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Effects of different pollinizer cultivars and pollination times on the fruit set and quality of pitaya cv. 'Bloody Mary' (*Hylocereus polyrhizus*)

GIZEM DEMIRKAPLAN[1], HAMIDE GUBBUK[2]* and RECEP BALKIC[3]

Akdeniz University, Antalya 07059, Turkey

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ABSTRACT

Pitaya (*Hylocereus polyrhizus* Britton and Rose) is a valuable crop due to its growing global demand, unique flavour, nutritional benefits, and potential for commercial production. An adaptation study of the 'Bloody Mary' pitaya cultivar conducted in subtropical regions confirmed its high adaptability. However, as a sterile cultivar, it requires cross-pollination for optimal fruit set and quality. The study was carried out during 2019 and 2020 at Akdeniz University, Antalya, Turkey to identify the most effective pollinizer cultivars and optimal pollination times for the 'Bloody Mary' cultivar of pitaya. The pollinizer cultivars evaluated were 'Cosmic Charlie', 'Red Jaina', and 'White Jaina', with pollination carried out at three times, 22:00 hrs, 24:00 hrs, and 06:00 hrs. Data on fruit set and key quality parameters were evaluated. The results showed that both fruit set rate and quality characteristics varied depending on the pollinizer cultivars and pollination times. Fruit peel chroma (C*) and fresh chroma (C*) were significantly affected by the pollinizer cultivars, while the fruit peel hue angle (h°) remained unaffected. 'Red Jaina' and 'White Jaina' were the most effective pollinizer cultivars for 'Bloody Mary', with optimal pollination times for fruit set at 22:00 h and 24:00 h. However, pollination times did not influence several quality parameters.

Keywords: Fruit set, Fruit quality, Interspecies pollination, Pitaya

Pitaya (*Hylocereus polyrhizus* Britton and Rose) can be grown including tropical, semi-tropical and sub-tropical regions. In tropical and semi-tropical conditions, pitaya is generally grown in the open field while in subtropical conditions, it is grown under cover or under the net with microclimate characteristics (Mizrahi *et al.* 2002). The first scientific adaptation study in Türkiye was carried out with 'Bloody Mary' and 'Cosmic Charlie' cultivars (Gubbuk *et al.* 2017) in plastic greenhouse. 'Bloody Mary' adapted better to subtropical conditions than 'Cosmic Charlie'. While 'Bloody Mary' is self-sterile cultivar, its commercial cultivation can be improved by planting it with pollinizer cultivars to overcome its self-sterility.

Cultural practices and understanding reproductive biology are crucial for improving yield and fruit quality in commercial pitaya cultivation. The main commercial pitaya genotypes include *Hylocereus undatus*, *Hylocereus polyrhizus*, *Hylocereus megalantus*, and *Hylocereus costaricensis*, along with their hybrids (Weiss *et al*. 1994, Tel-Zur *et al.* 2004). Pitaya cultivars are classified as self-fertile, partially self-fertile, or self-sterile. According to Crane and Baledi (2005), all pitaya species and cultivars can cross-pollinate with each other.

Pitaya flowers are large, hermaphroditic, nocturnal, and visually striking (Kakade *et al.* 2022). Blooming times and flower openness durations vary among studies. Kakade *et al.* (2022) observed that flowers begin to open between 6:30 and 7:30 pm, reaching full bloom by 10:00 pm, and start closing around 2:00 am. Zee *et al.* (2004) noted that flowers typically open between 6:40 and 7:00 pm with full bloom by 10:00 pm, and wilting begin at 2:00 am. On warm, cloudy days, flowers may open as early as 4:00 pm, while cooler temperatures can delay wilting until 10:00 am.

Dhiman *et al.* (2024) emphasized that key processes such as flower opening, anther dehiscence (pollen release), and pollination occur at night. Pollen is released between 4 pm and 2 am, and the stigma remains receptive from 8 pm to 7 am. Weiss *et al.* (1994) found that anther dehiscence starts 3–4 h before flower opening, around 10 pm, and lasts until 2 am, while the stigma remains receptive for 11–13 h, from 8 pm to 7 am. Muniz *et al.* (2020) reported that the stigma remains receptive from 8:00 PM. until the flower closes at 7:00 am or until 9:00 am on cloudy days. Li *et al.* (2022) noted that pitaya blooms from 8:00 pm to 8:00 am while precise pollination timing is essential for optimal fruit set, the pollen activity and stigma receptivity

[1]Directorate of the Fruit Growing Research Institute, Egirdir, Isparta, Turkey; [2]Akdeniz University, Antalya, Turkey; [3]Elmali Vocational School, Akdeniz University, Elmali, Antalya, Turkey. *Corresponding author email: gubbuk@akdeniz.edu.tr

remain underexplored. Paul (2014) stated that bees and insects pollinate in the morning, while bats, moths, and large butterflies pollinate at night in the plant's native habitat.

