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Research Article

MICROPROPAGATION OF DIFFERENT PITAYA VARIETIES

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ABSTRACT. Pitaya attracts attention due to its properties such as fruit color, minerals, antioxidant properties and rich nutrient content both in Turkey and in the world. Seed and cuttings are mostly used in the production of pitaya. One of the best methods for fast and disease-free production of pitaya is *in vitro* tissue culture. In this study, young shoots of different pitaya varieties were cultured in Murashige and Skoog (MS) basal medium supplemented with different plant growth regulators such as 6-benzylaminopurine (BAP), gibberellic acid (GA_3), Indole-3-butyric (IBA). The highest value of the multiplication coefficient (5.41) was found in Halley's Comet variety cultivated in MS medium supplemented with 2.0 mg/l BAP. The lowest value (1.84) was detected in Bloody Mary cultivar growing in MS medium supplemented with 2.0 mg/l BAP. According to the results of rooting studies, between 10-95% root formation was detected when the medium and pitaya varieties were compared. While the best medium for rooting, MS medium supplemented with 1 mg / l IBA is detected, It has been determined that MS medium without plant growth regulators (PGRs) can also be used for rooting. Based on our results, the healthy and large amount of pitaya seedlings could be obtained.

Keywords: *Pitaya, micropropagation, American Beauty, Halley's Comet, Vietnam White and Bloody Mary*

INTRODUCTION

Pitaya or dragon fruit, which has attracted attention due to its attractive appearance, taste, and nutritional content in recent years, is located in the genus *Hylocereus* of the Cactaceae family of the Caryophyllales order. It is known by many names in the world [1, 2, 3]. Some of those are pitahaya, night-blooming cereus, strawberry pear, dragon fruit (in Southeast Asia), and päniniokapunahou or päpipi pua (in Hawaii) [4]. Its homeland is Central America and Mexico. Nowadays, it is commercially produced in the Bahamas, Bermuda, Indonesia, Colombia, Israel, Philippines, Maymar, Malaysia, Mexico, Nicaragua, North Australia, Okinawa (Japan), Sri Lanka, South China, South Florida, Taiwan, Thailand, Vietnam, El Salvador, Venezuela, Colombia and West Indies [5, 6, 7].

Pitaya varieties consist of 3 main types: *H. polyrhizus*, *H. undatus*, *H. guatemalensis* and interspecific hybrids [8, 9]. One of the other important species is reported to be *H. megalanthus*. Different *Hylocereus* species can be distinguished according to the shape, color, and size of the fruit pulp. The most commercially grown species are *H. polyrhizus*, *H. megalanthus* and *H. undatus* [10, 11].

All dragon fruit varieties have flesh filled with a large number of edible black seeds. Its shoots are green and fleshy and can be three, four and five-pointed. There are aerial roots that hold on to trees and rocks. Apart from clinging, these roots collect nutrients and nutrients in the medium, helping the plant to nourish. Plants can be reproduced by cutting from the places where these roots are. Although fruit weights reach up to 900 gr, it is on average between 350 and 450 gr. Although Pitaya is mostly used as a fruit, it is also used

as health products and medicine. It has delicious fruits and can be used for making juice, marmalade, vinegar, jam, ice cream and wine. Its fruits are essential for health and contain vitamin C, phosphorus, iron, vitamin B1, vitamin B2, vitamin B3 and many other compounds. Ascorbic acid value per 100 g fresh dragon fruit has been reported as 25 mg for *H. undatus* [1, 7, 8]. A nitrogen-containing compound known as betalains or especially red betacyanin is effective in red color formation in fruit and peel [12, 13]. Betacyanins are used commercially as natural food dyes [3].

