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Morphological Characteristics and Pollination Requirement in Red Pitaya (*Hylocereus* spp.)

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***Abstract*—**This study explored the morphological characteristics and effects of pollination methods on fruit set and characteristics in 4 red pitaya (*Hylocereus* spp.) clones. The distinctive morphological recognition and classification among pitaya clones were confirmed by the stem, flower and fruit features. The fruit production season was indicated from the beginning of May to the end of August – the beginning of September with 6-7 flowering cycles per year. The floral stage took from 15-19 days and fruit duration spent 30–32 days. VN White, fully self-compatible, obtained high fruit set rates (80.0–90.5%) in all pollination treatments and the maximum fruit weight (402.6g) in hand self- and (403.4g) in open-pollination. Chaozhou 5 was partially self-compatible while Orejona and F_{11} were completely self-incompatible. Hand cross-pollination increased significantly fruit set (95.8; 88.4 and 90.2%) and fruit weight (374.2; 281.8 and 416.3 g) in Chaozhou 5, Orejona, and F_{11}, respectively. TSS contents were not much influenced by pollination methods.

Keywords*—Hylocereus* spp., morphology, floral phenology, pollination requirement.

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I. Introduction

PITAYA or dragon fuit (*Hylocereus* spp.) originated from the Americas [1] with a wide distribution in tropical and subtropical regions [10], [11]. It is increasingly gaining interest in many countries, including Taiwan due to their high economic potential as exotic fruit crops and their exceptional tolerance to extreme drought [11], [13], [15]. The benefits of dragon fruit for human health could be explained by its essential nutrients such as vitamins, minerals, complex carbohydrates, dietary fibres and antioxidants [7].

Pitaya cultivars being grown on commercial scale belong to four species: *H. undatus, H. monacanthus* (syn*. H. polyrhizus*), *H. costaricensis* and *Selenicereus megalanthus* (syn.) *H. megalanthus,* and their hybrids. [8], [14], [17], [18], [20]. There is great disagreement about specific circumscriptions in the genus. This is due, in part, to the fact that most species of *Hylocereus* were similar in stem and flower morphology. The main differences among several *Hylocereus* species were the size and color of the fruit, and the number and form of the spines [3]. The separation of species and varieties in the genus *Hylocereus* was difficult due to the high intra- and interspecific hybridization [17], which has caused some taxonomic confusion. Therefore, currently, in order to recognition and evaluation of new piatya varieties, International Union for the Protection of New Varieties of Plants (UPOV) proposed "The Guidelines for the Conduct of tests for Distinctness, Uniformity and Stability (DUS)" applying to Dragon fruit (*Hylocereus undatus* (Haw.) [6]. This document standardized and described the fully characteristics of pitaya as the stem, flower and fruit.

Regarding the reproductive biology, most studies reported that it had large, hermaphroditic nocturnal flowers, and belonged to the long day plant with natural flowering and production during warmer months [10], [11]. In Israel *H. polyrhizus* and *H. costaricensis* were indicated as self-incompatible (SI), requiring cross-pollination to set fruit while *H. undatus* and *S. megalanthus* were self-compatible (SC), setting fruit with self-pollination [12], [20]. In contrast with findings of [20], in the natural habitat in Mexico *H. undatus* had the highest fruit set both open and unmanipulated self-pollination [19]; however, up to now, no more comprehensive study on influences of pollination treatments and pollination requirement for *Hylocereus* spp. species as cultivars in naturally domesticated growing condition has not been conducted.

In Taiwan red pitayas (*Hylocereus* spp.) with white, red or purple pulp are widely cultivated plants. Because of their importance as exotic fruit crops, different species of the genera *Hylocereus* collected for study are being grown at the tropical fruit orchard, National Pingtung University of Science and Technology (NPUST). The present work investigated morphological characteristics, effects of pollination methods on fruit set and fruit characteristics in some promising pitaya clones in order to determine their pollination requirement and propose agro-managements that can improve the efficiency of pollination, fruit quality, and yield.

II. Material and Methods

A. Plant Materials and Experimental Treatments

The pitaya plants used were about 10 years old grown at the tropical fruit orchard, National Pingtung University of Science and Technology (NPUST), Taiwan. The experiment was carried out from April to September in 2013. Four pitaya clones: Vietnam (VN) White (*H. undatus*), Orejona (*H. monacanthus),* Chaozhou 5 and F_{11} (*Hylocereus* spp.) intercropped with together were examined. For investigating effects of pollination methods, four pollination treatments were applied.

