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Early fruit setting from tissue culture-derived mangosteen tree

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Abstract

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Vitro-plantlets of mangosteen derived from culturing young leaves were acclimatized in 1993. Small and large polybag seedlings were carefully raised under controlled environmental conditions until 1994 when they were ready to be transferred to the field. During this stage, morphological abnormalities of the seedlings were recorded. After transferring to the field for 5-6 years (1994-1999) at Yi Ngo District, Narathiwat Province and Klong Hoi Khong District, Songkhla Province, morphological characters of the plants were again observed in comparison with seed-derived plants. The results showed that tissue culture-derived plants were more bushy and started blooming 5 years after planting while the seed-derived plants still had tall canopy (not bushy) and were not bearing fruit in the same period of time. However, the blooming of cultured plants did not give the fruit setting in the first blooming year. All flowers dropped off completely. Heavy fruit setting was observed in the following year (2000). Tissue culture trees had smaller but healthier leaves whereas seed-derived trees had pale yellowish green leaves. Fruit qualities in terms of total soluble solids (TSS) and total acids (TA) were not much different between the two types of these mangosteen trees.

Key words : fruit setting, tissue culture, mangosteen

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บทคัดย่อ

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การให้ผลผลิตที่เร็วกว่าจากต้นมังคุดที่เพาะเลี้ยงเนื้อเยื่อ

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อนุบาลต้นมังคุดที่ได้จากการเพาะเลี้ยงเนื้อเยื่อเมื่อปี พ.ศ.2536 ดูแลต้นดังกล่าว และต้นที่ปลูกด้วยเมล็ดในถุงพลาสติกสีดำขนาดเล็ก และถุงสีดำขนาดใหญ่โดยควบคุมสภาพแวดล้อมจนถึงปี พ.ศ.2537 จนมีความพร้อมที่ย้ายลงแปลงปลูกได้ ในระยะนี้ได้ศึกษาความผิดปกติทางสัณฐานของต้นไปด้วย หลังย้ายไปปลูกในแปลงปลูกจริงได้ 5-6 ปี (2537-2542) ที่อำเภอยี่งอ จังหวัดนราธิวาส และที่อำเภอคลองหอยโข่ง จังหวัดสงขลา จึงได้ตรวจสอบลักษณะทางสัณฐานของต้นที่ปลูกด้วยเมล็ดเปรียบเทียบกับต้นที่ได้จากการเพาะเลี้ยงเนื้อเยื่อ ผลจากการศึกษาพบว่า ต้นที่ได้จากการเพาะเลี้ยงเนื้อเยื่อมีลักษณะพุ่ม เตี้ยกว่า และเริ่มออกดอกหลังปลูกเป็นเวลา 5 ปี ในขณะที่ต้นที่ปลูกด้วยเมล็ดมีลักษณะสูงชะลูดและยังไม่ออกดอก อย่างไรก็ตามดอกที่ออกในปีแรกจากต้นที่เพาะเลี้ยงเนื้อเยื่อไม่ติดผล ทั้งนี้เพราะร่วงหล่น การติดผลดกมากในปีต่อมา (2543) ต้นที่เพาะเลี้ยงเนื้อเยื่อมีใบขนาดเล็กกว่า แต่สีเขียวเข้มกว่า ในขณะที่ใบจากต้นปลูกด้วยเมล็ดมีสีเขียวอมเหลือง เมื่อพิจารณาคุณภาพของผลในรูปของของแข็งที่ละลายน้ำได้ทั้งหมด (total soluble solids: TSS) และกรด (total acids:TA) พบว่าไม่มีความแตกต่างกันมากระหว่างผลมังคุดจากต้นทั้ง 2 ชนิด

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Mangosteen is an economically important fruit both for domestic consumption and export. Thailand is the world's largest producer, with 62,000 t of fruit harvested in 1981. The area of production is estimated to be 12,000 ha, with most production coming from the eastern and southern provinces. The value of exports in 1991 was 5.6 million US$. Recently, the demand for this fruit has been gradually increasing both domestically and overseas. Production of good quality plants for planting must be considered. Mangosteen has been conventionally propagated by seeds. Even if they are formed from nucellar tissue and identical apomictic seedlings are produced, a long gestation period (vegetative growth) is inevitable. Most mangosteen trees in the southern part of Thailand begin to flower 7 years after planting. Tissue culture plants are an alternative source of plants which might shorten this period and give a good quality of both tree and fruit. Many authors have reported progress in micropropagation through direct shoot formation by culturing seeds (Te-chato and Aengyong, 1988; Normah *et al*., 1995; Goh *et al*., 1988), young leaves (Te-chato *et al*., 1992; Goh *et al*., 1994) and meristematic nodular callus (Te-chato *et al*., 1995a, b and c; Te-chato and Lim, 2000). Among these cultures, meristematic nodular callus has proven to be the most effective way for commercial production of mangosteen plants. Te-chato and Lim (2001) have improved the protocol for propagation of mangosteen through young leaf culture *in vitro*, and propagation of mangosteen through tissue culture technique has been well established. However, the following performance of the plants in the field has not yet been reported. This paper will present data on growing mangosteen in the field.

