Micropropagation of Mangosteen Through Young Leaf Culture

Sompong Te-chato and Mongkol Lim

Department of Plant Science, Faculty of Natural Resources, Prince of Songkla University

Hat Yai, Songkla 90112, Thailand

ABSTRACT

A four-step procedure was used for plant regeneration from *in vitro* grown laminae (5-15 mm long) of mangosteen. Step I medium was Murashige and Skoog (MS) with benzyl adenine (BA) at 0.5 mg/l in combination with thidiazuron (TDZ) 0.5 mg/l. Step II medium was woody plant medium (WPM) with BA alone at 0.1 mg/l. Step III medium was step II medium overlaid with half strength liquid MS with 0.06 naphthaleneacetic acid (NAA) and 0.03 mg/l BA. Step IV medium was WPM with 0.25 mg/l BA, 0.25% activated charcoal (AC) and or 5.6 mg/l phloroglucinol (PG). Lamina explants were cultured in medium I for 3-4 weeks to induce callus and the calluses were subcultured to the medium I for 2-3 times in order to multiplication. Step II medium was designed to induce leaf primordia. Medium III and IV were used to promote elongation of the shoots and induce roots, respectively.

INTRODUCTION

In vitro culture of mangosteen (*Garcinia mangostana* Linn.) were established using seeds (1, 10), young leaves from *in vitro* (13) and field grown seedlings and mature trees (2). Most of those reports described plant regeneration directly from cultured explants. Propagation of mangosteen by formerly mentioned protocols is less effective and leading to a failure in mass propagation of the trees. Recently, there are some reports on callus formation from young leaf culture (15). The calluses are classified to be compact nodule one. Histological studies revealed that it is composed of meristematic dome and a pair of leaf primordia. Origin of the nodules are from the two main sources of cells so called epidermis and vascular bundle (13). This success has been achieved by manipulating culture medium with thidiazuron (TDZ) (16).

TDZ at concentration higher than 1 μM was reported to promote callus formation, adventitious shoots or somatic embryos in many fruit crops (5, 7, 8, 9).

So far, there has no report on plant regeneration from meristematic nodule callus induced from any explant of mangosteen tree. This successful might be very helpful for mass propagation in a short term and improvement of this crop by genetic engineering technique in the near future. In this paper, we report clear distinguish steps routinely using in plant regeneration method for clonal propagation of mangosteen.

MATERIALS AND METHODS

Plant material:

In vitro shoot cultures of mangosteen were maintained using seed and leaf explant subcultured every 3-4 weeks. Seed- and leaf-derived shoots were excised and placed in half-strength liquid Murashige and Skoog (1/2MS). Culture conditions were similar to those reported previously (15, 16). Young purple or red and green leaves were collected from 3-6 week-old shoots and used for culture.

Meristematic nodule callus induction and multiplication:

The leaves with size approximately 5-15 mm in length were excised and transferred to step I medium. The medium was MS modified by supplemented with 500 mg/l polyvinylpyrrolidone (PVP), 3% sucrose, BA and TDZ at various concentrations showed in Table 1. The medium was solidified with 0.2% Phytagel (Sigma). Multiplication of the callus was carried out by regular subculture at 3 week-intervals to fresh medium of the same composition supplemented with BA and TDZ at an equal concentration of 0.5 mg/l during the initial 4 subcultures, number of nodules was counted at the end of each subculture and multiplication rate was recorded.

Shoot bud induction:

Shoot bud induction from the callus was performed in step II medium. The medium was woody plant medium (WPM) supplemented with 500 mg/l PVP, 3% sucrose, 0.3% Phytagel and BA at various concentrations. Three pieces of the callus were transferred to 25 ml of the medium containing in medium bottle (40 x 80 mm).

Elongation of the shoots:

The callus with tiny shoot buds which compose of meristematic dome and one pair of leaf was transferred to step III medium. The media used in step III were step II medium with two different quantities, 15 and 25 ml and two phases media. Lower phase medium is 10 ml of step II medium and upper phase medium is 1/2MS liquid medium supplemented with 0.06 mg/l NAA and 0.03 mg/l BA. Each culture bottle was contained one piece of the callus.

