UNIVERSITI PUTRA MALAYSIA

STUDIES ON INCOMPATIBILITY POLLINATION AND FLORAL MORPHOLOGICAL POLYMORPHISM OF CARAMBOLA (AVERRHOA CORAMBOLA L)

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STUDIES ON INCOMPATIBILITY POLLINATION AND AND FLORAL MORPHOLOGICAL POLYMORPHISM OF CARAMBOLA (*Averrhoa carambola* L.)

By

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STUDIES ON COMPATIBLE AND INCOMPATIBLE POLLINATIONS AND FLORAL MORPHOLOGICAL POLYMORPHISM OF PIN AND THRUM MORPHS OF CARAMBOLA (*Averrhoa carambola* L.)

By

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Chairman : Professor Dr. Wong Kai Choo

Faculty : Agriculture

The objectives of this study were to elucidate the physiological basis of self-incompatibility system and the floral morphological polymorphism in carambola (*Averrhoa carambola* L.).

Within 30 minutes after pollination, pollen degradation and hydration commenced on the stigma surface. Pollen tubes subsequently emerged from colpal furrow and crawled into apertures of vesicles. The pollen tubes reached the base of the transmitting tract of the style on legitimate pollinations in pin and thrum morphs

at 8.0 h after pollinations. Pollen tubes were uniformly inhibited at the middle of the style in pin illegitimate pollinations, however, tubes occasionally penetrated to the base of the transmitting tract of the style. In thrum illegitimate pollinations, tubes were inhibited at the junction between stigmatic and stylar tissues. Result of illegitimate pollination confirmed the partial loss of incompatibility in pin as opposed to the strong incompatibility in thrum as shown by both fluorescence microscopic observation and the field controlled pollinations study. Ripen fruits from pin illegitimate pollinations were significantly smaller and contained less seed compared to legitimate pollinations.

Carambola flower consisted of 5 rose-pink sepals, a pistil, 5 stamens and 5 staminoids. Pistil length was significantly longer in pin than in thrum morphs. On the other hand, the stamen length was significantly longer in thrum than in pin morphs. Pollen grain shape of pin and thrum morphs was oblate spherical and tricolporate. Pollen diameter was significantly larger in thrum than in pin morphs. Foveolate pattern of pollen exine sculpturing was more intense in pin than in thrum morphs. Each of the five stylar tissues terminated in a capitate stigma. The stigma receptive surface surrounded the stylar tip, except for a strip along the abaxial part between the middle and base of stylar tissues. Vesicle was a dewdrop-shaped cell. Stigma and vesicles sizes were significantly greater in pin than in thrum morphs. Result of measurements of the various floral morphological parts revealed highly significant variations even among the various clones within the same morphs.

Abstrak tesis dikemukakan kepada Senat
Universiti Putra Malaysia untuk memenuhi keperluan untuk Ijazah Master Sains

KAJIAN KEATAS KETIDAK SERASIAN PENDEBUNGAAN DAN POLIMOFISMA BUNGA BELIMBING (*Averrhoa carambola* L.)

Oleh

NOBUHIKO FUSHIMI

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Pengerusi : Professor Dr. Wong Kai Choo

Faculti : Pertanian

Objektif kajian ini ialah untuk memahami asas fisiologi dari sistem ketidakserasian sendiri dan polimorfisma morfologi bunga dalam belimbing (*Averrhoa carambola* L.).

Dalam masa tiga puloh minit selepas pendebungaan, penghidratan dan degradasi debunga bermula diatas permukaan stigma, dan diikuti dengan kemunculan tiub-tiub dari kerutan colpal yang kemudianya merayap masuk kedalam bukaan vesikel. Tiub-tiub debunga tersebut kemudianya sampai kekawasan penghantar dipangkal stil dalam masa lapan jam selepas pendebungaan berbantu. Tiub debunga secara seragam disekat dipertengahan stil bunga pada pokok pin yang

didebungakan secara terbuka, walau bagaimanapun, maseh ada diantara tiub-tiub tersebut yang menyerap masuk kekawasan perhantaran dipangkal stil. Dalam pokok thrum yang didebunga secara terbuka, tiub-tiub debunga tersekat ditisu yang menyambungkan diantara stigma dan stil. Hasil dari pendebungaan terbuka mengesahkan bahawa kehilangan separuh dari ketidakserasian berlaku dalam pokok pin, manakala ketidakserasian yang penuh terjadi dalam pokok thrum, dan ini juga telah diperjelaskan lagi dari kedua-dua permerhatian mikroskop fluoresen dan pendebungaan terkawal diladang. Buah matang yang terjadi pada pokok pin yang didebungakan secara terbuka lebih kecil dan mengandungi kurang biji berbanding dengan buah dari pokok yang didebungakan secara terkawal.

