Journal of Scientific and Innovative Research 2023; 12(2): 33-39
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2023; 12(2): 33-39

Received: 01-05-2022
Accepted: 23-05-2022

Kay Thi Oo
Tasoe Road, Kyaukse, Mandalay Region, Myanmar

Zin Mar Lynn
Tasoe Road, Kyaukse, Mandalay Region, Myanmar

Kyaw Zwa Oo
Tasoe Road, Kyaukse, Mandalay Region, Myanmar

Mya Yadanar Htwe
Tasoe Road, Kyaukse, Mandalay Region, Myanmar

Win Thein Htet
Tasoe Road, Kyaukse, Mandalay Region, Myanmar

Win Win Soe
Tasoe Road, Kyaukse, Mandalay Region, Myanmar

Wuttyi Tun
Tasoe Road, Kyaukse, Mandalay Region, Myanmar

Correspondence:
Dr. Kay Thi Oo
Tasoe Road, Kyaukse, Mandalay Region, Myanmar
Email: kaythi123@gmail.com

In vitro Propagation of Three Pitaya Varieties (*Hylocereus undatus, Hylocereus polyrhizus and Hylocereus megalanthus*) with the Use of Different BAP Concentrations

Kay Thi Oo, Zin Mar Lynn, Kyaw Zwa Oo, Mya Yadanar Htwe, Win Thein Htet, Win Win Soe, Wuttyi Tun

Abstract

The pitaya, also known as the dragon fruit, is a climbing vine that first gained popularity as a decorative plant before becoming a fruit crop. Stem cuttings are utilized as planting material because the seeds of preserved pitaya have a very low viability rate. Several research have looked at various pitaya propagation techniques, however there is relatively little data on the procedures for producing high-quality planting material using tissue culture. In the current study, we looked at the possibility of direct shoot regeneration of three different pitaya explants using stem segments from in vitro germinated seedlings in Murrashige and Skoog (MS) basal medium supplemented with five different concentrations of Benzylaminopurine (BAP): 0, 0.1, 0.2, 0.3, 0.4, and 0.5 mg/l. Following that, plantlets that had undergone direct organogenesis regeneration were rooted in the same MS basal medium. The newly regenerated shoots were started on explants using MS basal media supplemented with various BAP concentrations in less than 4 weeks. The highest number of shoot average per explant was obtained from MS medium containing 0.5 mg/l BAP and the least was found without using BAP in Yellow pitaya. The MS medium with 0.4 mg/l BAP produced the largest average number of shoots per explant, and the lowest were also seen with control treatment in the red pitaya variety. The Whitest pitaya shoots were produced in MS medium supplemented with 0.4 mg/l BAP, and the fewest were produced in the control treatment, which was higher than red pitaya. On their own, mature shoots from regenerated plants began to root in the same medium.

Keywords: Pitaya, Dragon fruit, BAP, Hylocereus, Shoot, Multiplication, Micropropagation.

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 [1]. It is known by many names in the world. Pitaya (dragon fruit) is the fruit produced by the plant with the same name belonging to the genus Hylocereus of the Cactaceae [2]. It contains glucose, betaines, vitamins, organic acids, soluble dietary fiber, phyto albumins and minerals [3]. Pitaya is not only utilized as fruit, but also can be taken as flower, vegetable, health products and medicine [4]. The fruit is delicious and it can also be processed into juice, jam, ice cream, pastries, vinegar and wine. Its plant is attractive due to its exotic appearance [5]. It was initially used as ornamental plant and latter due to its health benefits and market value emerged as a new fruit crop [6]. Owing to its rich nutrient contents and antioxidant properties, it is emerging as a super fruit worldwide. Belongs to family Cactaceae, basically, it is perennial semi epiphytic climbing cactus vine [7]. The cactus family are highly adaptable to a new environment. The plants are able to tolerate drought, heat, poor soil, and cold [8]. The modification of the stem for water storage, the reduction or absence of leaves, the waxy surfaces, and night-time opening of the tissues for carbon dioxide uptake (the CAM process), enable the plants to tolerate harsh conditions [9]. Terms used to describe plants with such adaptations include xerophyte and succulent [10]. These adaptations to survive dry, hot conditions, apply to the above-ground plant [11]. The roots are non-succulent and require small amounts of water and cooler temperatures [12]. Cacti will not tolerate saline or water-logged conditions, nor will they grow where there is an absence of plant life [13]. In their native lands, the plants were used for many purposes, but one of major importance is the fruit as a food source [14]. Fruit was collected from naturally established stands. Later, cuttings were taken from highly productive plants and grown around