(1) Hand self-pollination: Tagged mature flower buds were covered by bags before 2PM. Pollen from the same flower was applied to the stigma after flowers opened in the evening

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(9.00-12.00PM). To prevent open pollination, flowers were kept bagged except during hand pollination.

(2) Hand cross-pollination: Anthers of mature flower buds were removed and bagged before 2PM pollen shed to prevent unwanted pollination. Emasculated flowers in each clone were hand-pollinated with flesh pollen gathered from a different concurrently flowering clone in the same evening (9.00-12.00 PM) and then re-bagged.

(3) Automatic self – pollination: Flowers were covered with bagged throughout anthesis to prevent cross pollination.

(4) Open pollination: Natural pollination without interfering action from experimenters.

The pollination experiment design was in Randomized Complete Block Design (RCBD) with 3 replications and 20-25 flowers were randomly selected for one treatment.

B. Parameter Measurements

In order to characterize morphology, 5 developed stems (at the end of the year's growth), 5 flowers at mature bud and fully opening stages and 5 ripening fruits per each clone were randomly chosen and described characteristics. The average number in each parameter was of 4 - 5 measures. The method of measurements following to [6], [9] were recorded in Table I.

TABLE I
THE STEM, FLOWER AND FRUIT DESCRIPTORS OF THE RED PITAYA (*HYLOCEREUS* SPP.) CLONES

Descriptors	Method	Units
Stem length	Measure from basal insertion to apex of stem	Centimeters (cm)
Stem width	Measure on a flattest face of stem	Centimeters (cm)
Areole distance	Distance between a spine group and another group.	Millimeters (mm)
Spine number/areole	Average number of spines in 5 randomly areoles	Unit
Spine length	Measure on the longest spine of each randomly areoles	Millimeters (mm)
Rid margin form	3 categories: Concave, flat, or convex	
Spherical button shape	4-6 day spherical button, described by one of forms: narrow elliptical, medium elliptical, circular, oblate	
Mature bud size	Measure at just before flower opening: Bud length, pericarpel length, pericarpel width.	Centimeters (cm)
Sepal pattern	3 categories: none, edged or striped	
Petal color	5 categories: White, yellowish green, yellow, cream, red	
Stigma lobe color	2 categories: Cream, green	
Number of stigma lobes	Average number of stigma lobes in 5 randomly flowers	Unit
Anthers below stigma	The distance between anthers and stigma	Centimeters (cm)
Fruit shape	3 categories: Moderately elongated, medium, or moderately compressed.	
Peel color	7 categories: yellowish white, green, medium pink, dark pink, medium red, dark red, purple.	
Position of bracts towards peel	3 categories: addressed, slightly held out, strongly held out	
Number of bracts/fruit	Average number of bracts in 5 randomly fruits	Unit
Middle bract base width	Average number of 5 middle bracts in 5 randomly fruits	Millimeters (mm)
Middle bract color	4 categories: yellowish green, green, pink, red	
Longest apex bract length	Measure on the longest apex bract of each randomly fruit	Centimeters (cm)
Apical cavity form	3 categories: Shallow, medium, deep	
Pulp color	9 categories: White, dull white, light pink, medium pink, dark pink, medium red, dark red, purple, dark purple	

For indicating the flowering season, three plants for each clone were monitored to determine flowering time, total flowers/plant/year, the numbers of floral cycles/plant/year by basing on the flowers opening at the same day. Three to five randomly selected floral buds were labeled to identify time taken to reach floral and fruit stages.

The influences of pollination treatments were indicated at three parameters:

Fruit set rate (%) was calculated using the following formula:

$$= \frac{\text{Total number of fruitlets}}{\text{Total number of flowers}} \times 100$$

Fruit weight was measured with a electronic balance. Total soluble solid (TSS) content was measured by a manual refractometer (ATAGO, Tokyo, Japan) by squeezing from samples of the middle fresh-cut fruits and the result was expressed as ^{o}Brix.

C. Statistical Analysis

Morphological characteristics were described or measured by means for analyzing and comparing among clones.

Differences between the means in pollination treatments were ascertained with a multiple Duncan test [4], using Statistical SAS 9.0 software. The mean values for the parameters labeled with different letters are significantly different at $P \leq 0.05$ (*), $P \leq 0.01$ (**) and not significantly different $P > 0.05$ (ns).