Pitaya flowers are hermaphroditic, but some species and varieties are self-incompatible. Weiss *et al.* (1994) found that *H. undatus* and *H. polyrhizus* exhibit partial or total self-incompatibility, requiring hand pollination for commercial yields. Due to self-sterility, cross-pollination is necessary, and hand pollination ensures a 100% fruit set (Weiss *et al.* 1994, Kakade *et al.* 2022). Metz *et al*. (2000) recommended planting at least two different varieties in commercial orchards to enhance fruit set and size. Gunasena *et al*. (2007) also advised using multiple varieties and hand pollination to improve fruit set in pitaya orchards.

In pitaya pollination, both cultivar diversity and timing of pollen collection are crucial. Muniz *et al*. (2020) noted limited studies on pitaya pollination. Weiss *et al.* (1994) found *H. undatus* is self-compatible but only achieves 50–80% fruit set, while *H. polyrhizus* is self-incompatible and requires cross-pollination for high fruit set. Poor pollen viability in some *Hylocereus* spp. limits fertilization and fruit set (Dhiman *et al.* 2024). Lichtenzveig *et al.* (2000) reported *H. polyrhizus* pollen viability is typically high (up to 90%), but *H. megalanthus* has only 40%. Li *et al.* (2022) found the highest pollen germination (27.2–65.1%) occurs 2 h before to 6 h after blooming, peaking 2 h post-bloom. Storing pollen at 4°C for 24 h reduced germination from 65.2–35.5%. Tran *et al.* (2015) and Dag and Mizrahi (2005) showed that cross-pollination results in larger fruits and more viable seeds compared to self-pollination.

Pitaya cultivation faces challenges like self-incompatibility and self-sterility, leading to reduced fruit set when plants cannot effectively pollinate themselves or require pollen from other plants. Therefore, this study's objective was to identify the most suitable pollinizer cultivars and pollination times to enhance fruit set in the self-sterile 'Bloody Mary' pitaya cultivar.

MATERIALS AND METHODS

The study was carried out during 2019 and 2020 at Akdeniz University, Antalya, Turkey. Two years-old plants in 50 L pots (4 plants for each pot) containing a mixture of peat: perlite: pumice: soil in a ratio of 1:1:1:2 was used. Pot system was used as training. Spaghetti system (2 L/h) was used for irrigation and 2 spaghettis were placed in each pot. The irrigation regime was adjusted to 3 times a week in summer, 2 times a week in spring, and once a week in winter by considering the air temperature. During the season, 540 g N, 310 g P, 250 g K fertilizer/plot was applied (Chakma *et al*. 2014).

The self-sterile 'Bloody Mary' (*Hylocereus polyrhizus*) was used as mother cultivar and Cosmic Charlie (*Hylocereus undatus*), White Jaina (*Hylocereus undatus*) and Red Jaina (*Hylocereus polyrhizus*) cultivars were used as pollinizers.

Pollination was carried out at 3 different times, viz. 22:00 h, 24:00 h and 06:00 h in the morning. Pollen was collected from the pollinizer cultivars immediately before pollination and applied to the stigma of the mother plant flower using a brush.

Fruit set (number of fruits/plant), fruit weight (g), fruit width (mm), and fruit length (mm) were measured using a precision balance and digital calipers. The number of seeds/plant was also recorded. Fruit peel thickness (mm) was measured with a digital caliper. Fruit firmness (kg/cm²) was assessed at three different points using a penetrometer (FT 011; Effegi-Italy) with a 7.9 mm diameter tip. Total soluble solids (TSS) content (%) was determined using a digital refractometer (HI96801, Hanna, Rhode Island, USA). The colour of the fruit peel and flesh was measured at three different points using a Minolta Chroma Meter CR-400 (Minolta Camera Co., Ramsey, NJ). The obtained values were expressed in terms of L*, a*, and b* values, and the chroma (C*) and hue angle ($h°$) values were calculated according to McGuire (1992).

