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FLOWERING AND FRUITING PHENOLOGY, POLLINATION VECTORS AND BREEDING SYSTEM OF DRAGON FRUIT (*HYLOCEREUS* SPP.)

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SUMMARY

Dragon fruit (Hylocereus undatus), a species native to Central American rainforests is a climbing cactus species, which has recently received worldwide recognition as an ornamental plant and a fruit crop. It has been well established as a new crop in various tropical countries due to its precocious yielding ability and its acceptability in the market; hence considered as a new fruit crop for the future. Sri Lanka began to grow this species around 1992 as a small-scale crop with a mixture of genotypes including red skin-white pulp (Hylocereus undatus) and red skin-red pulp (Hylocereus spp.) fruit types. Although an understanding of the reproductive biology is fundamental to making sound decisions on conservation, management and genetic improvement of species, such information is limited in Sri Lanka for Dragon fruit. Consequently, the objectives of this study were to examine the flowering and fruiting morphology and phenology, pollination vectors and the breeding system in Dragon fruit at the plantation at Bulathsinghala.

Phenological studies revealed that flowering of Hylocereus spp. was seasonal and occurred from April to November with 4-7 cycles per year. Floral bud stage to anthesis took about 30 days. Bisexual flowers of Dragon fruit were open from 6.30 pm and closed by 10.00 pm on the same day. However, they remained open till 12.00 noon of the next day if they were not pollinated. During flower opening and the receptive period the stigma was positioned upright whilst after pollination *it turned downward. Synchronous flowering observed among individuals may increase the possibilities for cross-pollination. A detailed investigation of pollination vectors showed that the most likely pollinators of Dragon fruit in Bulathsinhala, Sri Lanka are Apis cerana (honeybee), Apis florae (dwarf honeybee) and Apis dorsata (giant honeybee). Fruiting season of Dragon fruit was from May to January, and may extend until February. Fruits ripened 30-50 days after flower opening (pollination). During the ripening, the green coloured skin change to red although scales remain green. Controlled pollination experiments and subsequent assessment of fruit set and fruit quality of two fruit types revealed that white-pulp type is self-compatible and red-pulp type is relatively self-incompatible. Although selfed flowers of both species also developed into fruits, their size and weight were significantly lower compared to out crossed flowers. In addition, fruits developed after artificial self-pollination were larger compared to fruits developed after open pollination suggesting inadequate pollination. Implications of these findings on utilization of genetic resources of Dragon fruit are discussed.*

INTRODUCTION

Dragon fruit (*Hylocereus undatus* (Haw.) Britton and Rose of the family Cactaceae, a species native to Central American rainforests is commonly cultivated climbing cactus species. In the recent past, it has received worldwide recognition as an ornamental plant and a fruit crop. At present, it has been well established as a new crop in various tropical countries and it is spreading rapidly, in many tropical and sub tropical countries mainly because of its precocious yielding ability and its acceptability in the market. It could be considered as a new fruit crop for the future. The main constraint in growing Dragon fruit is that the cost of establishing is high due to the use of trellises for climbing. The other agronomic practices are easy and less expensive due to low maintenance cost. The biggest advantage of this crop is that once planted it will grow for about 20 years, and one hectare could accommodate up to 800 plants. Dragon fruit is a fast return fruit crop with production in the second year after planting and full production within five years with regular bearing (Barthlott and Hunt, 1993; Bellec, 2004; Mizrahi and Nerd, 1996). Sri Lanka also began to grow this species around 1992 as a small-scale crop possibly with different *Hylocereus* spp. including *Hylocereus undatus* (red skin-white pulp) and *Hylocereus* spp. (may be *H. polyrhizus*, red skin-red pulp) at Bulathsinhala. Experience in Sri Lanka has also shown that the potential for expansion is very high in all climatic zones. Further, Dragon fruit has been identified as an obligate Crassulacean Acid Metabolism (CAM) pathway species, which is suitable for even dry conditions due to higher water use efficiency. Although, an understanding of reproductive biology is fundamental to making sound decisions on conservation, management and genetic improvement of plant species, such information is limited to Israel experiences where the species has been grown under controlled environmental condition (Lichtenzveig *et al*., 2000; Nerd and Mizrahi, 1997; Nerd *et al*. 1999). Consequently, the objectives of this study were to examine the flowering and fruit morphology and phenology, pollination vectors and breeding system of the Dragon fruit plantation at Bulathsinhala, Sri Lanka.

