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Research Article
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The effect of interspecific and intraspecific hybridization on seed germination of Pitaya (*Hylocereus* spp.)*

Pitayada (*Hylocereus* spp.) türler arası ve tür içi melezlemenin tohum çimlenmesi üzerine etkisi

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ABSTRACT

Objective: In this study, the effect of interspecific and intraspecific hybridization on seed germination of pitaya was investigated.

Material and Methods: Self-infertile 'Bloody Mary' (*Hylocereus polyrhizus*) was used as the maternal parent and 'Cosmic Charlie' (*Hylocereus undatus*), 'Red Jaina' (*Hylocereus polyrhizus*) and 'White Jaina' (*Hylocereus undatus*) were used as pollinators in this study. Flowers were pollinated at three different times (10: 00 pm, 00: 00 am, and 06.00 am). After pollination, seeds from each pollination combination were sown. The germination rate, average germination time, germination energy and germination index of the sown seeds were determined depending on pollination time and pollinator cultivars.

Results: The research findings indicated that the germination rate was 100% in all seed sowing combinations, depending on different pollinator cultivars ('Cosmic Charlie' and 'White Jaina' are in different species from the maternal parent) and pollination times. The results revealed that interspecific hybridization did not have a negative effect on seed germination. On the other hand, germination time, germination energy and index differed as per pollinator type and pollination hours.

Conclusion: According to the findings, it was concluded that interspecific hybridization in pitaya does not have problem in seed germination and results might offer an infrastructure for future breeding programme in pitaya.

ÖZ

Amaç: Bu araştırmada, türler arası ve tür içi melezlemenin pitayada tohum çimlenmesi üzerine etkisi araştırılmıştır

Materyal ve Yöntem: Araştırmada ana çeşit olarak kendine kısır bir çeşit olan 'Bloody Mary' (*Hylocereus polyrhizus*) ve tozlayıcı çeşit olarak ise 'Cosmic Charlie' (*Hylocereus undatus*), 'Red Jaina' (*Hylocereus polyrhizus*) ve 'White Jaina' (*Hylocereus undatus*) çeşitleri kullanılmıştır. Çiçeklerde tozlama işlemi üç farklı zamanda yapılmıştır (saat 22: 00, 00: 00 ve 06: 00). Tozlanmadan sonra, her bir tozlama kombinasyonundan gelişen meyvelere ait tohumlar ekilmiştir. Daha sonraki aşamada, ekilen tohumlarda çimlenme oranı, ortalama çimlenme süresi, çimlenme enerjisi ve çimlenme indeksi, tozlama zamanı ve tozlayıcı çeşitlere bağlı olarak belirlenmiştir.

Araştırma Bulguları: Araştırma bulguları, farklı tozlayıcı çeşit ('Cosmic Charlie' ve 'White Jaina' ana çeşit ile farklı tür içerisinde yer almaktadır) ve tozlama saatlerine bağlı olarak, tohum ekimi yapılan tüm kombinasyonlarda, çimlenme oranının %100 olarak gerçekleştiğini göstermiştir. Bu sonuç, türler arası melezlemenin tohum çimlenmesi açısından bir sorun yaratmadığını göstermektedir. Buna karşılık, çimlenme süresi, çimlenme enerjisi ve indeksi, tozlayıcı çeşit ve tozlama saatlerine göre farklılık göstermiştir.

Sonuç: Bu bulgular ışığında, pitayada türler arası melezlemenin tohum çimlenmesi açısından bir sorun yaratmadığı ve elde edilen sonuçların gelecekte pitayada yapılacak melezleme ıslahı çalışmalarına bir alt yapı oluşturacağı sonucuna varılmıştır.

