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博士學位論文
Ph.D. Dissertation

台農 2 號與紅妃番木瓜(*Carica papaya* L.)無性繁殖之研究

Studies on asexual propagation techniques of papaya (*Carica papaya* L.) cv. 'Tainung No.2' and 'Red Lady'

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INTRODUCTION

The cultivated papaya (*Carica papaya* L.) is the most economic important species in the family *Caricaceae*. Papaya grows year-round, is an elongated berry of various sizes with a smooth thin skin and a greenish-yellow color (Paull 2011). Its flesh is thick with a color ranging from yellow to red and offers a pleasant, sweet, mellow flavor (Devitt 2006, Fuggate 2010). Papaya is considered to be one of the most important sources of vitamins (A, B, C) (Bose 1990, Watson 1990), minerals, several proteins, and the industrially important enzyme papain (Barret 1985, Bose 1990, El Moussaoui 2001). Papaya fruit is low in sodium, fat, and calories, and contain no starch (Sampson 1986). Papain, whose proteolytic action is similar to that of pepsin and trypsin, is employed as a meat tenderizer in applications in the food industry, as well as in the textile, pharmaceutical, and cosmetic industries (Villegas 1997, Su 2009).

Papaya is one of the most economically important fruit crops in many tropical and subtropical countries. In 2016, total areas for fruit cultivation were 441,964 ha in the world which produced 13,050,749 tonnes (t) of fruit (Faostat 2017). In Taiwan, papaya is one of the top ten fruits of production (Fig.5). In 2015, papaya area harvested and production were 2,500 ha and 115,115 tonnes (Faostat 2017). Recently, the papaya production is affected by destructive diseases, specially, papaya ringspot (PRS). PRS is one the most destructive diseases of papaya and occurs in every region where papaya is grown. It has been reported to be a major limiting factor for commercial papaya production particularly in Hawaii, areas of Thailand, Taiwan, India, Mexico, Bangladesh, the Philippines, and the southern region of China (Chang 2003, Jayavalli 2011).

Papaya is a polygamous species with many forms of inflorescences. The species has three sex types: Staminate, pistillate and hermaphrodite (Dinesh 2001, Paull 2011). Papaya is one of the few fruit crops still mostly

propagated by seed for commercial production. Papaya seedlings propagated from seed is hindered by problems because of the sex reversal, inherent heterozygosity and dioecious nature of the crop (Teixeira 2007, Clarindo 2008). In the commercial plantations of most producing countries, male plants are useless and only hermaphrodite individuals are agreed by growers (Usman 2002, Hsu 2012). However, it was found that undesirable male plants prevail as high as 30% and sometimes over 50 % of trees planted in papaya fields (Jordan 1983). So, in actual commercial production, three or four seedlings are planted at each position, and when their sexes are determined, only hermaphrodites are kept. There are cases in which none of them are hermaphrodites. In addition, the plants grown from seeds show considerable variations in disease susceptibility, fruit quality, and yield (Reuveni 1990, Allan 1995, Teixeira 2007).

The main advantage of vegetative propagation is the certainty of keeping the characteristics of the mother plant (Hartmann 2002, Hartmann 2011). It had been reported the possibility of developing materials highly productive and resistant to diseases, which can be spread safely keeping intact the characteristics of the papaya mother plants through asexual propagation (San Jose 1988). Additionally, one can reduce transmissible diseases by choosing mother plants carefully. The case of gynodioecious cultivars, the bisexual types which produce fruits with shape, size, and flavor are preferred to round fruits of female plants as they fetch premium price in the market (Reuveni 1990, Teixeira 2009). Up to now, asexual propagation techniques, such as rooting of cuttings, grafting and micropropagation have been successful in papaya cultivars (Airi 1986 , Ramkhelawan 1998 , Teixeira 2007, Chong 2008 , Wu 2012, Setargie 2015).