MATERIALS AND METHODS

The study was carried out in the Dragon fruit plantation at Bulathsinhala owned by Sieco Agric Development Private Limited during 2004/5 and 2005/6 flowering seasons. The plantation area is located in WL_{1a} agroecological region at $6^0$41’42” N latitude and $80^0$09’43” E longitude, 20-24 m amsl. Seventy five percent expectancy of value of annual rainfall in the area exceeds 3,200 mm/year. Soil of the area is red yellow podzolic, well drained and acidic. The plants had been established from cuttings in 1998 at a spacing of 3.5 m x 3 m. Red skin-white pulp (*H. undatus*) and red skin-red pulp (*Hylocereus* spp, possibly *H. polyrhizus*) have been used in establishing the plantation although only 6 individuals of red skin-red pulp plants are available. Plants were trained on a 1.3 m high concrete trellis with iron bars and used tyre for support.

Flowering phenology was observed on 5 plants of red skin-white pulp type (*Hylocereus undatus*) and 2 plants of red skin-red pulp (*Hylocereus* spp.) from March 2004 to February 2006. Three to five randomly selected floral buds that emerge were labeled and their development was recoded to identify time taken to reach different floral and fruit stages. Number of floral and fruit cycles (flushes) per plant were identified based on number of flowers opened per plant per day and number of fruits harvested per time, respectively. Floral buds not developed to flowers were not included in the assessment. Floral morphology was observed on randomly selected 10 flowers from red skin-white pulp and 3 flowers from red skin-red pulp types. Colour, size, shapes, number of floral parts were observed on 2-3 dissected flowers at three flowering cycles. Opened flowers were also observed for availability and amount of floral rewards such as nectar and scent. Amount of nectar available was measured using capillary tubes.

Flower opening was recorded on fully matured floral buds from 4.00 pm till 12.00 noon on the next day at 5 occasions. During flower opening, potential pollinators were also observed and collected for identification. Collected specimens were identified by Dr. KS Hemachandra, Department of Agricultural Biology, Faculty of Agriculture, University of Peradeniya. During observation, pollinator’s movements within and between flowers and their activities including their ability to touch stigma were recorded. Captured insects were observed under a microscope (x10) to check the presence of pollen grains on body parts.

During bud stage, 3-4 floral buds were labeled and covered using paper bags (45 cm x 30 cm). Before anthesis (at about 3.00 pm), flowers were opened and anthers were removed by cutting using a scissor. Emasculated flowers were then controlled pollinated as W1 x W1, W1 x W2, W1 x R1, R1 x R1, R1 x R2, and R1 x W1 (where W, R and number referred as red skin-white and red skin-red pulp fruit types and plant number, respectively) and bagged again. Bags were removed on the next day after 2.00 pm. Non emasculated flowers were also labeled and kept for open pollination as control (W1 and R1 open pollination). Controlled pollinations were carried out between 8.00-9.00 pm by alternative brushing of collected pollen and receptive stigmas. Fruit development following controlled pollination was recorded. Once fruit ripened, fruits were harvested and fruit set was calculated, and total weight (using an electronic balance), length and width of fruit and length of scales (using a caliper) were measured. Number of scales per fruit was counted. Fruits were then opened and flesh and skin were separated, and skin weight was measured and flesh colour was recorded. Pulp to skin ratio was calculated. Flesh was divided into four equal parts and two randomly selected parts were used to count seed number, and multiplied by two to obtain seed number per fruit. Brix value of flesh was measured using a portable refractometer, where two readings were obtained for each fruit. Seeds obtained from controlled pollination experiments were germinated for further observations and experimentations on their genetic variation.