INTRODUCTION

Pitaya is one of the climbing cactus species, that first spread to this world as an ornamental plant, and then, its fruit began to be consumed as a fresh fruit (Gunasena et al., 2007). Today, it is used for many different purposes in the food industry. Pitaya fruits are source of essential micro-and macro-nutrients and contain higher level of essential minerals and vitamins (Stintzing & Carle, 2007; Wichienchot et al., 2010). *Hylocereus polyrhizus* contains more vitamin-C than *Hylocerus undatus*. Pitaya belongs to the family Cactaceae, under the order Caryophyllales. Pitaya has many subspecies and the most preferred subspecies widely distributed in the world are: *Hylocereus polyrhizus* (*H. monacanthus*), *Hylocereus undatus*, and *Selenicereus megalanthus* (*Hylocereus megalanthus*) (Mizrahi & Nerd, 1999). Vietnam, Thailand, Taiwan, Malaysia, and Israel are among the countries that produce pitaya commercially. Pitaya has been grown under protected cultivation in Turkey. Presently, protected cultivation of pitaya has been increasing in microclimate areas in the coastal part from Izmir to Hatay. The first adaptation studies of pitaya in Turkey started with Akdeniz University, Batı Akdeniz Research Institute (BATEM) and Antalya Provincial Directorate of Agriculture and Forestry collaboration (Gubbuk et al., 2017). This adaptation studies started with the cultivars 'Bloody Mary' and 'Cosmic Charlie'. Recently, the number of cultivars has increased rapidly. However, 'Bloody Mary' is still very adaptable cultivar in Turkey.

Pitaya can be propagated by two different methods, by seed and vegetative (Gunasena et al., 2007). Mostly, one of the vegetative propagation methods, stem cuttings, is preferred for commercial production (Le Bellec et al., 2006). Propagation of pitaya by seed is important for conventional breeding and conservation of plant genetic resources (Le Bellec et al., 2006; Ortiz et al., 2018). Germination of pitaya is affected by internal and external factors such as seed maturity degree, temperature, light, relative humidity, storage, substrate, pH level of the substrate, etc. (Rojas-Arechiga & Vázquez-Yanes, 2000; Ortiz et al., 2018; Fukuda et al., 2008). the effect of several conditions of seed germination of pitaya (*Hylocereus undatus*) was investigated (Fukuda et al., 2008). The highest germination rate was recorded with the seeds collected after 25 days of pollination. Optimum temperature range for the highest germination was from 20 to 35°C. The germinability of seeds was decreased when the seeds were stored 20°C. Lone et al. (2014), were examined the effects of different temperatures on seed germination of pitaya genotypes (*Hylocereus undatus*, *H. polyrhizus*, *Selenicereus megalanthus*, *H. undatus* x *H. costaricensis* and *H. costaricensis* x *H. undatus*). According to the experimental results, germination temperature of seeds varied as per genotypes. Ortiz et al. (2018), investigated the effects of different pH levels of seed germination of *Hyloceresus* spp. The results showed that the *Hylocereus* spp. seeds are sensitive to pH changes of the substrate. The optimum pH range was reported between 6.0 and 7.5 for the pitaya seed germination. Zerpa-Catanho et al. (2019), searched the effects of various conditions on seed germination of *Hylocereus* spp. The results indicated that seed germination increased with temperatures between 20 and 30°C. In addition, there is a positive effect under red, blue, and white led lighting on seed germination, but seed germination was affected negatively under 15°C and dark conditions.

There are both self-pollinated and self-infertile cultivars in pitaya. For example, 'Bloody Mary' is a self-infertile cultivar and it needs cross-pollination. For this reason, determination of the most suitable pollinator cultivar/cultivars in terms of yield and quality in self-infertile cultivars has a great importance in commercial cultivation of pitaya. High pollen content is important to select pollinator cultivars. In addition, some important vegetative characteristics, such as rapid growth and development, and resistance to biotic and abiotic stress of self-infertile cultivars can be used in breeding studies. Thus, determination of the effect of choosing the paternal cultivars from the same species or different subspecies on seed germination in the selection of pollinator cultivars is most important in pitaya breeding. Pitaya flowers open at 8: 00 pm, and they can remain open until the early hours of the morning. Pollen viability is very

