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The Industry and Progress Review on the Cultivation and Physiology of Wax Apple – with Special Reference to 'Pink' Variety

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ABSTRACT

The wax apple (*Syzygium samaragense*) is a tropical fruit tree and is very common in Southeast Asia, Taiwan, India and also grows well in Central and South America. The 'Pink' cultivar represents 95% of the planted area in Taiwan (as a result, almost all the research data presented in this paper are based on 'Pink' variety). Despite its name, this cultivar produces fruits varying from pink to deep red, depending on environmental and cultural conditions. Best prices are obtained with big, crispy, thick-fleshed, juicy, sweet and deep red fruits. Fruit quality is affected and improved by many factors, such as light, temperature, position on the tree, growing stage, leaf:fruit ratio, supplemental calcium and manganese applications. Due to successful off-season production techniques, fruits can be harvested all-year-round in Taiwan. Trunk girdling, canopy shading, root pruning and/or flooding create favorable conditions for flower forcing. The highest net carbon dioxide exchange rate for wax apples is about 9 μmol $m^{-2} s^{-1}$. Flooding decreases net carbon dioxide exchange rates, stomatal conductance, evaporation and maximum quantum yield of PSII (Fv/Fm), concentrations of chlorophyll and leaf water potential, however, it helps the reproductive phase of wax apples. A single sigmoid growth pattern was found in wax apple fruit development. The concentration of anthocyanin increases during fruit development. The major sugar species in the fruit are fructose and glucose. External sugars enhance red color development on the cultured skin discs. Paper bagging, a standard cultivation procedure, increases fruit hardiness, crispiness and color development.

Keywords: flowering, fruit quality, fruiting, production
Abbreviations: FAA, free amino acids; **PSII**, photosystem II; **SP**, soluble protein, **TPC**, total phenolic compounds; **SSC**, soluble solid concentration

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INTRODUCTION

Wax apple (S*yzygium samarangense*, other names are wax jambu and Java apple) is native to Malaysia to the Andaman and the Nicobar Islands where the trees grow in coastal rainforests (Morton 1987). This tropical tree can grow to a height of 5-15 m depending on environmental conditions. Flowers (**Fig. 1**) appear in March in south Taiwan and fruits ripen in May under natural condition. Fruits vary greatly in size, shape and skin color. The fruit size can be as small as

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Invited Mini-Review

Fig. 1 Flowers and fruits of wax apple.

about 4.3 cm long and 4.7 cm wide to more than 5.2 cm long and 5 cm wide (bell-shaped) or 7 cm long and 4 cm wide (elongated). Fruit mass ranges from 28 g to 100 g to the jumbo sized of more than 200 g per fruit. Fruit shape ranges from round to bell-shaped, oval or elongated and skin color diverges from white to pale green to dark green, pink to red to deep red. The fruit of wax apple are sweet and for eaten, fresh or cooked, and, for this purpose, is better than Malay apple and other species in the same genus. A small percentage of the fruit is used for sauces, jams and jellies. Wax apple trees are tropical and cannot tolerate temperatures below 7°C, preferring temperatures above 18°C (Kuo 1995; Huang *et al*. 2005). Fruits of wax apples prefer warm temperatures for normal growth and development. Low temperatures impede fruit growth and red color development, while high temperatures accelerate fruit growth and ripening yet inhibit red color development.

The wax apple is a heavy producer on well-fertilized good soils and can produce more than 200 clusters per tree, with 4-5 fruits in each cluster when mature. Average fruit weight of 'Pink' variety is about 100 g, however, a single fruit of 'Big Fruit', mutants of 'Pink', weights from150 g to more than 200 g (Chiu 2003).

STATUS OF PRODUCTION IN SOUTHEAST ASIA AND TAIWAN

The production area and production for the year 2003 in Indonesia were 13,454 hectares and 239,108 tons, respectively (Reza Tirtawinata, pers. comm.). Located at the center of origin, Indonesia has a huge amount of variety with great diversity. Wax apple is cultivated mainly as smallholdings ranging from 1 to 5 ha in Malaysia with its hectareage estimated 1,500 ha in 2005 (Zainudin Meon, pers. comm.). 'Pale Green', 'Dark Red', 'Light Red' and 'Green' are the four major Indonesian varieties. Fruit production is non seasonal, however, the peak periods are in March to April and November to December. The major wax apple cultivars in Thailand are 'Phet Ban Plew', 'Phet Sai Rung', 'Thun Klao', 'Phet Jin Da', 'Number One', 'Phet Sam Phran', 'Dang Indo', 'Phet Nam Pueng', 'Thub Thim Chan'. Fruits can be harvested almost year-round. There were 10,240 ha planted area and 70,310 metric tons fruits produced with a production value of $26.5 million US dollars in Thailand in 2004 (Chinnapan Thanarut, pers. comm.).