Materials and Methods

Plant material

In this experiment, the two different plant types, seedlings and vitro-plants obtained from young leaf culture, were prepared and grown in the field following the farmers' practices. Seedlings were generally obtained from sowing the seeds in a seedbed and transferring two-seedling-leaf plants to a medium or large polybag containing a soil-

compost mixture. Vitro-plants were prepared as described by Te-chato and Lim (1999, 2000). The plants were removed from culture bottles, the agar washed off, then acclimatized in a controlled environment for two weeks. Surviving vitro-plants were transferred to a polybag containing the same soil-compost mixture as that of the seedling growth. Both types of plants were raised in a greenhouse under 60-70% shading and watered two times daily for two years until the first branches appeared. The trees were then transferred to two different locations, Yi Ngo District, Narathiwat Province and Klong Hoi Khong District, Songkhla Province.

Morphological characteristics

Morphological characteristics in terms of leaf size, shape, position and architecture of the plants at green house and field level were examined and compared between the two different types of mangosteen trees.

Fruit bearing study

After transferring the two-year old mangosteen trees (from both types, seed- and tissue culture-derived) to the field, shading and watering were provided properly. The trees were spaced at the interval of 6x6 m, on a square planting grid and planted in holes measuring 50×50×50 cm. Rock phosphate, at a rate of 500 g/hole, was mixed in the planting medium. N:P:K 15:15:15 formulation at a rate of 0.5-1.0 kg/tree, together with a regular dressing of organic manures, was applied monthly. The time from planting (in green house) to first flowering was recorded and compared between the two different tree types. Fruits were collected and major characteristics, e.g. rind thickness, number of carpels and seeds and translucent percentage were examined.

Fruit quality study

After examining selected fruit characteristics, a fresh berry was removed and subjected to quality analyses. Total soluble solids (TSS) and total acids (TA) were chosen as parameters for comparison between the two different tree types.

Results and Discussion

Morphological characteristics

At early stages of growth, tissue culture-derived plants grew slower than the seed-derived plants. Plant height of the seedlings at one year after planting was approximately 2 times higher (Figure 1A). After year two the tissue culture-derived plants grew vigorously and rapidly and kept pace with the seed-derived plants. At this stage of planting the most significant difference between the seed and tissue culture-derived plants was branching, and the architecture of the plants from the two different sources was quite different. More lower stem branching was observed in the tissue culture derived plants. However, systematically arranged branches, forming a regular pyramidal-shaped crown, still appeared in the structure of seed-derived plants. This structure could be distinctly seen at 5 years after planting in the field (Figure 1B). From this point of view, the canopy diameter of tissue culture-derived trees was larger than seed-derived ones. In comparing leaf area, seed-derived plants were twice as large as tissue culture trees (Figure 2). However, tissue culture trees showed healthier leaves than seed-derived ones. This evidence was observed by the color of the leaves. Leaves from tissue culture trees had dark green color while seed-derived ones had light green color.

Fruit bearing study

Tissue culture trees started blooming in year 5 (1999) while seed trees still had only vegetative growth. The flowers produced at this period of time did not further develop into fruit, but shriveled and dropped off to the ground. In the following year (2000), tissue culture trees bloomed again and this time fruit set was seen. The fruits could be harvested and studied for fruit morphology and quality. As the seed trees at the same age had not bloomed, the fruits from other seed trees was collected and compared with the tissue culture ones. Basic data on fruit diameter or size, rind thickness and translucent percentage are shown in Table 1.

A

B

Figure 1. Morphological characteristics of one-year (A) and 6-year (B) mangosteen plant derived from seed (left) and tissue culture (right).

Table 1. Comparison of some fruit characteristics between two different types of mangosteen trees.

Source of tree	Fruit diameter (cm)	Rind thickness (cm)	Translucence (%)
Seed tree	5.21±0.05	0.78±0.005	0
TC#1	5.36±0.07	0.77±0.006	0
TC#2	5.25±0.08	0.81±0.017	0