Bunga belimbing mempunyai 5 sepal berwarna jingga-ros, 5 stamen, 5 staminoda dan satu pistil. Stil pokok pin secara bermakna lebih panjang dari stil pokok thrum. Disebaliknya pula stamen pokok thrum lebih panjang dari stamen pokok pin. Bentuk butir debunga dari kedua-dua pokok pin dan thrum adalah bulat oblate dan tricolporate. Garispusat butir debunga adalah secara bermakna lebih panjang dalam pokok thrum dari pokok pin. Corak 'foveolate' dari ukiran eksin dipermukaan butir debunga adalah lebih halus dalam pokok pin berbanding dengan pokok thrum. Semua kelima-lima stil berakhir dengan hujung yang reseptif dalam kesemua jenis morfs. Permukaan stigma yang sempurna adalah besar dan melingkugi hujung stil. Vesikel adalah satu sel yang berbentuk titisan embun. Saiz stigma dan vesikel secara bermakna lebih besar dalam pokok pin berbanding pokok thrum. Hasil ukuran dari berbagai bahagian-bahagian bunga menunjukan terdapatnya variasi sangat bererti diantara pelbagai klon-klon belimbing.

CHAPTER I

INTRODUCTION

Carambola (*Averrhoa carambola* L.) belongs to the family Oxalidaceae. It is believed to have been originated from Indonesia (Purseglove, 1968). It is a non-seasonal crop. As it is flowering throughout the year, fruit set may be found at any time and at different stages of development on the tree. The fleshy, five-angled fruit has sweet to sour taste and is eaten fresh or used for drinks. It is now found growing in the region of sub-tropics and tropics. In Malaysia, carambola is popular fruit, and is found growing in almost every part of the country. The crop is cultivated extensively with a total area of more than 1,900 ha in 1993 (Anon, 1994). It has become an important fruit crop in Malaysia, where it is now grown commercially for export. In 1994, Malaysia exported carambola to the amount of 8,218 tonnes, which was equivalent to RM$ 23.82 million. This values were equal to 11.6 % of fruit export in Malaysia (Anon, 1997). In recent years carambola fruits are becoming more popular in many countries especially in developed countries such as Japan, USA and Europe (Watson et al., 1988).

Carambola is a heterostylous species, with individual trees having either the long styled (pin) or the short styled (thrum) flowers which are compatible with each other but are self- and intramorph incompatible (Wong et al., 1994). Carambola

produces as many as 4,760-13,781 flowers per tree per month. Despite the production of large number of flowers, matured fruit set is considerably low, with only 5 - 198 flowers setting fruits (Saad et al., 1993). For satisfactory fruit production, it is necessary to plant a mixture of the two morphs, one of which acts as a pollenizer. The presence of pollenizer is sometimes achieved by bud grafting to a branch of an existing established commercial clone grown in the field. Such a practice can be an extra burden to the grower (Wong et al., 1994).

Heterostyly is a genetically-controlled floral polymorphism that is known in 155 genera under 24 families of flowering plants (Gander, 1979). The floral morphs differ reciprocally in style and stamen lengths. The flowers also differ in pollen size and production, and may differ in pollen exine sculpturing, pollen colour and presence of starch in pollen, stigma size, stigmatic papillae or corolla size or morphology (Dulberger, 1992). There are two morphs in distylous plants (distyly) and three morphs in tristylous plants (tristyly). The floral polymorphism is usually genetically linked with diallelic, sporophytic self-incompatibility system (Gander, 1979). In distylous plants the supergene determining floral morphology also controls a diallelic, sporophytic self-incompatibility system associated with floral polymorphism, so that pollinations between different morphs are compatible. Tristylous plants usually possess a two-locus diallelic, sporophytic self-incompatibility system associated with floral polymorphism, but whether or not a supergene is involved has not been demonstrated genetically (Gander, 1979).