The area, total production and production value for the wax apple industry in Taiwan were 7031 hectares, 69,234 metric tons and $189 million US dollars in 2006. 'Pink' has been the leading cultivar, representing 95% of the planted areas in Taiwan (Wang 1991), although the percentage decreased to about 85% in 2007 (Wang, pers. comm.).

WAX APPLE PRODUCTION SYSTEMS IN TAIWAN

There are 5 production systems, 1 regular and 4 forcing culture, for wax apple in Taiwan (**Fig. 2**). The 5 production systems were developed in chronological order as follows:

1. *Regular* - the original production system that most resembles to natural growth conditions. Fruits are harvested from May to August. This system is not used in the commercial production any more.

2. *Simple flower forcing* - an evergreen production system developed in the late 1970s. Similar to Regular, but flower forcing is carried out by pruning and/or chemicals in September. Fruits are harvested from January to May.

3. *Improved flower forcing* - an evergreen production system developed in early 1980s. With the aid of cultural and/or chemical treatments to retard vegetative growth, Cultural practices for retarding vegetative growth, such as trunk girdling and/or root pruning, are performed in early June and August, respectively. Then the trees are treated with flowering reagents in late August. Fruits can be harvested from December to March.

4. *Bald-cut* - Since fruits harvested from infertile soils after March have poor quality, farmers remove all the flowers, fruits, leaves and twigs in February. Cultural practices for retarding vegetative growth, such as trunk girdling and/ or root pruning, are done in early June and mid-August, respectively and flower triggering in late August. Fruits can be harvested from December to February (**Fig. 3**).

5. *Bald cut plus shading* - Bald cut in combination with shading (**Fig. 4**) for 40 days improves cluster as well as flower numbers.

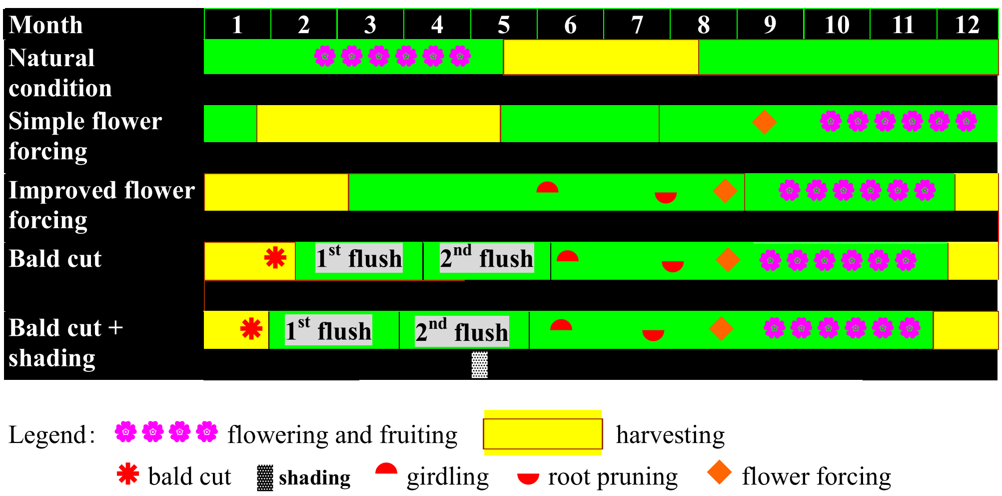

Fig. 2 Wax apple production systems in Taiwan.

Fig. 3 Bald-cut is a popular management system for wax apples in Taiwan.

Fig. 4 Shading – a method used for enhancing inflorescence formation.

PROGRESS REVIEW ON THE CULTIVATION AND PHYSIOLOGY OF WAX APPLE

Whole tree growth and development and possible techniques for manipulation

Flowers appear in March in south Taiwan and fruits ripen in May under natural conditions (Young 1951). However, 'Pink' blooms and sets fruit almost year-round after flower forcing (Wang 1991; Shü *et al*. 1996). As a result, fruits at different growing stages could be found in different orchards, different trees and even on the same tree. Despite its name, 'Pink' produces fruits with their color varying from pink to deep red, depending on environmental and cultural conditions. Best prices are obtained with deep red fruits. Fruit color is influenced by many factors, such as light, temperature, position on the tree, growing stage, and leaf: fruit ratio (Wang 1991; Shü *et al*. 1996, 1998; Shü 1999a, 1999b; Shü *et al*. 2001; Chang *et al*. 2003; Pan and Shü 2007). The details shall be depicted on the following pages.

