FLORAL STRUCTURE AND POLLEN LOAD IN RELATION TO FRUIT DEVELOPMENT AND PHYSICO-CHEMICAL CHARACTERISTICS OF DRAGON FRUIT [Hylocereus polyrhizus (F.A.C Weber) Britton & Rose] AT HARVEST

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By

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Chairman : Associate Professor Phebe Ding, PhD
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In Malaysia, dragon fruits are graded and priced according to their fruit weight. As dragon fruit is a type of berry, its fruit weight depends on the number of developing seeds. In order to obtain better quality and yield in term of fruit weight, a study was conducted on the floral structure and pollen load on fruit development of red-fleshed dragon fruit (*Hylocereus polyrhizus*). The first objective was to determine the pollination events of *H. polyrhizus*. Light and scanning electron microscopy (SEM) were used to examine pollen and stigma morphology and pollen-stigma interaction of red-fleshed dragon fruit at 0, 1, 2, 3 and 4 day after pollination (DAP). Fluorescence microscopy was used to detect pollen tube growth stained with aniline blue in the style at 0, 2 and 4 DAP. The flowers of *H. polyrhizus* bloomed at night in an upright position with a spatial different between the anthers and the stigma suggesting cross-pollination. The pollen grains were categorized as large with polar axis (P) to equatorial diameter (E) ratio of 0.97, oblate spheroidal-shaped and trizonocolpate with the exine covered with echinates. The stigma had multiple lobes (n = 28 ± 3) and belonged to the dry type. The style is long (27 ± 2.54 cm) and solid with one hollow canal. Pollen grains germinated within 2 h after landing on the receptive stigma and pollen tubes grow within the transmitting tract located beneath the hollow canal of the style to the ovary. Starch granules within the style tissue depleted as DAP progressed. Findings indicated that a few pollen tubes had started to arrive in the ovary cavity after 2 DAP. Successful fertilization of male gametes (from pollen tube) with female gamete (ovule) led to fruit set. The second objective was to evaluate the changes in cellular structure using light microscopy and SEM during fruit development of *H. polyrhizus* at every 5 DAP. Starch pattern during fruit development was examined using iodine-potassium iodide (I_2/KI). After successful fertilization, microscopic studies revealed that fruit development occurred in two distinct period of growth of cell division and cell expansion especially in the pulp region. Initially, parenchyma cells were small and rigid but

loosen as DAP progressed. The early phase of cell division occurred in the first 20 DAP followed by cell enlargement thereafter. These cellular structure changes occurred concomitantly with the decrease of starch granules and colour change in the pulp. Starch granules accumulated within the pulp parenchyma cells and hydrolysed to sugar once the pulp turned red at 25 DAP as indicated by iodine stain clearing. For the third objective, effects of different pollen load size on *H. polyrhizus* fruits in term of fruit size and postharvest qualities were studied. Freshly dehisced pollen grains collected during anthesis were weighed at 0.001, 0.05, 0.10, 0.15 and 0.20 g (representing pollen load of 0.01, 0.5, 1.0, 1.5 and 2.0 x, respectively). The usual pollen load applied by the grower was used as control (C). Fruits were harvested at every 5 DAP until day 35. Results indicated that pollen load of 0.01 x produced the smallest fruit in term of fruit weight, length and diameter, followed by 0.5 x. Fruits of other treatments produced similar fruit size as control. There were no significant differences in peel and pulp colour, respiration rate and chemical characteristic among treatments except for soluble solids concentration (SSC). Smaller fruit tends to yield higher SSC. A positive correlation between seed number and fruit weight was determined ($r = 0.931$, $P \leq 0.001$). In conclusion, flowers of *H. polyrhizus* are nocturnal bloomers with large pollen grains that are covered with echinates suggested zoophilous pollination. Fruits of *H. polyrhizus* are considered as fast crop as it took 30 days suggested by cellular structure and starch pattern change during fruit development. Findings indicated that fruit weight of *H. polyrhizus* could be manipulated by the amount of pollen grains as the pulp derived from the funiculli of developing seed.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

STRUKTUR BUNGA DAN KESAN DOS DEBUNGA KE ATAS PEMBENTUKAN BUAH DAN CIRI FISIKO-KIMIA BUAH NAGA [*Hylocereus polyrhizus* (F.A.C Weber) Britton & Rose]