Root induction:

Elongated shoots of more than 10 mm (in length) were excised and subjected to various treatments (Table 4). In case of treating, the basal cut end of shoots were wounded. The shoots were then dipped in the solution of 1,000 mg/l IBA which was sterilised by filtering through Millipore membrane with pore size 0.45 μm in the dark for 15 minutes. Each shoot was finally transferred to test tube (20 x 150 mm) containing 10 ml of step IV medium. The medium is WPM supplemented with 0.25 mg/l BA, 0.25% activated charcoal (AC), 5.6 mg/l phloroglucinol (PG), 3% sucrose and 1.0% Agar-Agar.

All the culture were maintained at 28°C under 2,000 lux illumination for 16 hour photoperiod. For callus induction and multiplication, 25 explants were placed in each Petri-dish. A minimum of 5 Petri-dishes were designed to each treatment. In case of shoot bud induction and elongation of the shoots, 3 pieces of the callus were placed in medium cultured bottle containing 25 ml of culture medium. A minimum of 25 vessels were designed to each treatment. Data on percentage of the callus formation, number of nodules and shoot regeneration were recorded. The data were statistically analysed using completely randomized design (CRD) and mean among each treatment was separated by Duncan's Multiple Range Test (DMRT). For root induction in step IV procedure, a hundred shoots were used to each treatment. Initially, the cultures were maintained under darkness for 2 weeks to induce root primordia. To promote elongation of the roots, the cultures were transferred to illuminated condition with 2,000 lux, 16 hour photoperiod at 26°C for further 2-4 weeks. At the end of culture period, percentage of root formation, number of root were scored.

RESULTS

Meristematic nodule callus induction and multiplication:

All concentrations of BA and TDZ tested in this experiment could provide meristematic nodule callus (Table 1). A lower (0.1 mg/l) or higher (1.0 mg/l) concentration of TDZ in combination with BA (0.5 mg/l) caused decrement percentage of meristematic nodule callus. An equal concentration of BA and TDZ at 0.5 mg/l gave the best results (Table 1).

Table 1 Effect of various concentrations of BA and TDZ on meristematic nodule callus formation

BA (mg/l)	TDZ (mg/l)	% meristematic nodule callus	avr. no. of nodule
0.5	0.1	15.0 ± 4.4 c	11.3 a
0.5	0.5	68.8 ± 20.1 a	10.3 a
1.0	0.5	66.7 ± 19.5 a	8.6 a
1.0	1.0	25.0 ± 7.3 b	5.6 b

Mean not sharing letter in common within column differ significantly by DMRT (P=0.05).

The result in Table 1 showed that step I medium used for callus induction is basal MS supplemented with 500 mg/l PVP, 3% sucrose, 0.5 mg/l BA, 0.5 mg/l TDZ and 0.2% Phytagel. The medium was also used for multiplication the callus. During four times of subculture, an average number of multiplication rate of the nodules was recorded to be 4 to 5 times within 3-4 weeks of culture (Table 2).

Table 2 Effect of subculture on multiplication of the nodules

no. of subculture	multiplication rate
1	4.7
2	4.8
3	4.2
4	4.1

Shoot bud induction:

The calluses induced in induction step I medium ceased to undergo further development. An additive or additional meristematic nodules was obtained when they were kept in the same medium. After transfer to WPM in the absence of TDZ, the nodules developed beyond globular-like stage. Yellow or cream immature nodules turns to mature green one. A great number of shoot bud formation was obtained in the medium supplemented with 0.1-0.5 mg/l BA (Table 3). A high concentration of BA (1.0 mg/l) reduced average number of shoot bud formation. According to the result, step II medium is WPM supplemented with 500 mg/l PVP, 3% sucrose, 0.1 mg/l BA and 0.3% Phytagel.

Table 3 Effect of various concentrations of BA in WPM on average number of shoot bud formation from the nodule

BA (mg/l)	avr. no. of shoot at length		
	0-5	6-10	>10 mm
0.1	9.27 a (2-19)	0.97 (0-7)	0.58 (0-6)
0.5	8.39 a (3-19)	0.94 (0-6)	0.48 (0-5)
1.0	5.90 b (1-13)	0.48 (0-5)	0.22 (0-5)

Mean not sharing letter in common within column differ significantly by DMRT (P=0.05).
Number in parenthesis shows the range of shoots.