Wax apple is a relatively fast-growing fruit species having 6 to 7 new flushes and 1 to 3 crops in a year (Young 1951; Wang 1991). As a result, pruning is a very important culture practice for canopy management for wax apples. The pruning of wax apple trees can be classified into heavy, medium and light. Heavy pruning is a standard practice, removing all or most of the twig, leaves, flowers or small fruits, used only in the bald-cut culture system in January through February. The light and medium pruning practices are used in the evergreen systems, including simple flower forcing and improved flower forcing, and the bald-cut culture systems for canopy management. As pruning affects bud breaking, shoot growth and development, canopy size and shape, light perception, it has been used as a standard canopy management technique in wax apple production (Wang 1991). Inflorescence formation percentage is not influenced by pruning. From intact to severe pruning, moderate pruning is proved to be the best for wax apple production (Hsiao 1996). Potted wax apple plants grown in a phytotron under 3 day/night temperature regimes, 35/25°C, 30/20°C and 25/15°C were investigated for their flowering physiology. High temperatures increase the SSC in the shoot and the percentage of inflorescence formation. Plants grown under 35/25°C had the highest (2%) SSC and lowest starch (3%), while those grown under 25/15°C had the lowest (0.5%) SSC and highest starch (32%) in the scaffold limbs. The trends are generally the same in either the leaves or branches, however, with some variation. Wax apple plants grown under 35/25°C and 30/20°C had higher inflorescence percentage (more than 20%) than those under 25/15°C (5.2%, Hsiao 1996). The highest net CO_2 exchange rate of 9 μmol m^{-2} s^{-1} was detected under light intensity at or over 700 μmol m^{-2} s^{-1}, leaf temperatures at 28-30°C, vapor pressure difference at 1 KPa (Chiou 1998). Flooding and root growth restriction are effective means for vegetative growth suppression which in turns promotes flower forcing and enhances fruit quality. However, flooding decreases net carbon dioxide exchange rates, stomata conductance, evaporation and maximum quantum yield of PSII (Fv/Fm), concentrations of chlorophyll and leaf water potential. The decreased physiological parameters recover after the emergence of adventitious roots, which replace the functions of the damaged root systems, on the trunk (Huang 2003).

Flower forcing

Girdling (**Fig. 5**), root pruning (**Fig. 6**), flooding (**Fig. 7**), and trunk hitting are the common methods for flower for-

Fig. 5 Girdling – a very popular method for new growth retardation.

Fig. 6 Root pruning – another method for new growth retardation.

Fig. 7 Flooding – for new growth retardation, but diseases may spread.

Table 1 Influence of shading on cluster and flower numbers of wax apple trees.

Treatment	No. cluster on shoot apical per tree	No. cauliflorous cluster per tree	No. flower per cluster	Total no. flower per tree
95 % shading	813.7 a*	403.7 a	15.3 a	18,626 a
60 % shading	779.0 a	397.3 a	16.2 a	19,056 a
Control	30.3 b	7.0 b	14.7 a	578 b

* = Mean separation within column by Duncan's multiple range test at 5% level.

cing in wax apple production in Taiwan in the 1980s and 1990s. Leaf net CO_2 exchange rate (9~10 μmol $m^{-2}s^{-1}$ for the control and 3 μmol $m^{-2}s^{-1}$ for the O_2-deficient), leaf water potential (-0.4~-0.5 MPa for the control and -0.6~-0.7 MPa for the O_2-deficient) and new shoot emergence (6.2 shoots for the control and 1.8 for the O_2-deficient) all decreased together with severe root damage and leaf yellowing and falling following the decrease of dissolved oxygen concentration, from 6.5~7.0 ppm to less than 4.5 ppm around root area for 58 days (Hwang 2003). Nevertheless, in contrast to the death rate of 38% for guava trees, no wax apple trees died during the flooding period. Flooding did not increase inflorescence formation rate for wax apples (Hwang 2003). Restricting root growth by wood boxes, from 1700 L, 730 L, 200 L, 90 L to 40 L, effectively decreases canopy width (from 2.75 m, 2.4 m, 2.2 m, 2.1 m to 1.75 m), total leaf number (from 4530, 2940, 1710, 1270 to 900) and leaf area (from 24.3 m^2, 17.1 m^2, 10.7 m^2, 6.9 m^2 to 4.8 m^2), total dry weights of shoots (from 6.0 kg, 4.5 kg, 3.1 kg, 1.9 kg to 1.2 kg) and roots (from 1.3 kg, 1.2 kg, 0.8 kg, 0.4 kg to 0.3 kg). However, root growth restriction did not decrease shoot/root ratio averaged at 4.1 (Hsu 1994, 1996).