RESULTS AND DISCUSSION

Floral and fruit morphology and phenology

The flowering in Dragon fruit (in both *Hylocereus* spp.) was seasonal and flower production in Bulathsinghala was from April to November, sometimes extending till December. In individual plants, flowering occurred in 4-7 cycles (flushes) per year. At the onset of flowering, 3-5 spherical buttons emerged from the stem margins and 2-3 of these may developed into flower buds in about 13 days. A longitudinal section of floral bud of Dragon fruit at the beginning of anthesis showing various floral parts is given in Plate 1 whilst pattern and position of flowering are shown in Plate 2. Plate 3 shows an opened flower with position of floral parts including stigma and anthers. The variation recorded of floral characters of Dragon fruit are given in Table 1. The light green cylindrical flower buds reached about 31 cm after 17 days when anthesis occurs. The extremely showy flowers (Plates 2 and 3) were hermaphrodite, bell shaped, large, white or cream white in colour, very fragrant, with a thick tube bearing several long linear green scales (Table 1 and Plates 1 and 2). Stamens were cream colored, and formed a showy fringe in the center and at the apex of the thick perianth tube. The hollow style was cream in colour and about 26 cm long. Stigma was also creamy white in colour and divided into average of 15 lobes. A single flower of Dragon fruit with numerous anthers produced a massive amount of pollen. Pollen grains were sticky and uniform in size (a diameter of 70-80 µm). After opening, flowers produced a musk smell and about 7 ml of nectar at the bottom of the floral tube (Plate 4). The flowers were nocturnal and open once, starting about 6.30-7.00 pm and was completing by about 10.00 pm. At about 2.00 pm the flower closed if artificially pollinated and thereafter petals begin to wilt. However, if flowers were not pollinated during the night, they remained open till 11.00 am of the next day. During flower opening and the receptive period the hollow stigma was positioned upright whilst after pollination it turns downward. The flower petals closed completely by daybreak.

Table 1. Variation of Floral Characters of Dragon Fruit at Bulthsinhala, Sri Lanka.

Character	*Mean ± SE*	*Range*
Length of mature floral bud (cm)	31.4 ± 1.19	20 - 36
Width of mature floral bud (cm)	20.0 ± 0.83	12 - 23
Diameter of fully opened flower (cm)	22.1 ± 1.33	10 - 30
Length of style (cm)	25.6 ± 0.91	18 - 30
Number of anthers	1140 ± 9.12	1100 - 1195
Number of stigma lobes	14.9 ± 0.61	12 - 18
Length of stigma lobes (cm)	3.1 ± 0.11	2 - 3.5
Length of ovary (cm)	6.0 ± 0.28	4 - 8
Nectar availability (ml)	6.9 ± 0.39	4 - 9
Smell		Musk
Number of flowers per plant		1 - 7

Note: N=10 flowers for red skin-white pulp and 3 flowers for red skin-red pulp fruit types. SE=standard error.

The normal fruiting season of Dragon fruit in Bulathsinhala Sri Lanka occurred from May to January, sometimes extending until February. Longitudinal sections of ovary of a flower and a ripened red skin-white pulp fruit are shown in Plate 4 whilst variation of fruit characters of Dragon fruit are given in Table 2. The fruit was a medium to large, round to oblong shaped berry. It was distinguished with green colour skin with relatively large green colour scales (Plate 4). The change in skin colour of the fruit was observed about 26 days after anthesis in both *Hylocereus* spp. and skin colour turns fully red 4-5 days after first colour change. Fruit firmness also decreased during this period. At peak ripeness, the skin colour of fruits became pink-red although the scales remain green in colour. Peak ripening was reached 40-50 days after anthesis (pollination). Thus, mature fruits can be harvested from average of 46 days after flower opening (pollination). Seeds were small (1 mm long), numerous and black and embedded within the pulp (Plate 4). The fruit pulp may be white (in *H. undatus*) or red (*Hylocereus* spp. possibly *polyrhizus*) and juicy. Floral and fruit characters observed in this study are similar to other studies conducted else where by Lichtenzveig *et al*. (2000), Nerd and Mizrahi (1997) and Weiss *et al*. (1994).

In this experiment, only 5-10 plants were used for monitoring of flowering and fruiting phenology. The Dragon fruit plantation at Bulathsinhala consists with 280 plants where details of flower and fruit productions of each plant are available. Analysis of such information will be useful in identification of selection criteria such as prolonged flowering and fruiting season, higher fruit set and higher number of fruits per plant for crop improvement and subsequent programs on production of quality planting materials.

Table 2. Variation in Fruit Characters of Dragon Fruit at Bulthsinhala, Sri Lanka.

Character	*Mean ± SE*	*Range*
Fruit shape	-	Round and oblong (common) or elongated ellipse
Length (cm)	17.3 ± 0.37	10 – 20
Width (cm)	9.6 ± 0.21	7 – 12
Girth (cm)	13.4 ± 0.42	10 – 18
Scale number	22.2 ± 0.89	10 – 32
Scale length (cm)	5.9 ± 0.21	2 - 7.5
Peel thickness (mm)	3.1 ± 0.11	2 – 4
Weight of fruit (g)	506 ± 19.25	220 – 840
Pulp colour	-	Red or White
pH value	5.1 ± 0.03	4.6 - 5.5
Brix value	15.3 ± 0.27	12 – 18
Time to pollination to fruit Ripening (days)	45.8 ± 0.41	40 – 50

Note: N=32 fruits for red skin-white pulp and 13 fruits for red skin-red pulp species.