III. RESULTS AND DISCUSSION

A. Morphological Stem Characteristics

For the identification of *Hylocereus* species, it was claimed that stem characteristics were the most important morphology [5], [9]. Our findings of 4 pitahaya genotypes were consistent with these previous conclusions. According to the stem descriptors in our study (Table II), the longer stems were recorded at VN White (111.8 ± 21.5cm) and F_{11} (120.6 ±

14.3cm) as compared to Chaozou 5 (80.6 ± 20.2cm) and Orejona (86.4 ± 25.9cm). The stem width also exhibited differences among four clones. VN White had the maximum value (54.3 ± 8.7mm), followed by Chaozou 5 and F_{11} with 52.6 ± 11.8 and 48.8 ± 3.5mm, respectively, and Orejona marked the minimum stem width (42.0 ± 7.2mm). VN White also recorded the longest distance between areoles (48.1 ± 8.4 mm) whereas the smallest distance value was measured in Orejona (28.8 ± 4.5mm). F_{11} and Chaozou 5 ranked second and third position with respective figures: 45.2 ± 1.8 and 42.9 ± 2.3mm. Regarding the spines, the number of spines per areole was found the maximum (4.5 ± 0.6) at VN White, the followed value (4.3 ± 0.5) at both Chaozou 5 and Orejona, the minimum (2.5 ± 0.6) at $F_{11.}$ The maximum spine length was of Chaozou 5 (4.4 ± 0.7mm), followed by F_{11} (4.2 ± 0.6mm), the same and minimum spine length degree was recorded at both VN White and Orejona (3.7± 0.7mm). Other morphological stem feature used for the distinction among clones is the form of rid margin. VN White and F_{11} belonged to the convex pattern whereas Chaozhou 5 was observation of the concave type and Orejona showed the flat form.

TABLE II
MORPHOLOGICAL CHARACTERISTICS OF THE STEM AMONG FOUR RED PITAYA CLONES (MEAN ± STANDARD DEVIATION)

Clone	Length (cm)	Width (mm)	Areole distance (mm)	Spine number/ areole	Spine length (mm)	Rid margin form
VN White	111.8 ± 21.5	54.3 ± 8.7	48.1 ± 8.4	4.5 ± 0.6	3.7± 0.7	convex
Chaozhou 5	80.6 ± 20.2	52.6 ± 11.8	42.9 ± 2.3	4.3 ± 0.5	4.4 ± 0.7	concave
Orejona	86.4 ± 25.9	42.0 ± 7.2	28.8 ± 4.5	4.3 ± 0.5	3.7 ± 0.7	flat
F_{11}	120.6 ± 14.3	48.8 ± 3.5	45.2 ± 1.8	2.5 ± 0.6	4.2 ± 0.6	convex

TABLE III
MORPHOLOGICAL CHARACTERISTICS OF THE FLOWER AMONG FOUR RED PITAYA CLONES (MEAN ± STANDARD DEVIATION)

Clone	Spherical button shape	Mature bud			Opening flower				
		Bud length (cm)	Pericarpel length (cm)	Pericarpel width (cm)	Sepal pattern	Petal color	Stigma lobe color	Stigma lobe number	Anthers below stigma (cm)
VN White	medium elliptical	28.6 ± 1.7	13.4 ± 2.0	3.2 ± 0.1	none	white	cream	28.7 ± 1.5	0.1 ± 0.3
Chaozhou 5	medium elliptical	29.7 ± 3.6	13.3 ± 1.2	3.2 ± 0.3	edged	white	cream	28.2 ± 1.6	1.1 ± 0.5
Orejona	medium elliptical	34.1 ± 1.7	14.5 ± 0.2	3.2 ± 0.3	edged	white	cream	21.7 ± 3.8	1.7 ± 0.5
F_{11}	medium elliptical	28.6 ± 1.6	12.1 ± 0.9	2.9 ± 0.1	edged	white	cream	28.0 ± 0.0	1.5 ± 0.4