Fruit seed oils are a good source of essential fatty acids and tocopherol with high stability [14]. It has been reported that some pitaya seeds contain essential fatty acids, especially linoleic acid, and linolenic acids for human health. When the % composition of fatty acids of *H. polyrhizus*, *H. undatus* and *H. meglanthus* are evaluated separately, it was reported as 23.5%, 21.0%, 18.8% for total SFA (saturated fatty acids) respectively and 26.3%, 23.9%, 14.3% for MUFA (monounsaturated fatty acids) respectively and 48.7%, 53.8%, 65.4% for PUFA (polyunsaturated fatty acids) respectively. The highest linoleic acid was reported in *H. meglanthus* with 65.4% and the highest oleic acid was reported in *H. polyrhizus* with 25.5% [3, 15, 16].

The production of Pitaya is traditionally performed through cuttings. Productions by cuttings are insufficient for commercial productions due to the lack of young nodes. Besides, Pitaya is also produced with seed but shows genetic expansion in seed production. Rapid, quality, and high-efficient micropropagation methods of some pitaya species is a very significant step for commercial production. Most researchers have focused on the cultivation technology of the plant [17, 18, 19, 20, 21, 22], extraction of components [23, 24, 25] and tissue culture methods [26, 27, 28, 29, 30, 31, 32, 33]. It can show different effects on pitaya varieties of medium supplemented with different plant growth regulators (PGRs) used in tissue culture studies. Researchers argued that this was due to the different genetic background and genetic condition that the pitaya has [34, 35, 36].

In this study, it is aimed to create a reliable and fast method for commercial production of different pitaya varieties that may be of commercial importance. *In vitro* proliferation and rooting of different pitaya varieties were evaluated using the plant tissue culture method, which is one of the important methods of plant biology, using MS basal medium [37] supported by combinations of different PGRs.

MATERIALS AND METHODS

This research was carried out in the Biotechnology Laboratory owned by Tekfen Agricultural Research Production and Marketing Inc. Adana, Turkey.

Plant material and Source of Explants

Plant materials were obtained from growers in Mersin, Turkey. Young shoot tips of pitaya varieties known as American Beauty, Halley's Comet, Vietnam White and Bloody Mary were used a source of explants for *in vitro* tissue culture.

Media and Culture Conditions

Initial plant materials were firstly sterilized by surface sterilization. Explants were subsequently kept under running tap water for 20 min and washed with dishware detergent and then rinsed by pure water. Then, explants were kept in 70% ethanol for 20

seconds and then 20 minutes in 1.5% (v/v) sodium hypochlorite. Finally, the explants were washed 3-4 times with distilled water.

MS medium supplemented with 2,0 mg/l BAP (M1) and 4.0 mg/l BAP (M2) was used for micropropagation of plants. For the rooting of the explants, MS medium supplemented with 1mg/l IBA (R2), 0.5mg/l GA_3 (R3), 0.5mg/l IBA + 0.5mg/l IBA (R4) and hormone-free MS (R1) medium used. Agar (7,5 g/l) was added to solidify the medium and the media pH was adjusted to 5.8 using 1 N HCl and 1 N NaOH. The prepared media were autoclaved at 121℃ at 15 psi for 20 min. and distributed in disposable plastic containers.

Explants with completed surface sterilization were cultured on MS basal media supplemented with different PGRs and aseptic plants were obtained. After the explants were transferred to the medium, they were transferred to plant growth chambers. All cultures were incubated under 36 W cool white fluorescent lights in a light / dark photoperiod for 16/8 hours and at 25 ± 2°C. Micropropagation trials continued by being subcultured every four weeks in a fresh medium. Micropropagation was evaluated at the end of 3 subcultures and the rooting studies at the end of 6 weeks.

Statistical Analysis

All trials were set up with three replications according to completely randomized design. All quantitative data calculated as percentage value were subjected to arcsine transformation before variance analysis. The data were analyzed using JMP-8 (SAS Institute Inc., NC, USA) statistical package program and variance analysis was performed. The differences between them were compared with the LSD (least significant difference) multiple comparison test.

In micropropagation trials, the multiplication coefficients were determined by considering the number of shoots per explant. In this context, both varieties and micropropagation mediums were evaluated. In rooting studies, root length (cm), % rooting, shoot length (excluding root) (cm) were evaluated (Figure 1).

