Short Communication: Conservation of Indigenous Thai Mango Cultivars by *In Vitro* Cultures

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ABSTRACT

While commercial cultivars of mango are renowned, cultivated area of indigenous cultivars become lower and will affect mango diversity in Thailand. This research aimed to conserve 8 indigenous mango cultivars by *in vitro* culture using nucellus tissue. Media and condition for browning control were tested and found that MS in the dark is the best. Then media was changed to MS + 0.1 mg/l NAA for a month and transferred into MS + 0.1 mg/l BA in the last 2 months. After experiments, plantlets were produced with the average shoot and root height at 3.14 ± 2.30 and 4.53 ± 2.33 cm in order.

Keywords: Indigenous Thai mango cultivars, *in vitro* culture, conservation

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INTRODUCTION

Thailand ranked fourth in the world for its mango production after India, China and Mexico (Tharanathan *et al*., 2006). The Office of Agriculture Economics (2015), Ministry of Agriculture and Cooperatives had recently reported that the costs of export were reached the maximum plateau in 2015 at 3,200 million baht (equivalent to 91 million US Dollars). Chachoengsao is one of Thailand's chosen provinces to cultivate mangoes for marketing and export. There are many varieties of both economic cultivars and indigenous cultivars but currently, various cultivars of indigenous mangoes are rare to find in local areas.

In the past, many kinds of indigenous cultivars were propagated throughout Thailand and were harvested in a large amount especially in summer. But nowadays, only famous cultivars are available. In 2012, Kanphong reported a few reasons that result to a rare condition of indigenous cultivars in local areas. Those are a decrease of market demand and many popular cultivars were imported such as mango from Australia called R2E2, which is readily grown all over Thailand. From above-mentioned, orchardists have not attempted to conserve the indigenous mango cultivars and that might bring a crisis of considerably lower cultivated area. As a matter of fact, many indigenous cultivars have specific benefits for example, Talapnak have individual sweet smell, Thongdam have lots of edible pulp but very lean seeds and trees are leafy and shady, Raet have exotic fruit shape and can be eaten ripe or unripe. This information would favor the preservation of indigenous variety.

Although mango ranked fifth in total world fruit production (FAO, 2010) and readily available to propagate in tropical areas, literature on mango in *in vitro* culture is still less. The

application of plant tissue culture is one of the most popular used strategies for commercial micropropagation. Embryogenesis is the most widely adopted regeneration system for mango (Jayasankar *et al*., 1999; Malabadi *et al*., 2011; Perez-Hernandez and Grajal-Martin, 2011). Samples usually used nucellar tissue from immature fruits (3–7 cm long or 45 days after flowering), they potentially offers a rapid propagation of somatic embryogenesis for mango genotypes (Chaturvedi *et al*., 2004; Ermayanti and Rantua, 2009; Nower, 2013). Tissue culture media for mango nucellus is various, some placed in MS, B5, half MS + half B5, LP and DKW (Patena and Barba, 2011; Li *et al*., 2012; Nower, 2013). While tissues were cultured for a week, browning usually occur around and over both mango tissue and media. Browning is a condition where in the media is getting brown due to the phenolic compounds of plants. It is evident that the growth of such compound suppress tissues. Many researchers reported solution to avoid such condition. Li *et al.* (2012) used 3 different antioxidant treatment solutions and found that 0.1 mg/ml L-cysteine HCl showed the less percent of browning. While Nower (2013) used 100 mg/ml ascorbic acid and some works (Ermayanti and Rantua, 2009; Patena and Barba, 2011) cultured in the dark to decrease browning and it has shown to be effective.

Aforementioned, the selected indigenous mango cultivars are Raet, Okrong, Thongdam, Tappet, Talapnak, Khaituek Boran, Faep and Phimsen. The experimental system described in this paper provide an affective protocol for browning elimination, somatic embryogenesis and plantlet regeneration for these cultivars in Thailand conservation.

MATERIALS AND METHODS

Immature mango fruits, around 30 days after flowering (4–7 cm in length), of eight indigenous mango cultivars were collected from mango orchards in Chachoengsao. These are Raet, Okrong, Thongdam, Tappet, Talapnak, Khaituek Boran, Faep and Phimsen. Mango fruits were washed in liquid detergent and left in running water for 10 mins. These were surface–sterilized using 15% (v/v) clorox for 15 mins and rinsed for three times using sterilized distilled water for 5 mins each. Before dissection, fruitlets were soaked in 95% ethanol, flamed for a few seconds and were bisected longitudinally to produce symmetrical halves (Figure 1). Zygotic embryos and seed coats were discarded, only nucellus tissues were used for culture.