B. Morphological Flower Characteristics

The flower characteristics, the followed appearances were used for distinguishing among clones [2], [16]. The 10-flower descriptors of 4 clones were described at Table III. Three similarities in all clones were confirmed, including the spherical button shape of the medium elliptical, the petal color of white, the stigma lobe color of cream. The differences, in part, among them were recorded at the dimension of mature bud and part structures of flowers. Orejona had the maximum mature bud size with 34.1 ± 1.7 cm long, 14.5 ± 0.2cm of pericarpel length and 3.2 ± 0.3cm of pericarpel width whereas these minimum values were measured at F_{11} with 28.6 ± 1.6cm, 12.1 ± 0.9cm, and 2.9 ± 0.1cm, respectively. The lowest number of stigma lobes was 21.7 ± 3.8 in Orejona, compared with 28.0 to 28.7 ± 1.5 in others. The flower structure relating to ability of pollen receptivity was the position of anthers and stigma. The upper part of the anthers below the stigma was of 1.7cm in Orejona, 1.5cm in F_{11} and 1.1cm in Chaozhou 5, whereas the anthers in VN-White was at the same height as the stigma. The different positions between two parts in Chaozhou 5, Orejona and F_{11} flowers was found to be similar to that of [20] who described that the upper part of the anthers in all the *Hylocereus* spp. were at least 2.0 cm below the stigma.

C. Morphological Fruit Characteristics

Fruit morphology, which is one of the taxonomic evidences, also exhibits the external quality of fruit. According to [3], the main differences among several *Hylocereus* species were the size and color of the fruit. Some fruit appearance traits were expressed at Table IV.

TABLE IV
MORPHOLOGICAL CHARACTERISTICS OF THE FRUIT AMONG FOUR RED PITAYA CLONES

Clone	Fruit shape	Peel color	Position of bracts towards peel	Number of bracts /fruit	Middle bract base width (mm)	Middle bract color	Longest apex bract length (cm)	Apical cavity form	Pulp color
VN White	moderately elongated	medium red	slightly or strongly held out	20.8 ± 1.5	3.5 ± 0.6	green	4.7± 2.4	deep	white
Chaozhou 5	moderately elongated or medium	dark red	slightly or strongly held out	27.0 ± 4.2	3.4 ± 0.5	yellowish - green	2.4 ± 2.2	medium or deep	dark purple
Orejona	moderately elongated	dark red	slightly held out	26.0 ± 4.4	2.4 ± 0.5	green	4.8 ± 0.1	shallow	dark red
F_{11}	Moderately elongated or medium	dark red	slightly held out	24.7 ± 7.1	3.3 ± 0.1	green	4.4 ± 0.8	deep	dark purple

The fruit shape of VN White and Orejona belonged to the moderately elongated whereas that of Chaozhou 5 and F_{11} was of the moderately elongated or medium. VN White exhibited medium red peel and white pulp as its name called and Orejona performed dark red color in both its peel and pulp. However, a similar color with dark red peel and dark purple pulp was observed in both Chaozhou 5 and F_{11}. The position of bracts towards peel of VN White and Chaozhou 5 was slightly or strongly held out whereas that of Orejona and F_{11} was only slightly held out. The number of bracts per fruit ranged from 20.8 ± 1.5 in VN White to 27.0 ± 4.2 in Chaozhou 5. The middle bract base width presented from 2.4 ± 0.5cm in Orejona to 3.5 ± 0.6cm in VN White. With the exception of the Chaozhou 5 having yellowish-green middle bracts, other clones kept their middle bracts green when fruit ripening. The variation of the longest apex bract length was from 2.4 ± 2.2 cm in Chaozhou 5 to 4.8 ± 0.1cm in Orejona. The apical cavity of fruits was classified in to 3 groups, the deep in VN-White and F_{11}, the medium or deep in Chaozhou 5 and the shallow in Orejona.

D. Flowering Phenology

Dragon fruit belonged to the long day plant with natural flowering and production during warmer months [10], [11]. In Israel flowering in the *Hylocereus* spp. occurred mainly during summer and early autumn (May to October) [20]. In our study condition, the floral season started the beginning of May in Chaozhou 5, Orejona and F_{11}, the middle of May in VN White and finished at the beginning of August in F_{11}, the middle of August in VN White, the end of August in Orejona and the beginning-middle of September in Chaozhou 5 (Table V).

Flowering occurred in 6 cycles in VN White, Orejona, F_{11} and 7 cycles in Chaozhou 5 with 22, 26, 32 and 29 flowers per plant per year, respectively. The flower duration took 15 days in VN White, 18 -19 days in others wheareas the fruit ripening time ranged between 30 days in Chaozhou 5 and 32 days in Orejona and F_{11}.