important for hybridization (Kazaz et al., 2020). Furthermore, intraspecific and interspecific hybridization also affect pollination. Because there are morphological differences between pitaya species. Interspecific and intraspecific hybridization can result in a higher genetic variability. Crane & Baleri (2005) reported that all species and cultivars of pitaya can be pollinated with each other. However, there are not any studies on the seed germination of fruits formed because of pollination in self-infertile cultivars, at different pollination hours and with cultivars belonging to different subspecies of pitaya. The germination status of the seeds formed in the fruits due to pollination performed at different times with different subspecies and cultivars belonging to the same subspecies with the self-infertile 'Bloody Mary' cultivar were investigated. For this reason, this study aimed to form a basis for future pitaya breeding studies.

MATERIALS and METHOD

The research was carried out in a plastic greenhouse having polyethylene curtains, belonging to the Department of Horticulture, Faculty of Agriculture, Akdeniz University, between 2019 and 2020. The research was performed in 50 L pots. A mixture of peat: perlite: pumice: soil at a ratio of 1: 1: 1: 2 was used as the medium in the pots. In the study where the steering training system was used, and 4 rooted plants were planted in each pot. A spaghetti tubing irrigation system was used, and 2 pieces of spaghetti tubing were placed in each pot. Considering the weather temperature, the irrigation regime was adjusted as 3 times a week in summer, 2 times a week in spring, and once a week in winter. Annually, 240 g phosphorus, potassium weighted, and 400 g balanced fertilizer was given per pot.

There are two registered cultivar (Bloody Mary and Cosmic Charlie) pitaya in Turkey. ‘Bloody Mary’ was selected as a maternal plant due to the more adaptable cultivar in subtropical condition. Pollinator cultivars (‘Cosmic Charlie’, ‘White Jaina’ and ‘Red Jaina’ were selected taking into consideration fruit color and species. In the research, 'Bloody Mary' was used as the maternal parent, and ‘Cosmic Charlie’, ‘White Jaina’ and ‘Red Jaina’ cultivars were used as pollinators. The characteristics of the cultivars are given below:

‘Bloody Mary’ (*Hylocereus polyrhizus*): The peel of the fruit color is red, and the external part of the fruit is covered with red-green scales. Fruit weight generally differs between 230 and 340 g (Pine Island Nursery Catalog, 2020).

‘Cosmic Charlie’ (*Hylocereus undatus*): Fruit peel color is pinkish, and external part is covered with red and green scales. Fruit flesh is light pink color. It is a self-fertile cultivar. Its fruit weight varies between 340 and 560 g (Pine Island Nursery Catalog, 2020).

‘White Jaina’ (*Hylocereus undatus*): Fruit peel color is pinkish, and external part is covered with red and green scales. ‘White Jaina's’ fruit flesh is white, and it is a self-fertile cultivar (Pine Island Nursery Catalog, 2020).

‘Red Jaina’ (*Hylocereus polyrhizus*): The color of the fruit peel is red, and the external of the fruit is covered with red-burgundy-colored scales. Fruit flesh is dark-red in color, and fruit weight varies between 230 and 340 g (Pine Island Nursery Catalog, 2020).

Three different pollination times were envisaged in the current study and pitaya flowers pollinated at 10: 00 pm, 00: 00 am, and 06: 00 am. Fresh pollen were used in pollination. After pollination, the seeds were extracted from randomly selected fruits. From each of the three pollination times, seeds were first passed through tap water, and then put on blotting paper to remove the water. The seeds were then sown in 45 cells seedling tray (30 cm x 50 cm x 5.8 cm) in a mixture medium containing 1: 1 peat and perlite in October 2020. The seedling trays were kept in the unheated glass greenhouse of the Horticulture Department of Akdeniz University during the seed germination and development.

Examined features are listed below.

Germination rate (%):

Germination rate in seeds was calculated according to the formula given.