The success of assexual propagation by cutting, grafting, tissue culture depends on numerous factors, among them the zone environment, the material and technique application, and the genotype (Hartmann 2002, Soundy 2008, Hartmann 2011, Mabizela 2017). In addition, the results can not apply to all

varieties and in all climatic conditions. So, scientists need conduct much more research on each variety under certain conditions.

In Taiwan, the papaya is mainly propagated by seed and by a method designed to reduce damage from viruses of insects. To date, there is hardly any information on cuttings, grafting and tissue culture propagation of the hybrid papaya cultivars ‘Tainung No.2’ and ‘Red Lady’. The ‘Tainung No.2’ papaya is the major cultivar with 90% of growing area and ‘Red Lady’ papaya is potentially one for spreading with fruit weigh of 1.5-2 kg, good fruit quality (flesh is thick, red, with 13% sugar content, and aromatic) and preferred by the local market (Agriculture and Food Agency, Council of Agriculture, Executive Yuan, R.O.C). So, on purpose of cloning good quality papaya varieties, we conducted researches on propagation of two papaya varieties (‘Tainung No.2’ and ‘Red Lady’) by grafting, cutting and tissue culture. The aims of this study were to investigate the effects of grafting, cutting and micropropagation techniques on commercial asexual propagation in 'Tainung No.2' and 'Red Lady' papaya. The specific objectives were:

- Research on grafting propagation of ‘Tainung No.2’ and ‘Red Lady’ papaya.
- Research on cutting propagation of ‘Tainung No.2’ and ‘Red Lady’ papaya.
- Research on tissue culture propagation of ‘Tainung No.2’ and ‘Red Lady’ papaya.

LITERATURE REVIEW

1. General of papaya plant

Papaya (*Carica papaya* L.) is a popular fruit native to tropical America. Papaya plant is grown for its melon-like fruit. It is a herbaceous perennial plant, bearing fruit continuously at the leaf axils spirally arranged along the single erect trunk. The papayas have common names, such as papaya, papaw or pawpaw, papayer (friench), melonenbaum (German), lechosa (Spanish), mamao (Portuguese), mugua (Chinese), and dudu (Vietnamese) (Paull 2011).

1.1. Taxonomy

Carica Papaya L., is the most important economic fruit, belongs to the *Carica* Genus, *Caricaceae* family. *Caricaceae* is a small family of dicotyledonous plant with five genera of tropical American origin (*Carica, Jarilla, Jacaratia*, *Horovitzia* and *Vasconcella)* and one from equatorial Africa *(Cylicomorpha)* (Paull 2011). There are 32 species described with distribution: *Carica,* 1 species, *Jarilla, 3* species, *Jacaratia, 5* species, *Horovitzia, 1* species, *Vasconcella, 20* species, and *Cylicomorpha, 2* species.

Carica and *Vasconcella* species are dioecious, except for the monoecious *Vasconcella monoica* (Desf.) and some *Vasconcella pubescens* and the polygamous *C. papaya*. Most species are herbaceous, single-stemmed and erect (Paull 2011).

1.2. Origin, distribution and production

Upto now, scientist has not found *Carica papaya* wild in nature. It is evidenced in distantly relation to the *Vasconcella* species by isozyme and AFLP analysis (Paull 2011). It is believed that Carica papaya is native to tropical America, Its origin region is southern Mexico and neighbouring Central America (Morton 1987). In the 16th century, spanish took papaya to the Caribbean and South East Asia. In the accounts of 18th century, seeds of

papaya had been taken from the Caribbean to Malacca and on to India (Paull 2011). Subsequent historical records indicate that from Malacca or Philippines the papaya distribution continued throughout Asia and to the South Pacific region. The factors such as a large number of the seeds in the fruit and their long viability have contributed to the wide geographical distribution of the fruit (Paull 2011).