Statistical analysis: The experiment was designed using a randomized factorial design with 3 replications, incorporating 3 pollinizer cultivars and 3 pollination times. In the pollination experiment, 10 flowers were used per each replicate, and for fruit analyses, 5 fruits were used per each replicate. Pairwise comparisons were conducted using Tukey's HSD (honestly significant difference) test to assess statistical significance. In addition, principal component analysis (PCA) was used to better visualize the relationships among the variables. The data were evaluated with three replications, and the PCA analysis was performed using the XLSTAT (NY, USA) software (Balkic *et al.* 2024)

RESULTS AND DISCUSSION

The effects of pollinizer cultivars on fruit set rate were found to be statistically important. The fruit set rate in 'Red Jaina' and 'White Jaina' 'Cosmic Charlie' is presented in Table 1. Considering the pollination times, the highest fruit set rate (95%) was determined at 24.00 h while the lowest fruit set rate (69.84%) was recorded at 22:00 h (Table 1). The impact of pollinizer cultivars and pollination times on fruit characteristics is given in Table 1. Fruit set was highest (100%) with 'White Jaina' and 'Red Jaina' as pollinizers for 'Bloody Mary,' compared to 42.62% with 'Cosmic Charlie.' Pollination timing also influenced fruit set, with the best results at 22:00 and 24:00 h. At 06:00 h, the stigma lost receptivity, reducing fruit set, aligning with Li *et al.* (2022). Fruit development took 30 days. 'Red Jaina' had the heaviest fruit (517.73 g), followed by 'White Jaina' (512.53 g) while fruit weight varied from 482.60 g to 513.28 g based on pollination time (Table 1). The largest fruit diameter (82.57 mm) was found in 'Red Jaina' with no significant effect from pollination time. Fruit length ranged from 123.68–128.15 mm, with 'White Jaina' producing the longest fruit and 'Cosmic Charlie' the shortest. Average fruit weight was 405–601 g, with pollinizer cultivar being the primary factor, consistent with previous studies (Weiss *et al.* 1994, Tran *et al.* 2015). The highest seed count (449.33) was in 'Cosmic Charlie,' while the lowest (334.33) was in 'White Jaina' (Table 1). The most seeds (445.33) were

Table 1 Effect of pollinizer cultivars and pollination times on various fruit physical characteristics

Treatments		FS (%)	FW (g)	FD (mm)	FL (mm)	SN
CC		42.62 b	458.62 b	75.50 b	125.08	449.33 a
RJ		100.00 a	517.73 a	82.57 a	128.15	445.00 a
WJ		100.00 a	512.53 a	79.06 ab	123.68	334.33 b
SLC		**	**	*	ns	**
22:00		69.84 c	493.01 b	78.16	127.35	361.67 b
24:00		95.00 a	513.28 a	79.52	125.31	445.33 a
06:00		77.78 b	482.60 b	79.73	124.24	421.67 a
SLPT		**	**	ns	ns	**
CC	22:00	9.53 d	405.42 e	70.64 c	115.21 c	390.00 bc
	24:00	85.00 b	500.91 cd	78.40 bc	132.18 ab	485.00 a
	06:00	33.33 c	469.56 d	77.45bc	127.86 abc	473.00 abc
RJ	22:00	100.00 a	601.71 a	86.87 a	136.72 a	385.00 cd
	24:00	100.00 a	520.57 bc	82.31 ab	129.86 ab	482.00 ab
	06:00	100.00 a	430.92 e	78.53 abc	117.86 bc	468.00 abc
WJ	22:00	100.00 a	471.90 d	76.98 bc	130.14 ab	310.00 d
	24:00	100.00 a	518.37 bc	77.87 abc	113.89 c	369.00 d
	06:00	100.00 a	547.31 b	82.32 ab	127.00 abc	324.00 d
SL C×PT		**	**	*	**	*

CC, Cosmic Charlie; RJ, Red Jaina; WJ, White Jaina; FPT, Fruit peel thickness; FF, Fruit firmness; SSC, Soluble solid content; SLC, Significance level of cultivar; SLPT, Significance level of pollination time; FS, Fruit set; FW, Fruit weight; FD, Fruit diameter; FL, Fruit length; SN, Seed count. Means with different letters in some columns were significantly different; *, ($p<0.05$); **, ($p<0.001$); ns, Non-significant.

recorded at 24:00 and 06:00 h, with the lowest (361.67) at 22:00 h, supporting the findings of Mizrahi and Nerd (1999).