(Total number of germinated seeds / Total number of seeds sown) x100

Mean germination time (days):

Germination time was calculated according to Ellis & Roberts (1981) by assuming total germination time to be 20 days in the ongoing studies.

$$MGT=\sum Ti\ Ni/\sum N$$

MGT: Mean Germination Time

Ti : Indicates the day after sowing the observation was made

Ni : Indicates the number of seeds germinated on the day of observation

N : Total number of germinated seeds

Germination energy (%):

It was calculated according to the formula below, considering the ratio of seeds germinating to the total germinated seeds from half the time from seed sowing to the end of germination (the total germination time was assumed to be 20 days) (Karaguzel et al., 2002)

$$GE = [\ \sum (T/2)\ Ni\ ]/\ [\ \sum Ni\ ]\ x\ 100$$

GE : Germination energy

Ni : Indicates the number of germinated seeds on the day of observation

T : The time from seed sowing to the end of germination

Germination index (Rapidity):

It was calculated according to the formula given below (Maguire, 1962):

$$GI = \Sigma\ n\ /\ d$$

GI: Germination index

n: normal number of seedlings obtained on day

d: Days counted from the start of the test,

Statistical analysis

The experimental design used was a randomized factorial design, with 3 replications and 60 seeds in each replication. The comparison of the means was made according to the LSD test.

RESULTS

The germination rates of seeds depending on the pollinator cultivar and pollination times, including the interaction, were recorded as 100%. However, mean germination time of seeds according to the cultivar x pollination hour interaction was found statistically different (Table 1). When examining the effects of pollinator cultivars on the mean germination time of seeds, the longest mean germination time (24.50 days) was found in Red Jania (R. Jaina) and the lowest (10.44 days) in ‘Cosmic Charlie’ (C. Charlie).

Table 1. Mean of the seed germination times depending on pollinator cultivars and pollination hours (days).

Çizelge 1. *Tozlayıcı çeşitler ve tozlama saatlerine bağlı olarak tohumlarda saptanan ortalama çimlenme süreleri (gün)*

Pollinator cultivars	Pollination hours			Mean pollination cultivars
	22: 00	00: 00	06: 00	
C. Charlie	9.85 e	8.96 e	12.52 d	10.44 c
R. Jaina	26.52 a	25.90 a	21.08 c	24.50 a
W. Jaina	20.39 c	11.62 d	23.11 b	18.37 b
Mean pollination hours	18.92 a	15.49 b	18.90 a	

LSD%5: cultivar x pollination hour: 1.369; cultivar: 0.790; pollination hour : 0.790

The effects of pollination hours on seed germination energy were also examined. The effects of pollinator cultivar x pollination hour interaction, pollinator cultivar and pollination hours on germination energy of seeds were found to be statistically significant (Table 2). When the effects of pollinator cultivars on germination energy of seeds were investigated, the highest germination energy (0.76) was recorded when the flowers were pollinated with C. Charlie pollen. While the lowest germination energy (0.45) was determined when the flowers were pollinated with R. Jaina pollen. Considering the mean pollination hours, the highest germination energy of seeds with 0.76 was recorded at 00: 00 am. Moreover, the lowest one was recorded at 06: 00 am (Table 2).

Table 2. Seed germination energy determined depending on pollinator cultivars and pollination hours (%)

Çizelge 2. *Tozlayıcı çeşitlere ve tozlama saatlerine bağlı olarak tohumlarda saptanan çimlenme enerjileri (%)*

Pollinator cultivar	Pollination hour			Mean pollination cultivars
	22: 00	00: 00	06: 00	
C. Charlie	0.81 b	0.93 a	0.56 cd	0.76 a
R. Jaina	0.40 e	0.41 e	0.54 cd	0.45 c
W. Jaina	0.60 c	0.94 a	0.51 d	0.68 b
Mean pollination hours	0.60 b	0.76 a	0.54 c	

$LSD_{\%5}$: cultivar x pollination hour: 0.061; cultivar: 0.035; pollination hour: 0.035

The effects of pollination cultivar x pollinator hour interaction, pollinator cultivar and pollination hours on germination index of seeds were statistically significant (Table 3). The highest germination index of seeds was determined when the flowers were pollinated with C. Charlie cultivar, followed by 'White Jaina' (W. Jaina) and R. Jaina cultivar, respectively. Pollination hours on seed germination index were also found statistically. The highest germination index was found when the flowers were pollinated at 00: 00 am (Table 3).