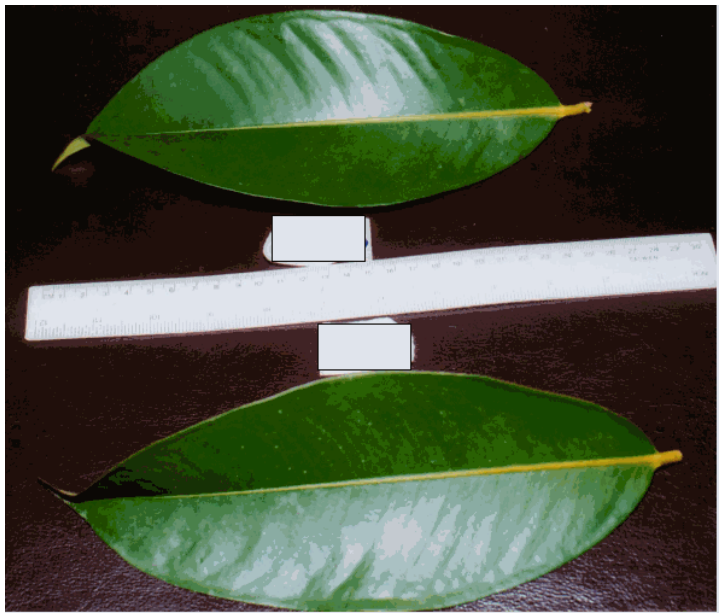

Figure 2. Leaf morphology of tissue culture (upper) and seed (lower) derived plant.

Table 2. Number of carpels of mangosteen fruit between two different types of mangosteen tree.

Source of tree	% of fruit with 4-8 carpels					Average no. of seed/fruit
	4	5	6	7	8	
Seed tree	0	16.7	50	33.3	0	0.3
TC#1	0	36.4	63.6	0	0	0.5
TC#2	0	50	0	50	0	0.5

Table 3. Comparison of some fruit qualities between two different types of mangosteen trees.

Source of tree	TSS	TA
Seed tree	16.35	0.768
TC#1	17.05	0.797
TC#2	17.15	0.755

TSS = Total soluble solids
TA = Total acids

$$\%TA = \frac{\text{N base} \times \text{molecular mass of citric acid} \times \text{tritrated volume} \times 100}{\text{volume of distilled water (ml)}}$$

N base = 0.1 N
Molecular mass of citric acid = 0.06404

The resulting data were not different in any parameters observed. The number of carpels per fruit was also not clearly different between the two sources of trees (Table 2). The number of carpels was from 5 to 7, while 4- and 8-carpel fruit was not found. According to the results obtained in this investigation, tissue culture trees tended to produce more seeds per fruit than that of the seed trees (Table 2).

Fruit qualities in terms of TSS and TA were not distinctively different between the two types of trees. However, tissue culture trees tended to provide higher TSS and TA than that of the seed trees (Table 3).

There are several limitations to propagation of mangosteen by seed, e.g. delicate root system of young seedlings, extremely slow growth of the plants and long period required for fruit bearing. In general, mangosteen seedlings require 8-10 years to begin producing fruit. However, under very favorable conditions, the time may be reduced to 6-7 years. In the case of vegetatively propagated trees, time required until bearing fruits is recorded as 5-6 years, but this time does not include a maintenance period of about 2 years in the green house. So the total of 7-8 years must be accounted from planting to flowering. Tissue culture-derived trees in this present study demonstrated the shortest pre-fruiting period (5 years). Moreover, the bushy plant structure (branching at early stage of planting), made it easier for harvesting and other maintenance.

Vegetatively propagated mangosteen has been carried out using top grafting on mangosteen rootstock and grown in some orchards, but it is not popular due to incompatible growth. The tree structure is not balanced and it readily collapses in the strong wind. Plants propagated from suitably related rootstocks, which have a more vigorous root system, are generally sturdier and grow more rapidly (Popenoe,1974; Halijah, 1988; Alexander *et al*., 1982; Gonzalez and Anoos, 1951; Richards, 1990) but incompatibility between rootstock and mangosteen scions must be faced. Success in early stages in a green house by many vegetative methods has been reported, but there are no reports of further development of these trees in the field. Tissue culture plants do not require rootstocks and thus there was no problem as described above. As the plants were cultured in the medium containing growth regulators, especially cytokinin, for a long time the juvenile phase of the plant may be reduced. This allows the tree to flower in a shorter time in comparison with seedlings and conventionally vegetative-propagated trees. Furthermore, this growth regulator also plays a significant role in branching of the plants, leading to a bushy type of mangosteen tree, and branching seems to affect the leaf area. A large number of leaves were flushed from tissue culture trees, which might have caused the leaves produced from tissue culture trees to be far smaller (approximately 2-fold) than those produced from seed trees (Figure 2). Te-chato and Lim (unpublished data) noted the effect of two cytokinins, BA and 2i-P, on branching of related species. To date, no attempts have been made on using these growth regulators in mangosteens at the seedling stage. In the case of fruit quality, there was no significant difference between seed and tissue culture trees. However, the latter tended to have higher total soluble solids (TSS). This proves that the tissue culture technique can produce true-to-type mangosteen plants as in the results of DNA analysis reported by Te-chato (2001). From the results obtained in this study, the authors believe that planting mangosteens by using tissue culture trees is the most feasible method for producing high quality fruits for export in the future.

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