The physiological function of various morphological components of the heterostylous syndrome is not well understood. Darwin (1877) suggested that the

complementary placing of stigmas and anthers in the floral morphs is a mechanical device for promoting insect-mediated pollinations between the morphs, which is reinforced by a distinct physiological mechanism of illegitimacy. Alternatively, Mather and De Winton (1941) suggested that the real significance of the morphological differences in pistils and stamens shown by heterostylous flowers was that they were causally and developmentally related to physiologically self-incompatibility system. Furthermore, Dulberger (1975a) has argued that heteromorphic characters of stigma and pollen grains participate in the physiological mechanism of incompatibility. She has hypothesized that the differential growth of styles and stamens in the morphs mediated the production of the incompatibility substances, and that metabolism related to cell wall extension played an important role in incompatibility. However, the significance of this association has been unclear (Dulberger, 1975a).

Richards(1986) has suggested that incompatibility in some heterostylous species, i.e., *Primula sp, Linum sp, Armeria sp* is controlled by factors, perhaps glycoproteins, which arise from the tapetum on the male side, and are located on the stigma surface on the female side. Furthermore, Dulberger (1992) has recently concluded that if the association between pollen size heteromorphism and differing inhibition sites has a functional basis, then neither the pollen storage product nor the pollen grain size proper are likely to be involved, although it must be strongly linked with pollen morph-specific size or pollen dry weight. She has suggested that a protein or growth factor deriving from an early premeiotic stage of microsporogenesis, during differential growth of the sporogenous cells or the pollen mother cell, may be involved.

Since the earliest report of Darwin (1862), the phenomenon of heterostylous syndrome has attracted considerable attention to many biologists (Gander, 1979). Most of researches on heteromorphic incompatibility have been devoted to studies on the genetics and ecology of natural populations in an effect to understand the evolution, adaptive, significance and breakdown of heterostylous syndrome. Very little is known about the physiological basis of incompatibility in heterostylous plants.

The present study is, therefore, carried out to provide a comprehensive understanding of the incompatibility system of the distylous carambola. Floral morphological polymorphisms are investigated in an attempt to relate their participation in the physiological mechanism of the in compatibility system.

Objectives

The objectives of this present study are :

1. To study the compatibility relationship among different morphs of carambola,
2. To study floral morphological polymorphisms with objectives of relating their participation in the physiological mechanism of the self-incompatibility system in carambola.

Hypothesis

Self-incompatibility in heterostylous species is closely associated with floral morphological differences between mating types. These morphological polymorphisms play an important role in the mechanism of self-incompatibility in the carambola.

CHAPTER II

REVIEW OF LITERATURE

The Carambola

General

Averrhoa carambola, locally known as "belimbing besi" or starfruit or carambola has been grown in Malaysia for more than 100 years. It is believed to have been originated from Indonesia (Purseglove, 1968). It is now found growing in the region of sub-tropics and tropics. It has been reported to be cultivated in Taiwan (Green, 1977), Australia (Watson et al., 1988), USA (Campbell et al., 1985, Crane, 1992), Brazil (Donadio, 1990), Guyana (Ramsammy, 1989), Trinidad, Canary Island (Galan-Sauco et al., 1989), Tabago (Andrew, 1989) and Surinam (Lewis and Groeizam, 1989).

In Malaysia, carambola is a popular fruit. It is found growing in almost every part of the country. The crop is cultivated extensively with a total area of more than 1,900 ha in 1993 (Anon, 1994). The main carambola cultivation areas include Sungai

Besi - Serdang - Seri Kembangan - Kajang (Selangor region), Seremban - Port Dikson (Negri Sembilan region), Muara - Batu Pahat (Johore region) and Kuching (Sarawak region) (Bong, 1984).

In recent years carambola is becoming more popular as a tropical fruit especially in developed countries such as Japan, USA and Europe (Watson et al., 1988). It has become an increasingly important fruit crop in some tropical countries, in particular Malaysia, where it is now grown commercially for export. In 1994, Malaysia exported carambola amounting to 8,218 tonnes, which was equivalent to RM$ 23.181 million. This value was equivalent to 11.6 % of fruit export in Malaysia (Anon, 1997).

Tree Characteristics

Carambola belongs to the Oxalidaceae family. The Oxalidaceae family contains primarily herbaceous genera. It is pyramidal in shape when young but rounded at the tops with an open canopy when mature (Watson et al., 1988). Carambola is a low tree or arborescent shrub, 5-12 m height, with a short or crooked, low branched trunk, 20-35 cm in diameter (Ochese et al., 1961). Carambola is normally vegetatively propagated by bud-grafting but tissue culture technique was also investigated as an alternative propagation method (Litz and Conover, 1980).

Flower Characteristics

Oxalidaceae is known as one of the families in heterostylous species for having both distyly and tristyly (Ganders, 1979). Carambola is a distylous species, with