Elongation of the shoots:

Shoots obtained from all concentrations of BA elongated very slowly. Transfer a cluster of tiny shoot buds to a thick layer of step II medium (25 ml) or overlaid thin layer medium with 10-15 ml of liquid 1/2MS containing 0.06 mg/l NAA and 0.03 mg/l BA promoted a rapid elongation of shoots (Figure 1). Step III medium is the medium used for overlaying on step II medium.

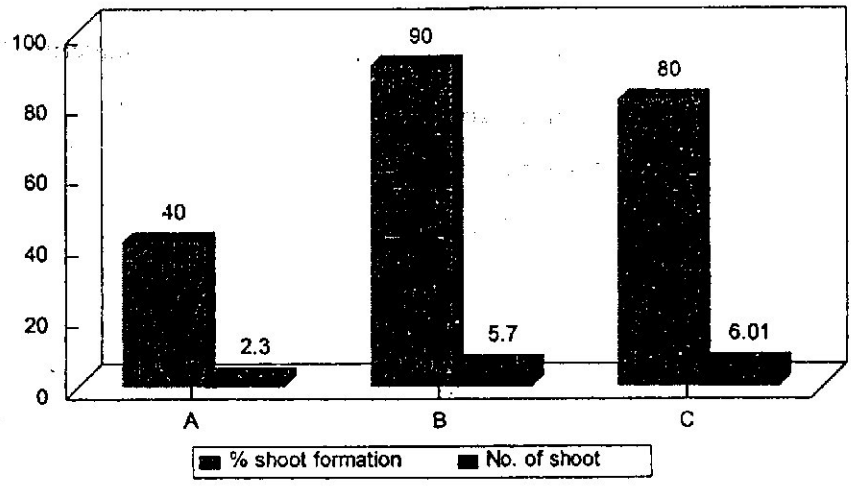

Figure 1 Effect of step III medium on shoot bud formation. A: thin, B: thick layer of step II medium and C: overlay with step III medium.

Root induction:

The highest percentage of root induction was obtained from micro-shoots which was wounded and treated with IBA solution (Table 4). Addition PG to the medium caused a slightly decrease in percentage of root induction. However, in some experiment addition PG promoted a 100% root induction. Wounds applied to the basal part of the shoots play a significant role on root induction. Without wounding, percentage of root induction was severely decreased. Percentage of root induction in the absence of wound and IBA treatment was 0% (Table 4).

Table 4 Effect of various treatments to micro-shoots on root induction

treatment	wound	IBA	PG	% root	# root
1	+	+	-	68.24 a	0-2
2	+	+	+	50.23 b	0-3
3	+	-	+	38.36 c	0-2
4	-	+	+	4.44 d	0-1
5	-	-	+	0.00 d	0

Mean sharing letter in common within column differ significantly by DMRT (P=0.05).

DISCUSSION

Generally, cultured young leaves of mangosteen onto WPM or MS supplemented with 5 mg/l BA has been reported to produce 2-50 micro-shoots (3, 13). Addition of TDZ in step I medium provided meristematic nodule callus formation (Table 1). By regular subculture the callus to fresh medium at 4 week-interval, more than one million (3^{17}) of plant will be produced a year from one lamina. Contrary to the results obtained by Goh *et al.* (3) they reported that as many as 45 shoot buds were obtained from a single seedling leaf. Each leaf from an *in vitro* shoot could yield up to 8 shoot buds. They calculated that about 1,500 plantlets could be obtained in the first year from one pair of seedling leaves. They also reported that a wound response in the presence of BA was necessary to trigger shoot bud differentiation. The present results demonstrated that those response was not found to trigger callus formation. TDZ has been reported to be the most active cytokinin-like substance for shoot induction in woody plant tissue culture (4). The plant growth regulator in combination with CPPU (N-(2-chloro-4 pyridyl)-N'-phenylurea (forchlor-fenuron) stimulated callus formation in common bean and faba bean (6). From our results it appears that callus formation in mangosteen leaves is controlled by TDZ and BA. However, TDZ produced an inhibitory effect on shoot bud formation so that it is necessary to transfer the callus to shoot bud formation medium or step II medium (Table 3). This evidence has also been reported on proliferation of guava (7). The use of TDZ in proliferation

of guava induce abnormal shoot growth. Shoots were stunted with yellowish leaves, and this effect increased with increasing TDZ. Some of these shoots grew normally upon transfer to the medium with BA.