TABLE V
FLOWERING PERIOD, FLOWER CYCLES AND NUMBERS PER PLANT, TIME OF FLOWER AND FRUIT STAGES AMONG FOUR RED PITAYA CLONES

Clone	Flowering season	Flower cycles/ year	Flowers / plant/ year	Flower duration (day)	Fruit duration (day)
VN White	14/5-15/8	6	22	15	31
Chaozhou 5	30/4-9/9	7	29	18	30
Orejona	2/5-31/8	6	26	18	32
F_{11}	30/4-5/8	6	32	19	32

E. Effects of Pollination Methods

Fruit set (FS), fruit weight (FW) and total soluble solid (TSS) content of four pitaya clones were differently influenced by pollination types (Table VI). As can be seen from the data, VN- was fully self-compatible, obtained high and similar percentages of fruit set (80.0 – 95.2%) in all pollination treatments while Chaozhou 5 was partial self-compatible due to no fruit formation in automatic self-pollination, a lower fruit set rate (52.2%) in hand self-pollination than open- and hand cross-pollination with 71.1 and 95.8 %, respectively. On the contrary to two clones above, Orejona and F_{11} revealed completely self-incompatibility, only setting fruit by hand cross-pollination (88.4 and 90.2%), by open-pollination (61.1 and 90.7%), respectively.

For fully self-compatible clone VN-White, the heaviest fruits were obtained from hand self- and open-pollinations with 402.6 and 403.4g of weight, respectively whereas automatic self-pollination produced the lightest fruit and hand cross-pollination resulted in the middle values. In contrast, other three partially or completely self-incompatible clones revealed significantly higher fruit weight in hand cross-pollination than that in open-pollination. These figures ranged from 281.8–416.3g, compared to 145.0–295.8g, respectively.

Except for VN-White clone, hand cross-pollination led to higher fruit set rates and heavier fruit weight than open pollination in other clones. Our findings are in agreement with those of [20], who reported that self-incompatible species obtained the highest fruit set and fruit weight by cross-pollination. However, the dissimilar results were found in self-compatible species *H. undatus* which the earlier study indicated that much lower rates of fruit set resulted from open pollination (12.5–43.2%) and automatic self-pollination (0%). These dissimilar results may be attributable to the shorter distance of the anther below stigma (0.1cm) in our study, compared to at least 2cm in [20] studies, indicating that the stigma had a better ability of pollen receptivity in VN White. The anthers was at the same height as the stigma, touching it when the flowers closed, which also explain about the clone VN-White belonging to the most culltivated species *H. undatus* performed completely autogamous as the same as reports of [10] and [19].

TABLE VI
EFFECTS OF POLLINATION METHODS ON FRUIT SET (FS), FRUIT WEIGHT (FW) AND TOTAL SOLUBLE SOLID (TSS) CONTENT IN FOUR RED PITAYA CLONES [1]

Clone	Parameter	Hand cross-	Hand self-	Auto. self-	Open-	Sig.
	FS (%)	85.6	95.2	80.0	90.5	ns
VN White	FW(g)	326.1 [ab]	402.6 [a]	275.1 [b]	403.4 [a]	**
	TSS (°brix)	18.8 [ab]	18.2b [b]	19.4a [a]	18.2 [b]	*
	FS (%)	95.8 [a]	52.2 [c]	0.0	71.1 [b]	*
Chaozhou 5	FW(g)	374.2 [a]	251.4 [b]	-	295.8 [b]	*
	TSS (°brix)	16.7 [b]	18.4a [a]	-	18.7 [a]	*
	FS (%)	88.4 [a]	0.0	0.0	61.1 [b]	*
Orejona	FW(g)	281.8 [a]	-	-	149.2 [b]	*
	TSS (°brix)	17.5	-	-	17.0	ns
	FS (%)	90.2	0.0	0.0	91.7	ns
F_{11}	FW(g)	416.3 [a]	-	-	145.0 [b]	**
	TSS (°brix)	17.1	-	-	17.7	ns

1. Means with different letters in row are statistically different at significance (Sig.) of levels: $P < 0.05$ (*), $P < 0.01$ (**) respectively, according to Duncan‘s multiple range test

There was not significantly difference in TSS content between hand cross- and open-pollination in Orejona (17.5 and 17.0°Brix) and F_{11} (17.1 and 17.7 °Brix), respectively. However, other clones tended to increase TSS content in smaller fruits. VN-White fruits contained 19.4 °Brix in auto self-polliantion compared with 18.2 °Brix in both hand self- and open pollination whereas Chaozhou 5 fruits obtained higher values which accounted for 18.4 °Brix in hand self- and 18.7 °Brix in open-pollination, than in cross-pollination (16.7 °Brix).

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