Table 2 presents the effects of pollinizer cultivars and pollination times on fruit physical and chemical properties. The interaction between these factors significantly influenced certain traits. The thickest fruit peel (2.98 mm) was observed in 'Red Jaina,' while the thinnest (2.24 mm) was found in 'White Jaina.' Peel thickness varied from 2.40–2.77 mm depending on pollination time. The highest fruit firmness (3.43 kg/cm²) was observed with 'Cosmic Charlie,' while the lowest (2.57 kg/cm²) was found with 'White Jaina.' Pollination time had no significant effect on fruit firmness. Thinner peels are preferred for fresh consumption, while thicker peels are ideal for storage and presentation. Fruit firmness, which affects shelf life, was highest with 'Cosmic Charlie,' with no significant difference between 'Red Jaina' and 'White Jaina'. Gunasena *et al.* (2007) observed that reduced firmness can shorten shelf life. 'Red Jaina' had the highest soluble solids content (SSC) at 15.76%, followed by 'White Jaina' at 15.09%. The highest SSC (15.39%) was recorded at 24:00 and 22:00 h, while the lowest (14.47%) occurred at 06:00 h. Merten (2003) reported an acceptable SSC range of 12–13%, and Jiang *et al.* (2015) found SSC levels of 17–18% in red-fleshed cultivars in Vietnam. Our results surpassed these values.

Table 2 Effects of pollinizer cultivars and pollination times on some fruit physical and chemical characteristics

Treatments		FPT (mm)	FF (kg/cm^2)	SSC (%)
CC		2.59 c	3.43 a	13.85 b
RJ		2.98 a	2.63 b	15.76 a
WJ		2.24 b	2.57 b	15.09 a
SLC		*	**	*
22:00		2.40 b	2.98	14.83 b
24:00		2.77 a	2.88	15.39 a
06:00		2.68 a	2.76	14.47 b
SLPT		*	ns	*
CC	22:00	2.28 de	3.43 a	13.46 d
	24:00	2.71 c	3.45 a	15.36 b
	06:00	2.79 bc	3.41 a	12.73 d
RJ	22:00	2.83 bc	2.72 b	17.14 a
	24:00	3.13 a	2.76 b	15.35 b
	06:00	2.98 ab	2.42 c	14.80 bc
WJ	22:00	2.08 e	2.80 b	13.90 cd
	24:00	2.46 d	2.43 c	15.48 b
	06:00	2.27 de	2.47 c	15.90 ab
SL C×PT		*	*	*

CC, Cosmic Charlie; RJ, Red Jaina; WJ, White Jaina; FPT, Fruit peel thickness; FF, Fruit firmness; SSC, Soluble solid content; SLC, Significance level of cultivar; SLPT, Significance level of pollination time. Means with different letters in some columns were significantly different; *, ($p<0.05$); **, ($p<0.001$); ns, Non-significant.

Table 3 shows the fruit peel and flesh chroma (C*) and hue (h°) values. The interaction between pollinizer cultivars and pollination time had no significant effect on the hue and chroma of the fruit peel, but it did impact the fruit flesh. The highest fruit peel C* values were observed in 'Cosmic Charlie' and 'Red Jaina,' ranging from 41.38 to 44.09 depending on pollination time. The darkest fruit peel colour (highest h° value of 14.84°) was recorded at 24:00 and 22:00 h. The highest C* value for fruit flesh (4.24) was observed in 'Cosmic Charlie,' while the lowest (3.79) was found in 'White Jaina.' Fruit flesh C* values ranged from 3.86–4.04, depending on pollination time. The highest value for fruit flesh was 10.83 for 'White Jaina,' followed by 10.75 for 'Red Jaina,' with values varying between 9.20 and 9.40 based on pollination time. No previous studies have reported the impact of pollinizer cultivars on the C* and h° values of pitaya peel and flesh. Our findings indicate

Table 3 Effects of pollinizer cultivars and pollination times on fruit peel and flesh colour