Goh *et al.* (3) reported that percentage of root induction was obtained about 80% by transferring shoots of 10-15 mm in rooting medium without any treatment. Different results were obtained in our results. Wounding plays the most important role in induction roots followed by IBA treatment (12). Wounding in combination IBA treatment promoted the best results (Table 4). Even PG was reported to promote a 100% root induction (11), but in this evidence we used the young generation shoots. The same concentration of PG which was used in the former experiments might be higher enough to cause a harmful effect on young tissue. Regardingly, percentage of root induction obtained in the presence of PG in the present studies was significant lower than absence of PG. However, time comsumed for root induction was the same. Root primordia induction and root elongation took place 2 weeks in the dark and 2 weeks in illumination condition, respectively. Total time consumed for root induction was 4 weeks shorter than that reported by Goh *et al.* (3).

REFERENCES

Goh, H. K. L., Rao, A. N. and Loh, C. S. 1988. *In vitro* plantlet formation in mangosteen (*Garcinia mangostana* L.). Annals of Botany 62: 87-93.

Goh, H. K. L., Rao, A. N. and Loh, C. S. 1990. Direct shoot bud formation from leaf explant of seedlings and mature mangosteen (*Garcinia mangostana* L.) trees. Plant Science 68: 113-121.

Goh, C. J., Lakshmanan, P. and Loh, C. S. 1994. High frequency direct shoot bud regeneration from excised leaves of mangosteen (*Garcinia mangostana* L.). Plant Science 101: 173-180.

Huetteman, C. A. and Preece, J. E. 1993. Thidiazuron: a potent cytokinin for woody plant tissue culture. Plant Cell, Tissue and Organ Culture 33: 105-119.

Matsuta, N. and Hirabayashi, T. 1989. Embryogenic cell lines from somatic embryo of grape (*Vitis vinifera* L.). Plant Cell Reports 7: 684-687.

Mohamed, M. F., Read, P. E. and Coyne, D. P. 1992. Dark preconditioning, CPPU and thidiazuron promote shoot organogenesis on seedling node explants of common bean and faba bean. J. Amer. Soc. Hort. Sci. 117: 668-672.

Mohamed-Yasseen, Y., Barringer, S. A., Schnell, R. J. and Splittstoesser, W. E. 1995. *In vitro* shoot proliferation and propagation of guava (*Psidium guajava* L.) from germinated seedlings. Plant Cell Reports 14: 525-528.

Neuman, M. C., Preece, J. E., Gaffney, G. R. and van Sambeek, J. W. 1988. Production of embryoid-like structure from immature cotyledonary tissue of black walnut (*Juglans nigra* L.). HortScience 23: 807.

Singha, S. and Bhatia, S. K. 1988. Shoot proliferation of pear cultures on medium containing thidiazuron and benzylaminopurine. HortScience 23: 803.

Te-chato, S. and Aengyong, W. 1988. Microplant propagation of mangosteen (*Garcinia mangostana* L.). Songklanakarin J. Sci. Technol. 10: 1-7.

Te-chato, S., Lim, M., Chumroenruk, M. and Suranilpong, P. 1994. Effect of phloroglucinol on root induction of mangosteen (*Garcinia mangostana* L.) *in vitro*. Khon Kaen Agric. 22: 127-132.

Te-chato, S., Lim, M. and Muangkaewngam, A. 1992a. Enhanced efficiency root induction of microplant of mangosteen *in vitro*. Plant Biotechnology Newsletter 20: 4-6.

Te-chato, S., Lim, M. and Muangkaewngam, A. 1992b. Enhanced efficiency micropropagation of mangosteen through young leaf culture. Songklanakarin J. Sci. Technol. 14: 1-7.

Te-chato, S., Lim, M. and Muangkaewngam, A. 1992c. Tissue culture of mangosteen: Root induction and establishment of vitro-plants to soil. Songklanakarin J. Sci. Technol. 16: 1-5.

Te-chato, S., Lim, M. and Suranilpong, P. 1995a. Embryogenic callus induction in mangosteen (*Garcinia mangostana* L.). Songklanakarin J. Sci. Technol. 17: 115-120.

Te-chato, S., Lim, M. and Suranilpong, P. 1995b. Type of medium and cytokinin in relation with purple leaf and callus formation in mangosteen. Songklanakarin J. Sci. Technol. 17: 121-127.