Oleh

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April 2011

Pengerusi : Profesor Madya Phebe Ding, PhD
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Di Malaysia, harga dan gred buah naga ditentukan berdasarkan jisim buah. Seperti buah beri yang lain, jisim buah naga dipengaruhi dengan bilangan biji yang terbentuk. Bagi mendapatkan hasil buah yang berkualiti tinggi terutamanya dari segi saiz buah, suatu kajian telah dijalankan ke atas struktur bunga dan dos debunga ke atas pembentukan buah naga isi merah (*Hylocereus polyrhizus*). Objektif kajian pertama ialah untuk mengenalpasti aktiviti pendebungaan *H. polyrhizus*. Kajian morfologi debunga dan stigma serta interaksi debunga dengan stigma semasa pendebungaan dikaji dengan menggunakan mikroskop cahaya dan mikroskop pengimbas elektron (SEM) pada 0, 1, 2, 3 dan 4 hari selepas pendebungaan (HSP). Mikroskop pendafluor digunakan untuk mengkaji pertumbuhan tiub debunga di dalam stil bunga yang diwarnai dengan anilin biru pada 0, 2 dan 4 hari selepas pendebungaan (HSP). Bunga *H. polyrhizus* mekar pada waktu malam dalam keadaan tegak. Penempatan cepu debunga dan stigma bunga adalah jarang dan ciri ini mencadangkan pendebungaan silang. Saiz debunga adalah besar dengan nisbah paksi polar (P) kepada diameter equatorial (E) bersamaan dengan 0.97, berbentuk sfera buntal (*oblate*) dan trizonokolpat dengan permukaan exine debunga diselaputi dengan deduri (*echinates*). Stigma tergolong dalam kategori kering (*dry*) dan terdiri daripada 28 ± 3 jejari yang bertempat di atas stil bunga yang panjang (27 ± 2.54 cm). Stil bunga bercirikan pejal dan mempunyai satu saluran kosong (*hollow canal*). Dua jam selepas pendebungaan, tiub debunga dari debunga kelihatan tumbuh menerusi tisu-tisu transmisi pada stigma dan bahagian bawah saluran menuju ke ovari. Granul-granul kanji yang terdapat di dalam sel-sel parenkima stil berkurangan apabila bilangan HSP berlaku. Interaksi antara debunga dan stigma mengambil masa 1 HSP. Segelintir tiub debunga tiba di ovari bunga pada 2 HSP. Hasil fertilisasi antara gamet jantan (dari tiub debunga) dan gamet betina (ovul) mengakibatkan pembentukan buah. Objektif kedua ialah mengkaji perubahan struktur sel yang berlaku semasa pembentukan buah *H. polyrhizus*. Buah dituai pada setiap 5 HSP. Perubahan struktur sel pada setiap peringkat dikaji dengan menggunakan mikroskop cahaya dan SEM. Kehadiran kanji di

dalam buah juga diuji dengan menggunakan iodin/kalium iodida (I_2/KI). Selepas fertilisasi, pembentukan buah terdiri daripada dua fasa iaitu divisi dan pembesaran saiz sel terutamanya pada isi buah. Pada peringkat awal, sel parenkima adalah kecil dan mempunyai dinding sel yang teguh tetapi bertambah besar dan longgar apabila buah semakin matang. Divisi sel berlaku pada 20 HSP yang pertama diikuti dengan pembesaran saiz sel pada HSP seterusnya. Perubahan struktur sel berlaku seiring dengan pengurangan kanji dan perubahan warna isi buah pada 25 HSP. Granul-granul kanji yang didapati dalam sel parenkima pada bahagian isi buah menghasilkan kawasan biru hitam apabila diuji dengan larutan I_2/KI pada 5 - 20 HSP. Pelunturan I_2/KI bermula pada bahagian isi buah yang menjadi merah pada 25 HSP. Objektif ketiga ialah untuk mengkaji kesan dos debunga ke atas buah H. polyrhizus dari segi pembentukan buah, saiz buah dan kualiti buah selepas tuai. Butiran debunga yang segar dikumpul dari bunga mekar dan dos debunga ditentukan dengan menimbang debunga yang dikumpul kepada 0.001, 0.05, 0.10, 0.15 dan 0.20 g. Setiap rawatan mewakili dos debunga yang terdiri daripada 0.01, 0.5, 1.0, 1.5 dan 2.0 x. Dos debunga yang biasa digunakan oleh peladang dijadikan sebagai kawalan (K). Buah dituai secara rawak pada 5 HSP sehingga hari ke-35. Keputusan kajian menunjukkan rawatan 0.01 x menghasilkan buah yang paling kecil dari segi jisim, panjang dan diameter, diikuti dengan 0.05 x, berbanding dengan rawatan yang lain. Rawatan lain menghasilkan buah yang sama seperti kawalan dari semua aspek yang dikaji. Tiada perbezaan bererti ditemui dalam kadar respirasi dan fizikal-kimia antara rawatan kecuali bacaan jumlah kandungan pepejal terlarut (KPT). Buah bersaiz kecil dari rawatan 0.01 x didapati mempunyai nilai KPT yang tinggi berbanding dengan rawatan yang lain. Jisim buah berkolerasi positif dengan bilangan biji yang terhasil di dalam buah ($r = 0.931$, $P \leq 0.001$). Kesimpulannya, bunga *H. polyrhizus* mekar pada waktu malam serta ciri debunga besar yang diselaputi dengan duri merupai ciri pendebungaan zoofili. Buah *H. polyrhizus* mencapai tahap pemakanan yang optimal pada 30 HSP. Kenyataan ini disokong dengan perubahan struktur sel buah dan corak kanji semasa pembentukan buah. Isi buah *H. polyrhizus* dibentuk oleh funikuli biji yang berkembang menunjukkan bahawa jisim buah naga isi merah dapat dimanipulasi dengan mengawal dos debunga yang diguna semasa pendebungaan.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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°C	celcius
C*	chroma
C_2H_4	ethylene
CO_2	carbon dioxide
cm	centimeter
CPD	critical point dryer
DAP	day after pollination
FAA	formaldehye-acetic acid-alcohol
FID	flame ionization detector
h	hour
h°	hue
g	gram
L*	lightness
L	liter
K_3PO_4	potassium phosphate
LSD	least significant difference
mL	milliliter
n	Newton
NaOH	sodium hydroxide
r^2	correlation coefficient
SAS	statistical analysis sytem
SEM	scanning electron microscope
SSC	soluble solids concentration
TTA	titratable acidity