In the past decade, papaya has attained great popularity because it can be intensively cultivated, its rapid returns and the increased demand for the fresh fruit as well as its processed products. Papaya is commercially cultivated between 23º North and 32º South latitude (Paull 2011), an area which includes many tropical and sub-tropical countries of the world. From 2006 to 2016, Papaya area harvested and production had been developing quickly in the world (Figure (Fig.) 1). The highest papaya production was obtained in Asia (49%) followed by Americas (37.7%) and by Africa (13.2%). The lowest production (0.2%) is recorded Oceania (Fig.2). Top ten countries of papaya production (2006-2016) are India, Brazil, Indonesia, Negeria, Mexico, Dominican Republic, Democratic Republic of the Congo, Kenya, Thailand, Colombia, Philippines (Fig.3) (Faostat 2017).

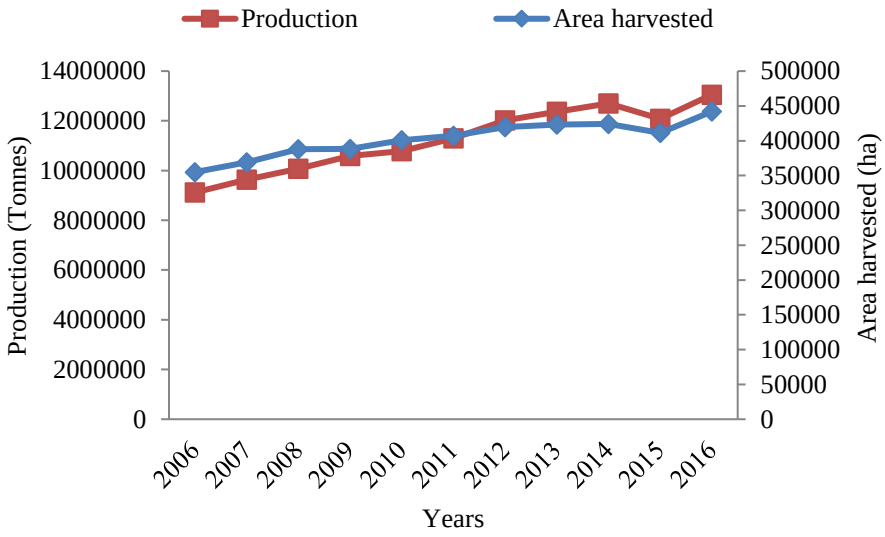

Figure 1. Production of papaya in the world 2006-2016 (Source: Faostat, 2017)

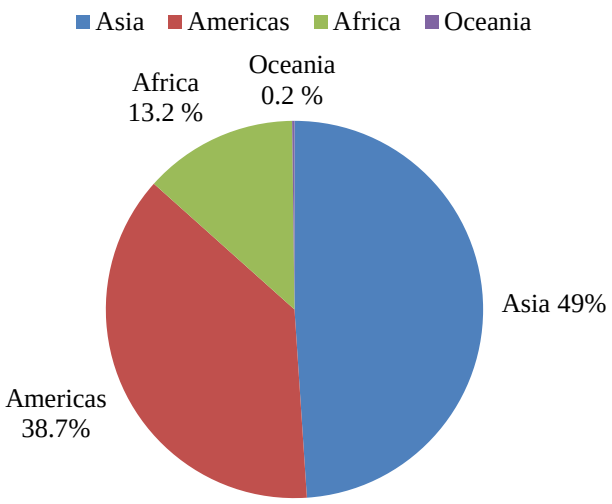

Figure 2. Production share of papaya by region (Source: Faostat, 2017).