Treatment		FPC (C*)	FPH (h°)	FFC (C*)	FFH (h°)
CC		43.41 a	13.91	4.24 a	6.35 b
RJ		43.31 a	14.53	3.90 b	10.75 a
WJ		40.78 b	14.52	3.79 b	10.83 a
SLC		*	ns	**	**
22:00		42.02 b	14.73 ab	4.03	9.20
24:00		41.38 b	14.84 a	4.04	9.40
06:00		44.09 a	13.33 b	3.86	9.33
SLPT		*	*	ns	ns
CC	22:00	43.13	14.96	4.52 a	3.10 e
	24:00	40.70	15.19	4.35 ab	5.17 d
	06:00	46.41	11.59	3.85 d	10.79 ab
RJ	22:00	42.72	14.60	3.70 cd	12.27 a
	24:00	42.87	14.91	3.85 bcd	12.20 a
	06:00	44.34	14.08	4.17 abc	7.77 c
WJ	22:00	40.22	14.64	3.88 bcd	12.23 a
	24:00	40.60	14.59	3.94 bcd	10.82 ab
	06:00	41.55	14.33	3.56 d	9.44 b
SL C×PT		ns	ns	*	**

CC, Cosmic Charlie; RJ, Red Jaina; WJ, White Jaina; FPC, Fruit Peel Chroma; FPH, Fruit peel hue angel; FFC, Fruit flesh chroma; FFH, Fruit flesh hue angel; SLC, Significance level of cultivar; SLPT, Significance level of pollination time.

Means with different letters in some columns were significantly different; *, (p<0.05); **, (p<0.001); ns, non-significant.

that the colour of the fruit peel is primarily influenced by the mother cultivar, while the pollinizer cultivar has a more significant effect on the fruit flesh color. Martinelli *et al.* (2021) observed different C* and h° values for pitaya in *Hylocereus polyrhizus*, but our results differ, likely due to variations in cultivars and environmental conditions.

Principle component analysis (PCA) analysis was used to show the relationships between examined features and the treatments. The results showed the relationships between all treatments (pollinizer cultivars and pollination times) and the examined features are shown in Fig. 1. As a result of the PCA analysis, the first two components (PC1 and PC2) explained a total of 67.57% of the variance (Fig. 1). Positive correlations were found between fruit set and fruit weight, fruit diameter, fruit length, and soluble solid content. Our findings, which affect yield, align with the results reported by Oliveira *et al.* (2024), Morillo-Coronado *et al*. (2021). On the other hand, a negative correlation was observed between the C* and h° values in both fruit peel and flesh. Pollination of Bloody Mary with 'Red Jaina' and 'White Jaina' had a more pronounced impact on the examined criteria than pollination with 'Cosmic Charlie'. Additionally, fruit set, fruit weight, fruit width, and fruit length, all of which directly influence yield, showed a more pronounced response to pollination at 22:00 and 24:00 h.

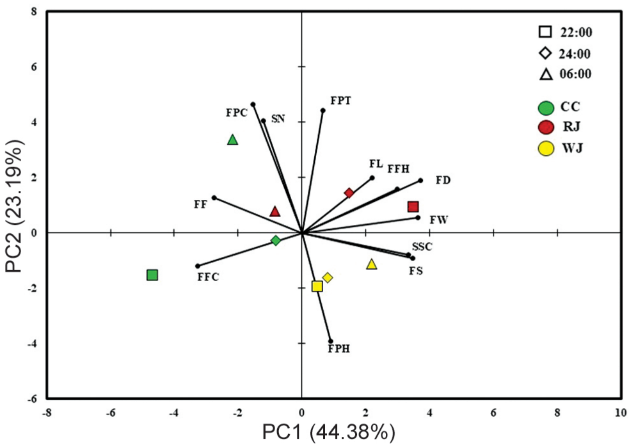

Fig. 1 Biplot graph by fruit set (FS), fruit weight (FW), fruit diameter (FD), fruit length (FL), seed number per fruit (SN), fruit peel thickness (FPT), Fruit Firmness (FF), soluble solid content (SSC), fruit peel chroma (FFC), fruit peel hue angel (FPH), fruit flesh chroma (FFC), fruit flesh hue angel (FFH) according to pollinizer cultivars × pollination times.

In conclusion, the study showed that both pollinizer cultivars and pollination times significantly impact fruit set and quality in 'Bloody Mary' pitaya. 'Red Jaina' and 'White Jaina' were the most effective pollinizers, with the best pollination times at 22:00 and 24:00 h. While pollination times had little effect on quality parameters, the choice of pollinizer notably influenced the chroma values of both fruit peel and flesh. These findings highlight the importance of selecting suitable pollinizers and timing pollination for optimal fruit production in 'Bloody Mary' pitaya. As a result, we recommend selecting the right pollinizer cultivars and optimizing pollination timing to maximize fruit yield and quality in pitaya cultivation.

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