Pollination vectors and breeding system

Many small (less than 2-3 mm long) nocturnal insects visited the flowers from about 9.30 pm till 11.00 am the next day. Early in the morning bees commonly visited and walked on flowers for either pollen or nectar collection

(Plate 3). During the visits, in contrast to small insects, bees were active, their body parts covered with a mass of pollen grains, touch the stigma. They visited many flowers from different plants and spend about even 1-2 minutes per flower. In addition, activities of bees may cause dislodging of pollen from anthers and such pollen may also fall on the stigma. The insects involved in the effective pollination process were identified as *Apis cerana* (Honeybee), *Apis florae* (Dwarf honeybee) and *Apis dorsata* (Rock bee) of the family Apidae. Although Weiss *et al*. (1994) suggested that bees are not efficient pollinators of flowers of *Hylocereus* spp. due to the larger size of the flower and the arrangement of its parts, the observations of this study revealed that their body parts were covered with a mass of pollen grains and assisted in the pollination process. Although bats or hawk moths have been identified as natural pollinators in native range of Dragon fruit (Weiss *et al*., 1994), no bats or moths were observed visiting flowers during the nights. Further, in general bats have not been observed visiting the field by watchers of the plantation.

In red skin-white pulp type (*H. undatus*), fruit set was 100% following selfing treatment. However, in selfed-treatment although fruit set was 100%, size of fruits was lower compared to outcrossing treatment (Table 3). In contrast, in red skin-red pulp type, comparatively smaller number of flowers developed to fruits (fruit set was only 40%) following selfing (Table 3). As in the case of red skin-white pulp type, fruits developed following selfing were significantly smaller compared to fruits developed following outcrossing. These evidences are suggesting the occurrence of a relatively strong self-incompatible mechanism in red skin-red pulp fruit type and weak self-incompatible mechanism in red skin-white pulp fruit type. It is clear from Table 3 that number of seeds per fruit also associated with type of pollination. Results of fruit-set experiments also revealed that in both fruit types, fruits developed from artificially self-pollination were larger compared to open pollinated fruits suggesting inadequacy of pollination under open pollination conditions in Bulathsinhala. Weiss *et al*. (1994) also reported similar results for *Helocereus* spp. and *Selenicereus* spp. in Israel under controlled environmental condition. Red skin-red pulp type used in this experiment may be *H. polyrhizus* because it has been identified as self-incompatible species where pollen tube germination following selfing has been arrested at the ovary (Lichtenzveig *et al*., 2000). However, further studies are required to identify the incompatibility mechanism of the red skin-red pulp fruit type in Bulathsinhala with controlled pollinations and subsequent observations of pollen tube on stigma and in style and ovary. Cross compatibility was higher in red skin-white pulp type compared to red skin-red pulp type (Table 3). Cross compatibility in different *Helocereus* spp. has also been observed by Lichtenzveig *et al*. (2000), Tel-Zur *et al*. (2004) and Weiss *et al*. (1994).

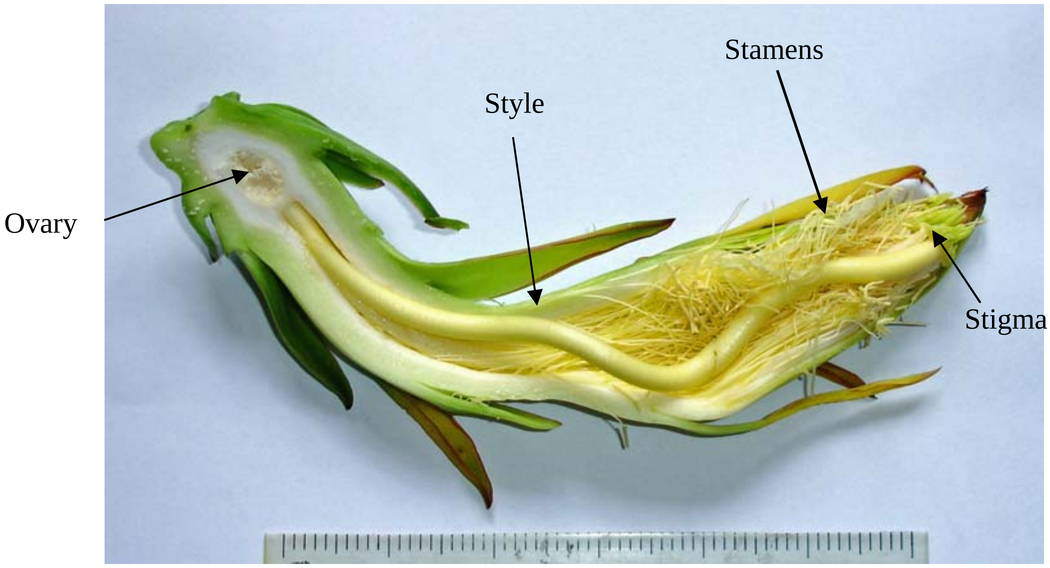

Plate 1. Longitudinal Section of Floral Bud of Dragon Fruit at the Beginning of Anthesis.