RESULTS AND DISCUSSION

Contamination was not observed in any of the cultures and successful results were observed. Production of plants by *in vitro* tissue culture method has been investigated. *In vitro* micropropagation and rooting of different pitaya varieties on MS medium supplemented with different combinations of PGRs were evaluated. As a result of our studies, different types of pitaya responded differently to different mediums. For this reason, it is especially important to develop protocols for each type and this research has the data to provide this.

In this study, the highest shoot average per explant was obtained from M2 coded medium containing 4.0 mg/l BAP. The average multiplication coefficient was found to be 3.34 statistically. The highest shoot development average per explant was observed in Halley's Comet variety with 5,27. The highest multiplication coefficient was determined with M1 medium and Halley's Comet type with 5.41. The lowest multiplication coefficient was found in M1 medium and Vietnam White variety with 1.84 (Table 1). Similarly, Khalafalla et al. [38] stated that MS medium supplemented with plant growth regulators [(benzyladenine (BA), kinetin (Kin), naphthalene acetic acid (NAA)] has a positive effect on the shoot development of *Opuntia ficus-indica*. They found the highest shoot multiplication in MS medium supplemented with 5.0 mg/l BA. Researching with

different PGRs, Mohamed-Yasseen [27] observed the best shoot proliferation of *H. undatus* in medium containing 0.5mM TDZ and 0.5mM NAA (naphthaleneacetic acid).

Table 1. *The effect of different media on the efficiency of multiplication of in vitro propagated different pitaya varieties*

	Medium	Multiplication coefficient
American Beauty	M1	2.38d
	M2	4.12c
Halley's Comet	M1	5.41a
	M2	5.14b
Bloody Mary	M1	1.26g
	M2	2.12e
Vietnam White	M1	1.84f
	M2	2.00ef

*LSDvariety: 0,122***, LSDmedium: 0,086***, LSDvariety x medium: 0,173****

Suárez Román et al. [39] conducted studies on the reproduction of Yellow (*Selenicereus megalanthus*) and Red (*H. polyrhizus*) pitaya by *in vitro* tissue culture. It has been observed that the mean of the multiplication coefficient of the red pitaya (3.2) is higher than the yellow pitaya (3.0). In our study, this value was found between 1.69 and 5.27. When the researchers evaluated the medium, the highest mean multiplication coefficient was found in MS medium containing 2mg / l BAP + 2mg / l Kinetin and the lowest multiplication coefficient was found in MS medium with (1.4) 5mg / l BA + 0.05mg / l NAA (naphthaleneacetic acid). In our study, it was found 3.34 in M2 medium and 2.72 in M1 medium.

In root trials, as in micropropagation, Halley's Comet was the best variety according to Shoot Length with an average of 3.17 cm. When the medium and the pitaya variety are evaluated together, the highest value (4.02 cm) was determined in the Halley's Comet variety developing in the R2 medium. In general, shoot length was determined to be between 3.17-1.07 cm in plant varieties (Fig. 1) (Table 2).

Fig. 1. *Root length and shoot length measurement of Halley' s Comet rooted in R2 medium under in vitro culture conditions*

Considering the root lengths, Bloody Mary (3.18 cm) is the type that gives the best results according to the average root length. Although there was no statistically significant difference between R1 and R2 mediums, it was determined that the two medium were successful in terms of root length. Accordingly, it was observed that the hormone-free MS medium might be sufficient for the lengthening of the roots. Similarly, Clayton et al. [40] found that 11 rare or endangered cactus species provide successful rooting in hormone-free MS medium.