TBO	toluidine blue O
TCD	thermal conductivity detector
UV	ultraviolet
μm	micrometer

CHAPTER 1

GENERAL INTRODUCTION

Pitaya or dragon fruit belongs to the Cactaceae family which is a native of the tropical regions of Mexico and Central and South America (Anderson, 2001). In South East Asia, the fruit is commonly known as dragon fruit as the peel of the fruit is covered with bracts which resembles the 'scales' of the mythological dragon (Wu et al., 2006). Dragon fruit is also known as thanh long, pitaya, pitahaya, pitajaya, pitajuia or pitalia in different parts of the world (Le Bellec et al., 2006). The plants are characterised by elongated stems (usually three winged) with aerials roots, large white monoecious flowers and spineless edible berry fruit with many edible seeds (Tel-Zur et al., 2004). The peel colour varies from pinkish to red while the pulp colour varies from white to red or purplish, depending on varieties. There are only few varieties of dragon fruits currently found cultivated in Malaysia such as the red fleshed varieties; *Hylocereus polyrhizus, H. costaricensis* and the white fleshed; *H. undatus*.

Fruit weight of dragon fruit is positively correlated with the number of viable seeds (Weiss et al., 1994). Hence, achievements of uniform and profitable yields depend entirely on hand pollination. As a result, variation of weight and size are produced. The optimal time to harvest dragon fruit is within 28 to 30 days after flower anthesis at full colour development (Nerd et al., 1999). The average weight for a mature fruit ranged between 350 – 900 g. The price of dragon fruit is paid according to fruit weight and varieties.

Fruit weight, like fruit colour, plays an important role in the consumer purchase process. For example, in order to gain access to Europe market, the preferred average weight for white-fleshed dragon fruits in Vietnam is 300 g (Hoa, 2008). In Malaysia, the Department of Agriculture graded and priced the dragon fruits into different catergories of: Grade A = more than 450 g, Grade B = 300-450 g and Grade C = less than 300 g (Ministry of Agriculture, 2006**)**.

The fruit is unique since it contains betacyanins pigments with antioxidant properties; a rare properties which is hard to find in other fruits. Thorough examination of the floral structure and its relation to fruit development would provide useful data for the plantation industry. Moreover, researches done for dragon fruits are mostly carried out in Israel where the environment and planting conditions were different in Malaysia. In addition to that, as pollen load was found to affect the fruit size and weight of cherimoya (Gonzalez et al., 2006) and cranberries (Cane and Schiffhauer, 2003), a study on the pollen load effect on dragon fruits were also carried out in order to optimize the fruit yield.

Therefore, the objectives of this study were (i) to investigate the pollination process of red-fleshed dragon fruit, (ii) to elucidate the changes in cellular structure during fruit development and (iii) to evaluate the effects of pollen load size on fruit set, size and postharvest qualities of the red-fleshed dragon fruit.

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