Plate 2. Night blooming of Dragon fruit flowers.

Plate 3. Potential Pollinators of Dragon Fruit at Bulathsinghala Plantation.

Plate 4. Longitudinal Sections of Ovary of a Flower and a Mature Red Skin – White Pulp Fruit.

Table 3. Percentage Fruit Set, Pulp Colour, Fruit Weight, Brix Value and Number of Seeds Following Selfing and Crossing of *Hylocereus* spp.

Cross	*Fruit set (%)*	*Weight (g) Mean ± SE*	*Pulp: peel*	*Brix Value*	*Number of seeds*
W1 x W1	100 (W)	580.8 ± 3.03^{b}	4.2:1	15	10,670
W1 x W2	100 (W)	644.4 ± 4.10^{a}	4.6:1	15	11,460
W1 x R1	100 (W)	640.6 ± 4.64^{a}	5.2:1	16	8,100
W1 open	100 (W)	441.6 ± 2.04^{c}	3.1:1	18	7,920
R1 x R1	40 (R)	146.5 ± 2.47^{e}	1.1:1	13	4,420
R1 x R2	80 (R)	164.5 ± 3.90^{f}	1.9:1	13	2,980
R1 x W1	80 (R)	$367.5 \pm 11.79_{d}$	3.1:1	18	2,580
R1 open	60 (R)	61.3 ± 4.73^{g}	0.8:1	10	160

Note: N=8 fruits for red skin-white pulp type (W) and 5 fruits for red skin-red pulp type (R). W1=*Helocereus undatus*, R1=*Helocereus* spp. possibly *polyrhizus*. In fruit set column, letters in parenthesis indicate pulu colour of fruit. SE=Standard error. Mean fruit weight values with different superscript letters are significantly different at $P<0.05$.

It is reported that in many countries where the crop is grown as a new crop, pollination is poor due to lack of natural pollinators found in the native environments. Hence, hand pollination has been suggested in order to increase fruit set and fruit weight. Hand pollination although laborious, can be done easily by alternate brushing of anthers or collected pollen and stigma. After anthesis, pollen can be collected on to a plate using a fine paintbrush. Weiss *et al*. (1994) stated that pollen is most viable at the time of anthesis. Israeli scientists have developed a procedure for pollen storage to guarantee high yields. In this procedure pollen collected in the evening or morning and dehydrated in desiccators until the moisture content is reduced to 5-10% and stored at 4 ^{0}C for 3-9 months. It is reported that the weight of fruits produced from frozen pollen is similar to that of fresh pollen in commercial orchards (Metz *et al*., 2000).

In Israel hand pollination of *H. undatus* and *H. polyrhizus* is essential to increase fruit set as they are self-incompatible (Lichtenzveig *et al*., 2000). Thus, hand pollination with different genotypes (may be species) will help in increasing fruit set and fruit size (weight). Bellec (2004) and Lichtenzveig *et al*. (2000) also reported that cross pollination with different genotypes and species will help not only in increasing fruit set but also increase fruit size. Therefore, efficient pollination could be achieved by artificially cross-pollination of compatible clones. Hence, to avoid low fruit set and smaller fruits, mixed plantings with several genotypes and hand pollination can be recommended. For artificial pollination process, a mixture of pollen can be collected to plates and their application is advised due to the partial self-incompatibility of the species.

Two fruit types (possibly two *Hylocereus* spp.) studied in this experiment cross readily. Thus, it would be possible to produce new variations with different fruit qualities including pulp colour, sweetness etc. by planned controlled crossing experiments with identified plus mother plants. Implementation of these findings are important to produce high fruit set of marketable size. In future, as Lichtenzveig *et al*. (2000) suggested development of self-compatible types with acceptable fruit size is an important long term goal of breeding and improvement programs of Dragon fruit which require less labour for hand pollination and thus to reduce cost of production.

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