Table 2. *Effect of different mediums on root formation, shoot length and root length (Values in parentheses are angular transformation values of percentage of response)*

	Medium	Shoot Length (cm)	Root length (cm)	Rooting percentage (%)
American Beauty	R1	2.34 d	3.48 d	35 fg (36,23)
	R2	2.85 c	2.16 f	90 ab (71,95)
	R3	1.60 ef	1.20 g	25 hı (29,92)
	R4	1.45 f	1.09 g	55 c (47,87)
Halley's Comet	R1	3.36 b	5.51 b	50 cd (45,00)
	R2	4.02 a	3.80 d	95 a (76,89)
	R3	3.60 b	1.20 g	30 gh (32,96)
	R4	1.70 ef	1.04 g	30 fgh (33,59)
Bloody Mary	R1	1.73 e	5.35 b	20 ı (26,88)
	R2	1.62 ef	6.11 a	90 b (70,78)
	R3	0.44 h	0.10 ı	10 j (20,40)
	R4	0.90 g	1.18 g	40 ef (38,63)
Vietnam White	R1	1.11 g	2.60 e	45 de (41,73)
	R2	1.56 ef	4.55 c	90 b (71,33)
	R3	1.07 g	0.55 h	10 j (19,21)
	R4	0.56 h	0.91 gh	25 hı (30,20)

*Shoot Length: LSD variety: 0.126***, LSD medium: 0.126***, LSD variety x medium: 0.253***, Root length LSD variety: 0.183***, LSD medium: 0.183***, LSD variety x medium: 0.367, Rooting percentage: LSD variety: 2.655***, LSD medium: 2.655***, LSD variety x medium: 5.311****

El Finti et al. [41] studied *in vitro* micropropagation of *Opuntia ficus-indica* and observed rooting both in media containing PGRs and in media without PGRs. They detected 100% rooting in all mediums. They observed the best root formation in medium containing indole-3-butyric acid (IBA) and indole-3-acetic acid (IAA). In our study, rooting success varied considerably (10-95%). This variability is thought to be due to both different pitaya varieties and the PGRs used. Emphasizing that plants need essential nutrients in addition to phytohormones in their growth and development, Zhe Cheng et al. [42] examined the effects of waste coconut water and sucrose on micropropagation of *H. polyrhizus*. They used MS medium containing 0.03 mg/l BAP and 0.01 mg/l NAA supplemented with different concentrations (0%, 2%, 4% and 6%) of waste ripe coconut water and sucrose (0%, 1%, 2% and 3%). They found that waste coconut water had a positive effect on the elongation of the shoots while it did not have any effect on root

induction. In our study, different nutrients or elicitors were not used. However, successful results were obtained in both shoot elongation and rooting. In a different study, Sheng et al. [43] examined the effects of plant growth regulators on germination of pitaya seeds in vitro conditions. In their study, they investigated the germination percentage of seeds in semi-solid MS medium supplemented with 1 ppm BAP and 3 doses of IBA (0.0-0.50.8 ppm). They reported that the application with the highest germination rate (93.33%) was a combination of 1 ppm BAP and 0 ppm IBA. They obtained the highest callus induction percentage (75%) for *H. costaricensis* from the combination of 3.6 ppm 2.4D and 1.8 ppm BAP. In our study, there was no study with pitaya seeds since genetic variability may occur. Callus formations were constantly observed in in vitro cultures but were not taken into consideration. It is clear that callus culture studies in pitaya tissues can be used to produce secondary metabolites. Our study to meet commercial production demand and establish health micropropagation protocols based on varieties will guide other researches.

CONCLUSION

This study provides a protocol for micropropagation and rooting of different pitaya varieties. The effects of the media prepared with different PGRs on the micropropagation of different pitaya varieties were evaluated. Media and pitaya varieties were analyzed in terms of growth factor, root formation, root length, shoot length. When the multiplication coefficients were examined, the highest value (5.41) was determined in Halley's Comet cultivar grown in MS medium supplemented with 2.0 mg/l BAP. Halley's Comet has been observed to be successful in root formation as in micropropagation. M2 coded medium containing 4.0 mg/l BAP was determined as the best medium according to the multiplication coefficient averages. R2 medium containing 1 mg / l IBA was observed to be successful in rooting. Vietnam White has been found to be more unsuccessful in both micropropagation and rooting than other pitaya varieties. It is predicted that successful results will be obtained from different studies. The results obtained will provide highly efficient reference protocols for micropropagation of pitaya.

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