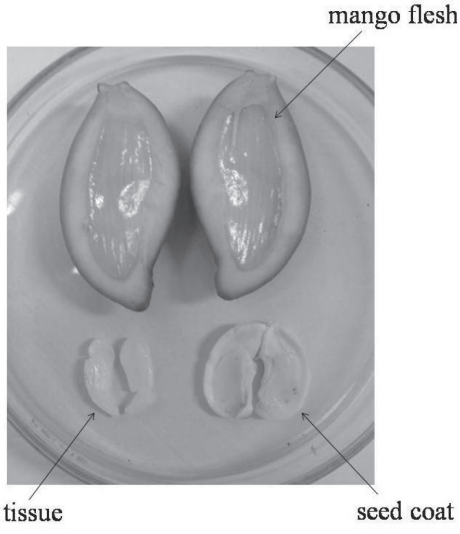

Figure 1 A longitudinally bisected mango fruit

Raet mangoes were selected to be the model for browning-inhibition testing because this cultivar is the most abundant in local areas. Sterilized explants of Raet mangoes were cultured on different 6 conditions (MS-L = MS media with light, MS-D = MS media with dark, AS-L = MS media+100 mg/l ascorbic acid with light, AS-D = MS media+100 mg/l ascorbic acid with dark, CH-L = MS media+1000 mg/l activated charcoal with light and CH-D = MS media+1000 mg/l activated charcoal with dark) in 12 days to observe the results. Conditions that affect the least level of browning were used in the next step.

Table 1 Average level of browning in different media and conditions after 12 days in culture (n = 10)

Days	MS-L	MS-D	AS-L	AS-D	CH-L	CH-D
2	1.67	0.33	0.33	1.00	0.67	1.67
4	1.67	0.67	1.00	1.33	1.00	1.67
6	1.67	1.00	1.33	1.33	1.33	1.67
8	2.00	1.00	1.67	1.33	1.33	1.67
10	2.33	1.00	1.67	1.67	1.33	1.67
12	2.67	1.00	2.00	2.00	1.33	1.67

In the second month, media was changed from MS to MS + 0.1 mg/l NAA and the last 2 months MS + 0.1 mg/l BA were utilized for embryo maturation. All the media were adjusted to pH 5.6–5.8, before adding agar and sterilized by autoclaving at 15 psi 121°C for 15 mins. Cultures were incubated at the temperature of 23 ± 2°C throughout the 4-month experiment.

RESULTS AND DISCUSSION

After first month, the results showed that ascorbic acid and activated charcoal were not effective for browning elimination but MS media gave less browning level. Dark condition also overcome the exudation of phenolic compounds from the cut ends of mango explants than culture in light. This treatment combination had the least browning level. Then MS and dark condition was applied to other 7 cultivars.

Although explants were cultured in MS in the dark condition in the first month, some of them gradually became brownish or blackish. When explants started to turn brown, sub-cultured to fresh medium was beneficial to control browning.

After that, cultures were transferred to be exposed to light and media was added as a growth regulator 0.1 mg/l NAA to induce somatic embryos. Embryos with developed roots and visible plumules of 8 indigenous cultivars occurred in the 2nd month of cultures.

After 4 months with MS media added 0.1 mg/l BA were utilized for embryo maturation, Phimsen gave the least amount of embryogenesis percentage at 3.85 but this cultivar showed obvious rate of shoot and root growth at 8.50 ± 8.63 and 8.25 ± 8.93 cm length average respectively. While percentage of embryogenesis of Okrong is the highest percentage at 77.78 but growth rate of both shoot and root remain less (2.27 ± 1.11 and 3.40 ± 1.92 cm respectively). The cultivar that has lowest size of plantlet was Raet as shown in Table 2. As Scheffe's method statistical analysis, Raet and Phimsen plantlet shoot and root sizes were different in significant at the 0.001 and 0.05 level respectively.

Table 2 Plantlet sizes and percentage of somatic embryogenesis of eight indigenous cultivars after four months in culture (MS in the dark for one month, MS media supplemented with 0.1 mg/l NAA in the light for one month and MS media supplemented with 0.1 mg/l BA for the last 2 months.)