Table 3. Seed germination index based on pollinator cultivars and pollination hours

Çizelge 3. *Tozlayıcı çeşitler ve tozlama saatlerine bağlı olarak tohumlarda saptanan çimlenme indeksleri*

Pollinator cultivars	Pollination hours			Mean pollination cultivars
	22: 00	00: 00	06: 00	
C. Charlie	6.75 b	7.39 a	5.76 c	6.63 a
R. Jaina	3.23 f	3.54 ef	4.61 d	3.79 c
W. Jaina	4.09 de	6.27 b	3.80 e	4.83 b
Mean pollination hours	4.69 b	5.85 a	4.72 b	

$LSD_{\%5}$: cultivar x pollination hour: 0.554; cultivar: 0.319; pollination hour: 0.31.

DISCUSSION

When the 'Bloody Mary' cultivar was used as the female parent, the germination rate of seeds was recorded as 100% depending on the pollinator cultivars and pollination hours. The results showed that subspecies differences in pollinator cultivars and pollination hours did not affect the germination rate. The finding is very important to select pollinator cultivars for future breeding studies. Seed germination started in 4 days after sowing of seeds in each combination, and it was noted that the average germination ranged from 8 days to 26 days. The differences in terms of germination times of seeds were the result of pollinator cultivars. The shortest germination time of seeds was observed when C. Charlie cultivar was used as pollen. In addition, the shortest germination time of seeds was observed when the flowers were pollinated at 00: 00 am. Gunasena et al. (2007), reported that the initiation of the seed germination took between 3 and 5 days, just as our results. The number of viable seeds is found higher because of cross pollination. Our results were in close agreement with those of Dag & Mizrahi (2005). According to our results, pollinator cultivars and pollination hours significantly affected the germination energy (%) and germination index of seeds. When the germination energy was evaluated in terms of pollinator cultivar, it was determined that the germination energy of seeds was the highest value (0.76) when the flowers were pollinated with C. Charlie cultivar, and the lowest (0.45) when the flowers were pollinated with R. Jaina cultivar. If taking into account of the pollination hours, the highest to the lowest germination energies were determined when the flowers were pollinated at 00: 00 am, 10: 00 pm and 06: 00 am, respectively. Pollinator cultivars had significant effect on germination index of seeds. Germination energy (%) is thought to be related to germination time. It was observed that the germination index of seeds was found to be the highest when the flowers were pollinated with C. Charlie pollen. Because C. Charlie is not the same subspecies with maternal parent. On the other hand, the germination index of the seeds was the lowest (3.79) when the flowers were pollinated with R. Jaina (in the same subspecies as the maternal parent). Considering the pollination hours, the highest germination index of seeds was found when the flowers pollinated at 00: 00 am. Resultant response might be due to female organ was in the most suitable condition in terms of accepting pollen at 00: 00 am. Furthermore, the female organ was not fully receptive in the pollination at 10: 00 pm. Moreover, the stigmas in the female organs were found to be withered in the pollination at 6: 00 am. It is therefore, thought that the differences in germination energy and duration at the pollination hours are due to these abnormal and incompatible conditions.

CONCLUSION

As a result, C. Charlie is recommended as a pollinator cultivar for Bloody Mary because of its short germination time, high germination energy, and high germination index exerted for self-infertile Bloody Mary pitaya cultivar. The highest germination energy was determined when the flowers were pollinated at 00: 00 am, results also showed that hybridization should be possible with different subspecies in pitaya to obtain new hybrid for future breeding programs.

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