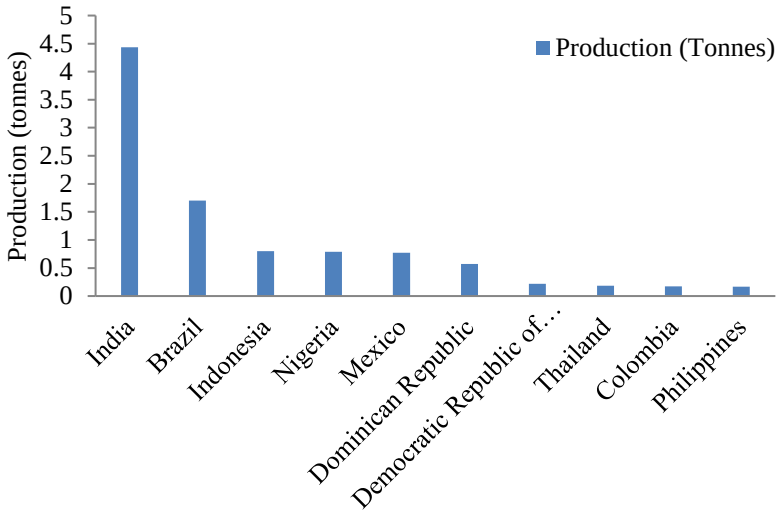

Figure 3. Top papaya production countries (Source: Faostat, 2017)

Papaya production in Taiwan

In 17th century, papaya travelled from the west Indies to Asia where at the end of the Qing Dynasty it was introduced to Taiwan and be developed forcefully upto now. In 2016, papaya area harvested and production achieved 2,584 ha and 118,661tonnes, respectively (Faostat 2017). Periods of 1994-2016, papaya area harvested and production were varied in Taiwan and they were increasing trend in 2014-2016 (Fig. 4). Papaya is in top 10 fruit growing in Taiwan with 4% of fruit production in 2013 (Fig.5).

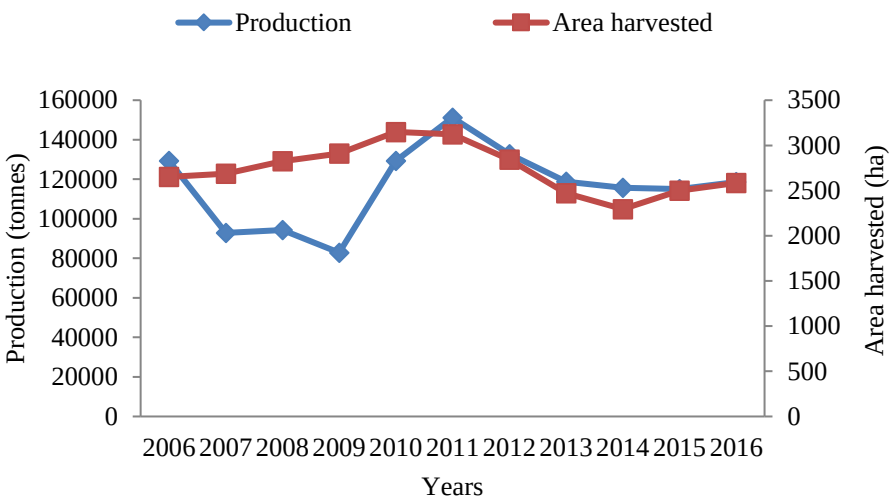

Figure 4. Area harvested and production of papaya in Taiwan (Source: Faostat, 2017)

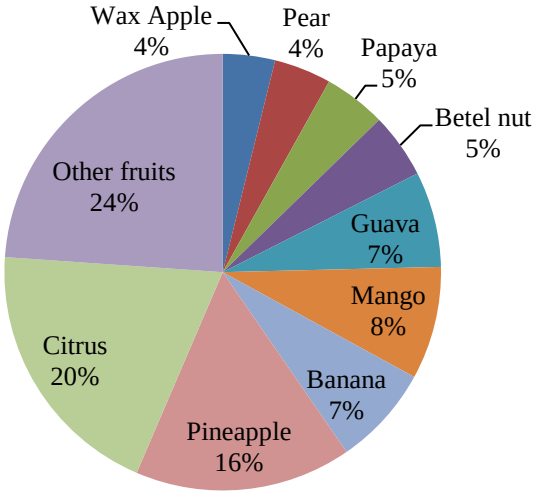

Figure 5. Fruit production in Taiwan in 2013 (Source: COA, TIER, 2014)

1.3. Characteristic of papaya plant

*** Stem**

The papaya plant is a large, mono-axial herbaceous plant with an erect stem terminating with a crown of large leaves and can attain by 9 m heights (Fig. 6). Although there are occasional lines or cultivars that produce and

abundance of lateral branches, especially during the juvenile period, the main stem normally growth of the axillary branches does occur when the trees become 3-5 years old (Paull 2011). The stem is semi-woody and hollow and a major site of starch storage. The bark is smooth, grayish, with large and prominent leaf scars. When the stem is wounded, a thin milky sap oozes from the wound (Fig. 6).