Cultivars	Average shoot length (cm)	Average root length (cm)	Percentage of somatic embryogenesis
Raet	1.05 ± 0.74***	1.17 ± 0.42*	33.33
Okrong	2.27 ± 1.11	3.40 ± 1.92	77.78
Thongdam	2.33 ± 0.80	3.55 ± 2.57	45.45
Tappet	2.62 ± 1.55	7.12 ± 5.71	14.71
Talapnak	3.82 ± 2.52	4.33 ± 1.44	64.52
Khaituek	2.57 ± 1.47	5.60 ± 2.85	38.71
Faep	1.94 ± 0.88	2.87 ± 2.27	60.87
Phimsen	8.50 ± 8.63***	8.25 ± 9.83*	3.85

*** The mean difference is significant at the 0.001 level (Scheffe's method)
* The mean difference is significant at the 0.05 level (Scheffe's method)

It was noticed that plantlets that regenerated from nucellus somatic embryos had abundant secondary roots (Figure 2) and elongated as reported in Patena and Barna (2011)'s work especially amongst Phimsen, Tappet and Khaituek. Embryos germination or embryos with developed roots and visible plumules of these cultivars were observed throughout on MS basal media supplemented with 0.1 mg/l NAA and MS media supplement with 0.1 mg/l BA in 3–4 months or 9–12 weeks. These results were little earlier than a report of Malabadi (2011) that embryos could be developed after 12 weeks and germinated, each with a distinct shoot meristem and radical end. While Patena and Barba (2011) took only 4–8 weeks. It was noticed that plantlets derived from mature seeds of mangoes grown in soil and from nucellus explant derived from somatic embryo are similar in morphology except that the latter has more secondary roots.

In similarity of condition, media and culture period, Okrong and Faep germinated only somatic embryo at cotyledonary stage as Figure 2, while a work of Indonesia reported that commercial cultivar from Thailand, like Namdokmai cultivar, couldn't produce any somatic embryos in 3M medium (Ermayanti and Rantua, 2009).

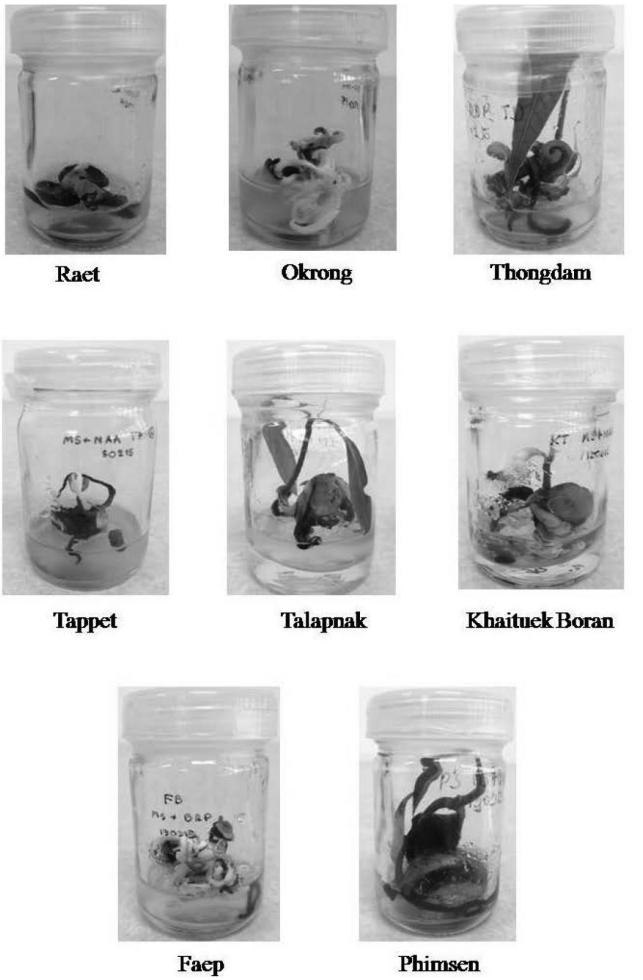

Figure 2 Somatic embryo induction from nucellar explants of eight traditional-cultivar immature mango fruits after four month-culture

In order to reach the embryo's desired size, optimum treatment such as proper concentration of growth regulators, liquid state of medium, and low content of nitrogen are required (Chturvedi *et al*., 2004). For other growth regulators, 2,4-D was tested in 0.0, 0.5, 1.0, 1.5 and 2 mg/l. The highest somatic embryogenesis number was obtained on the medium without 2,4-D (Nower, 2013).

CONCLUSION

A method for somatic embryogenesis from immature nucellus (around 30 days after flowering) of 8 indigenous mango cultivars has been developed. Browning of nucellus explants were effectively controlled by pre-culturing in MS medium in the dark at 23 ± 2°C for a month. After that, growth regulators were added and cultured in light condition. After the 4 month culture, different cultivars showed different results in the same media and condition. Average shoot and root sizes of mango plantlet were 1.05 ± 0.74 to 8.50 ± 8.63 cm and 1.17 ± 0.42 to 8.25 ± 9.83 cm respectively, while plantlet sizes of Raet and Phimsen were different in statistically significant.

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