plantlets were transferred to grow in the MS medium supplemented with 3 mg/l BAP, 3 g/l activated charcoal and 5 different concentrations of L–lysine for 4 weeks, and subsequently transferred to pots for 3 months. The plantlets from the medium supplemented with 40 μM L-lysine could reach the average height of 133 cm while those from the medium with 0, 5, 10,

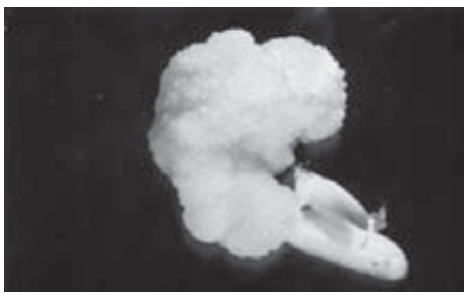

Figure 1 Characteristic of compact callus.

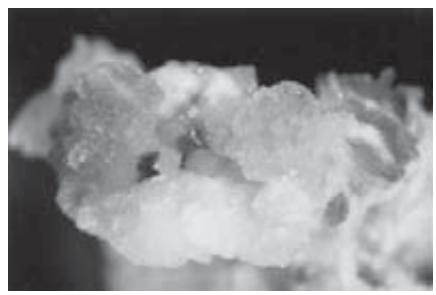

Figure 2 Characteristic of green spots on the surface of callus.

Figure 3 Shoot formation on callus.

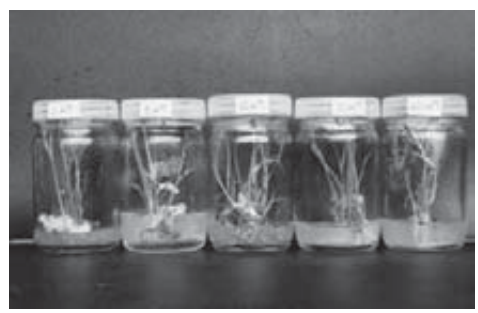

Figure 4 Plantlets regeneration from callus.

Table 1 Effect of L-lysine at different concentrations on the percentage of callus formation derived from KDML105 seed.

L-lysine concentration (μM)	No. of seed culture per one experiment	Replication				Average
		1	2	3	4	
0	150	93.18	83.33	97.22	90.36	91.02
5	150	93.22	80.00	97.62	97.62	92.12
10	150	98.25	77.08	100.00	100.00	93.83
20	150	98.15	82.50	100.00	100.00	95.16
40	150	93.65	83.33	100.00	98.25	93.81

Table 2 Average percentages of plantlet regeneration, green spot, root and black callus formation from different calli cultured on different regenerated media.

1/Group of callus	Regenerated media containing lysine (μM)	Plantlet formation (%)	Green spot formation (%)	Root formation (%)	Black callus formation (%)	No. of plantlets per callus
Group 0	0	17.48	25.37	5.38	51.69	7.00
	5	24.21	0	16.82	59.89	12
	10	33.27	14.84	15.92	35.89	5.11
	20	54.32	0.00	5.38	41.09	7.22
	40	26.43	9.05	0.12	64.29	9.33
Group 5	0	10.00	13.10	7.03	69.79	7.50
	5	20.32	11.83	18.47	49.24	5.80
	10	29.77	2.39	6.78	50.94	6.79
	20	33.52	13.27	0.12	52.94	4.75
	40	26.99	4.61	16.82	51.49	4.22
Group 10	0	26.49	29.49	12.83	31.20	5.87
	5	47.44	25.07	12.83	16.53	9.79
	10	55.77	10.68	10.26	23.31	8.72
	20	59.30	16.03	9.29	15.40	10.46
	40	39.31	0.00	12.53	48.13	8.22
Group 20	0	45.51	11.04	9.95	33.50	9.06
	5	49.15	9.78	4.33	36.73	9.38
	10	58.02	17.82	7.40	16.77	9.70
	20	63.87	8.06	8.06	20.00	9.99
	40	59.74	14.01	11.73	14.50	11.06
Group 40	0	29.10	6.35	15.72	50.17	5.91
	5	36.38	20.66	23.43	19.23	12.22
	10	43.96	31.53	21.00	3.50	6.03
	20	46.99	12.35	11.36	28.63	9.98
	40	36.51	20.04	14.50	27.60	6.85

1/ The number of group referred to the concentrations of L-lysine (μM) in the medium.