After transplanting, shoot growth is initially slow, though considerable root growth is taking place, extending out well beyond the canopy drip line. Stem growth is then rapid up to flowering, increasing in circumference up to 2 mm per day (Paull 2011). Growth rate peaks at flowering then declines as the tree starts bearing. The rate of growth is influenced by nitrogen and phosphorus supply, irrigation and temperature (Paull 2011).

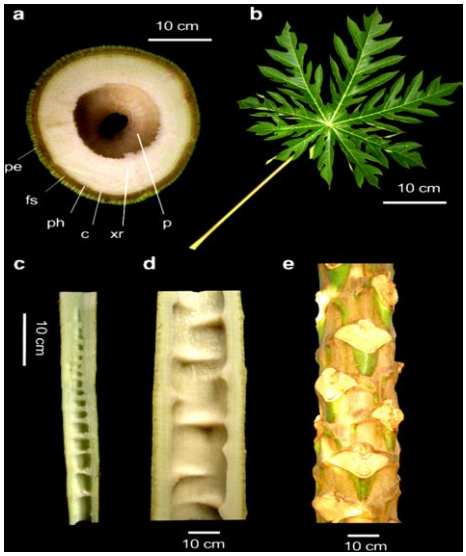

Figure 6. Vegetative parts of the papaya plant. (a) Cross section of a 1-year-old papaya stem: periderm (pe), fiber sheath (fs), phloem (ph), cambium (c), xylem rays (xr), pith (p). (b) Leaf lamina and petiole. (c) Longitudinal section of a 3-month-old papaya stem showing hollow pith cavity. (d) Longitudinal section of a 1-year-old papaya stem showing complete pith cavity. (e) Stem of a 1-year-old papaya plant showing conspicuous petiole scars.

*** Leaves**

A cluster of leaves occurs at the apex of the plant and along the upper part of the stem and makes up the foliage of the tree (Fig. 6). New leaves are constantly formed at the apex and old leaves senesce and fall. Leaves are palmately lobed with prominent veination and can measure 40-50 cm or more in diameter and have an individual leaf area of 1625 cm^2, with 15 mature leaves per plant (Paull 2011). In the tropics, new leaves appear two to three a week (Chan 1984). Petioles are cylindrical, hollow and length of 60 – 90 cm, depending upon the cultivar. The most recently matured leaf's fresh weight (about 10^{th} leaf from 2.4 cm juvenile leaf) varies from ca 50 to 170 g. The leaf petiole dry mass increases at a rapid rate until flowering the increases more slowly, peaking after fruit bearing starts (Paull 2011).

*** Floral organization and flowers**

Papaya has three types of distinctly different flowers, male, female and hermaphrodite, which give rise to fruits (Bose 1990) (Fig. 7). The flowers are found in the auxiliary pendulous in the inflorescence. Male flowers are yellowish, 2-4 cm long with petals fused to form a long tube, with 10 fertile stamens and a rudimentary ovary (Fig.7 A, D). Female inflorescence is much shorter, 3-4cm long and sits alone or in small groups in leaf axils. Female flowers are larger, usually white or cream in color, with five free petals and a large ovary with 5 fan shaped stigmas without stamens (Fig. 7 C, F). Hermaphrodite flowers have either 5 or 10 stamens and a prominent ovary (Fig. 7 B, E).

Five major floral structures namely pistillate, pentandria, elongate, staminate and intermediate have been identified of papaya (Bose 1990).