Shading, partial or whole canopy (**Fig. 4**), using black-colored plastic nets is also effective in the flower forcing of wax apples. This non-destructive technique was developed in the late 1990s. After shading for 40 days using 60% and 95% shading, vegetative growth was successfully retarded and the number of panicles (37, 1176 and 1222 panicles for the control, 60% and 95% shading, respectively) and flowers (578, 19056 and 18628 flowers for the control, 60% and 95% shading, respectively) increased. Net CO_2 exchange rates, and carbohydrate concentrations in the leaf decreased, while nitrogen concentration increased during the shading period (Lai 1996). **Table 1** shows that both shading treatments had about 30 times more clusters than the control trees (Lai 1996).

Fruit growth and development

The length of wax apple fruits increased from 1.5 cm to 5 cm, width from 1.2 cm to 6 cm, weight from 2 g to 150 g during the 80 days growing period from anthesis. Either the length, width or weight of the fruit were found to be in a single sigmoid growth pattern while firmness decreases when fruits mature (Shü *et al*. 1998). Reverse phase-HPLC analysis revealed there are 4 anthocyanin species, mainly cyaniding and peonidin, in the skin of wax apple fruits (Xue 2005). With the decrease of chlorophyll and increase of anthocyanin during fruit development, fruit color of wax apple fruits changes from green to yellow green to pink to red and finally deep red. Sugar concentrations in the fruit increase as fruit gets mature with fructose and glucose being the main components. Sucrose has only 3/5 the amount of fructose. Starch reaches its peak at 11% and decreases thereafter (Shü *et al*. 1998).

Fruit quality improvement

Position on the tree influences quality of wax apple fruits

The fruit on the trunk-lower had the heaviest fruit weight and largest fruit volume among the 9 positions. Upper inner fruits had the smallest weight and volume but reddest color. For high soluble solids concentrations, it is desirable to harvest wax apples from the lower, inner position. Within-tree management practice is suggested to reduce the variation of fruit quality in wax apple (Shü 1999).

An in vitro culture system used to study the environmental effects on wax apple fruit quality

To avoid the interference of environmental factors, an *in vitro* culture system was adopted using isolated fruit skin discs, harvested from mature stage fruit of about 60 days after anthesis, cultured in a controlled environment. Ten discs each were placed on a paper bridge in 250 ml culture vessels, containing a 10-mL 6% sugar solution. The vessels were placed in a growth chamber at a continuous 20°C in the dark for 36 h and were then transferred to culture vessels containing different sugar concentrations, under different light intensities and/or temperatures (Liaw *et al*. 1999; Shü *et al*. 2001; Chang *et al*. 2003; Pan and Shü 2007). No plant growth regulators or any other additives were added to the system. To measure anthocyanin, five skin discs 10 mm in diameter were put into 5 ml 1% HCl in methanol and incubated in the dark at 4°C. Optical density at 530 nm was measured 36 h later (Shü 1999b).

Temperature affects the characteristics of wax apple fruits

Temperature was found to have pronounced effects on quality attributes of wax apple fruit discs. Anthocyanin and SSC were greatest in the 20°C treated discs under constant temperatures. In the slow-increase and fast increase treatments, quality attributes in discs were better in treatments with a final temperature of 25°C than of 30°C. The concentration of FAA, SP, SSC, starch and TPC all decreased with increasing temperature. Transient shifting to high temperature of 30°C for 1-day had no effect on pigmentation but treatment periods from 3- to 5-days had a substantial adverse effect. At 30°C for 5-days, exposed discs had the lightest weight and shortest diameter as well. Both SSC and TPC decreased in the 3- and 5-day treatments. When temperature was shifted from 20 to 30°C for 2 to 11 days, the widest and heaviest discs were found in the 5-day treatment. Anthocyanin and SSC concentration decreased following increased length of exposure to high temperature. Pigmentation of discs exposed to high temperature treatment was worse than in uncultured controls. Both FAA and protein concentrations decreased after culture (Pan and Shü 2007).