20 μM lysine were 132, 129, 128 and 126 cm, respectively. However, the highest number of tillers per plant (5 tillers/ plant) was obtained from those grown in the medium supplemented with 10 μM L-lysine (Table 3) while the lowest number (3.33 tillers/plant) came from that of 5 μM L-lysine supplement.

Only 2 out of 80 plantlets grown under the greenhouse condition could reach the flowering stage in August. They both were exposed to different concentrations of L-lysine. One had been on 5 μM L-lysine of callus induction medium and flowered 24 days after transferring to the pot (Figure 5) while the other one had been on 40 μM L-lysine medium and flowered 26 days after being grown in the pot. Since August is a long photoperiod month which is unsuitable for normal KDML105 to flower, the occurrence of this flowering

Table 3 Plant height and number of tillers per plant cultured on the modified MS medium containing different concentrations of L-lysine.

L-lysine concentration (μM)	The first month		The second month		The third month	
	Plant height (cm)	No. of tillers per plant	Plant height(cm)	No. of tillers per plant	Plant height (cm)	No. of tillers per plant
0	96.58	3.11	124.69	4.17	131.83	4.89
5	98.71	2.81	126.50	3.57	129.10	3.33
10	95.65	3.39	120.00	5.13	127.87	5.00
20	94.16	3.19	120.00	4.56	126.38	4.31
40	93.45	2.89	126.05	3.95	133.16	4.00

suggested the somaclonalvariation resulted from the supplementation of L-lysine in the culture medium. L-lysine itself could naturally turn into pipecolic acid which is known to have certain effect on flowering in plants. The work of Fujioka and Sakurai (1992) indicated that flowering of *L. paucicostata* 151 grown under 9 hr/day illumination for 8 days was inhibited but adding L–pipecolic acid to the culture medium reverted the blockage resulting in numerous budding of flowers.

IV. Fingerprinting of extracted DNA

The two flowering plants gave 6 healthy seeds, and these seeds were further cultured to the full–grown rice plants. Their leaves were extracted and subjected to DNA fingerprinting using SRAP (sequence–related amplified polymorphism) technique. The fingerprinting showed distinctly different band patterns from those of wild type KDML105 (Figure 6). This might result from the effects of L-lysine on callus formation and regeneration as well as during its growth and flowering.

Figure 5 The 30 day-old panicle of the mature rice plant derived from the plantlet grown in 5 μM L- lysine medium.

CONCLUSIONS

1. The suitable medium for callus induction from mature seeds of rice c.v. KDML105 was MS medium supplemented with 2 mg/l 2,4-D, 1 g/l L–proline, 1 g/l casein hydrolysate and 20 μM L-lysine.

2. For the development of plantlet from callus, MS medium supplemented with 1 mg/l kinetin, 1 g/l L–proline, 300 mg/l casein hydrolysate and 20 μM L-lysine gave the best result.

3. Only 2 out of 80 plantlets of L-lysine-treated culture could survive and flowered in the natural field condition. One was from the 5 μM L-lysine-supplemented culture and another was from

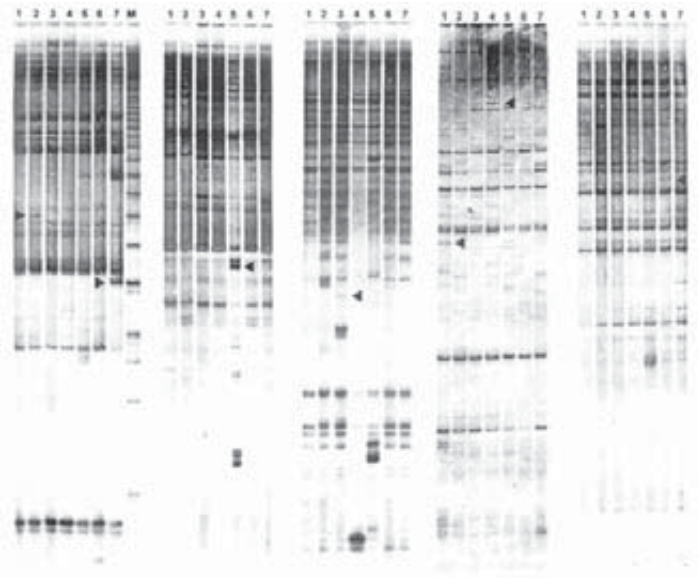

Figure 6 DNA fingerprinting of 6 rice plants derived from using sequence-related amplified polymorphism (SRAP) technique.

40 µM L-lysine. The total of 6 seeds were obtained from these 2 matured plants.

4. The SRAP fingerprints of these 6 surviving plants were distinctly different from the original patterns found in wild type KDML105.

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