Sugars improve characteristics of wax apple fruits

Anthocyanin contents in the cultured fruit skin discs, an *in* *vitro* culture experiment system as mentioned above, increase with the addition of external sugars, i.e. fructose, glucose, maltose, and especially sucrose at the concentrations of either 3%, 6%, 9%, or 12%. The enhancement was very well correlated to sugar concentrations at $p < 0.001$ under either linear or quadratic equations (Liaw 1993; Liaw *et al*. 1999). Phenylalanine, a precursor of anthocyanin at either 25 µM, 50 µM, or 100 µM, also increased anthocyanin biosynthesis. In contrast, the addition of nitrogen, either potassium nitrate or ammonium sulfate at 5 mM, 10 mM or 20 mM, decreased the contents of anthocyanin (Liaw 1993; Liaw *et al*. 1999).

Combined effects of light, temperature and sucrose

Skin disks were cultured in a factorial arrangement of two light levels [dark or light (300 $\mu mol \cdot m^{-2} \cdot s^{-1}$)] as subplots and three sucrose concentrations (0%, 3%, or 6%) as sub-sub-plots within three temperature levels (20, 25, or 30°C) as whole plot treatments. Weight, diameter, SSC, and anthocyanin content were measured 2 weeks after incubation. Light increased SSC and anthocyanin, but reduced the increase in weight and diameter. Increasing the temperature limited increase in diameter and anthocyanin content. Weight, SSC, and anthocyanin contents increased in a linear fashion with concentration of sucrose in the culture solution. However, none of the three factors played a unique role in anthocyanin synthesis in wax apple. Among the 18 combinations, light/20°C/6% sucrose gave the highest SSC and anthocyanin content, while dark/20°C/6% sucrose produced the largest diameter (Shü *et al*. 2001).

Developmental stages affect characteristics of wax apple fruits

Using the above-mentioned *in vitro* culture system, fruit discs from the rapid growth stage to the red stage, i.e. from 4 to 8 weeks after anthesis, had greater anthocyanin induction potential than the other stages when cultured with 6% sucrose (Chang *et al*. 2003). The diameter, weight, chlorophyll, SP and TPC decreased in the sucrose-added fruit skin discs. In contrast, the concentration of anthocyanin and SSC increased at the same time. The biosynthesis of anthocyanin requires carbohydrates and proteins (or amino acids) as energy source, building blocks and the enzymes that catalyze the process (Saure 1990). The sucrose concentration in the culture solution decreased from 6 to about 5% during the 15.5-day cultural period (Shü 1999b). Although sucrose concentrations at the end of the cultures in this study were not determined, there should still be adequate sugar in the cultural solution for anthocyanin biosynthesis. However, the required amino acids, protein or phenolic compounds for anthocyanin biosynthesis must have come from the skin disc itself. This may partially explain the decrease of the weight, diameter, chlorophyll, protein and free amino acids content.

Plant growth regulators affect fruit size and quality of wax apple fruits

The highest weight and volume of the fruit treated with CPPU (*N*-(2-chloro-4-pyridyl)-*N′*-phenylurea) was about 1.35 times that of the control. However, their L/D ratio decreased, i.e., fruit were wider than the control. Flesh was 0.5 cm or even 1.0 cm thicker in the CPPU-treated fruits than BA (6-benzyladenine)-treated or control fruits (Shü and Yeh 1998).

Manganese improves quality of wax apple fruits

The whole wax apple trees were sprayed with manganese sulfate solutions, at 0%, 0.5%, 1.0%, 1.5% and 2.0%, 4 times at an interval of 14 days 3 weeks after petal fall. Anthocyanin concentration at 1.42 $\mu mole/cm^2$ and 1.3 $\mu mole/cm^2$ were the highest for the 0.15% and 2.0% treatments than the other concentrations. However, there were no difference in fruit weight and soluble solids concentrations (Lee 2003).

Fig. 8 Bagging – although laborious and costly but important for protecting from fruit fly injuries.

Bagging is essential for wax apple production in Taiwan

Bagging is used to prevent attacks from fruit flies and pesticides contamination (**Fig. 8**). In comparison to paper bags, plastic bags increase fruit size (+27%) and cracking (+60%), however, decrease SSC (-11%), sugar/acid ratio (-17%), crispiness (-12%) and firmness (-23%) (Wu 2002).

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