houses [15]. A similar process is now in place in several countries around the world to establish plantations of cacti with edible fruit, from column, shrubby and climbing types [16]. The fruits are scooped out with a spoon, much like a kiwi fruit. The flesh is firm and crisp, with a delicately sweet and lingering flavor [17]. The juicy flesh can be used in marmalade, jellies, ice creams and soft drinks [18]. Dragon fruits do not contain cholesterol, saturated fat. Therefore, regular consumption will help manage blood pressure and control cholesterol levels [19]. The seeds have a high in polyunsaturated fatty acids (omega-3 and omega-6 fatty acids), reduced triglycerides and lower the risk of cardiovascular disorders [20, 21]. Fruits are high in fiber (regular consumption can help avoid constipation, improve digestive health and help to reduce weight), rich in vitamins C, B (B1, B2, and B3), calcium, iron, lycopene, and antioxidants that help in human health [22]. The fruit as a food substitute for rice and as a source of dietary fiber. Fruits contain phytoalbumins, which have antioxidant properties that help prevent the formation of cancer cells (Ruzainah *et al*. 2009) [23]. The flower buds of dragon fruit are used to make soups or mixed with salads and the red pulp of the dragon [24].

There are four types dragon fruit based on their colour [25]. But in this study, we only use three types of dragon fruit varieties.

1. *Hylocereus undatus* i.e., red colour fruit with white colour flesh.

2. *Hylocereus polyrhizus* i.e., red colour fruit with red colour flesh.

3. *Hylocereus megalanthus* i.e., yellow colour fruit with white colour flesh.

Taxonomic Hierarchy of Three Pitaya varieties

Kingdom	Plantae
Subkingdom	Viridiplantae
Infrakingdom	Streptophyta
Superdivision	Embryophyta
Division	Tracheophyta
Subdivision	Spermatophytina
Class	Magnoliopsida
Superorder	Caryophyllanae
Order	Caryophyllales
Family	Cactaceae Juss.
Genus	*Hylocereus*
Species	*Hylocereus undatus* (Haw.) Britton & Rose *Hylocereus polyrhizus* (F.A.C.Weber) Britton & Rose *Hylocereus megalanthus* (K.Schum. ex Vaupel) Moran

The plants take 3 years to grow from seed [26]. Thus, the multiplication of dragon fruits under in vitro conditions is a superlative method [27]. Tissue culture provides an alternative solution for producing many genetically similar, phytosanitarily and physiologically high-quality plantlets within a limited period [28].

Correspondence: Kay Thi Oo is Deputy Director, Plant Tissue Culture Laboratory, Department of Biotechnology Research, Kyaukse Township, Mandalay Region, Myanmar (e-mail; kaythi123@gmail.com)

MATERIALS AND METHODS

Plant Materials and Source of Explants

Seeds of Dragon fruit were extracted from fresh ripe fruit. Red and white ones of which have been purchased from local fruit sellers in Kyaukse, Myanmar and the yellow one obtained from Mawlamying, Myanmar. Seeds of three dragon fruit varieties were used as source of explants for *in vitro* micropropagation. Aseptic cultures of Dragon fruit or Pitaya were established via *in vitro* plantlet regeneration.

Preparation of explants

All three cultivars of the plant were regenerated *in vitro* to produce stem explants, which were harvested from aseptic seedlings that were 4 weeks old. After being separated from the flesh, the seeds were sterilized using a modified version of the procedure described by De Feria *et al.* [29].

Establishment of seed cultures

After being separately extracted, seeds were surface-sterilized by spraying with 70% ethanol for two minutes and dipping in a solution of 1% sodium hypochlorite and 6% Tween 20 for ten minutes. Following proper cleaning with autoclaved distilled water, sterilized seeds were grown on MS medium containing 3% sucrose and 0.6% agar and maintained at 25–27°C with a continuous light under fluorescent tube light (50 mol m-2s-1). Each glass vessel contained 10 surface-sterilized seeds and 20ml of basal MS media. After culture for about 4 weeks of incubation, the sterilized explants were obtained.

Preparation of Culture medium

In the present experiment, basal MS [30] media without any plant hormone were used for seed germination and MS media supplemented with five different concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5 mg/l) of BAP (6-benzyl-amino- purine) and control were used for shoot proliferation and root development. Agar (6 g/L) was added to solidify the medium and the pH was adjusted to 5.8 with 1N HCL or 1 N NaOH 20 ml of prepared culture medium was poured into each glass culture vessel (250 ml capacity) and then they were covered using plastic lid [31]. These culture vessels were autoclaved at 121°C for 20 min at 15 psi subsequently they were kept to cool before the explant inoculation.

Cultural Environment and Culture Condition

All cultures were incubated under continuous cool and white fluorescent tube light (1500 Lux) and 27±1° C temperature controlled with air-conditioning system at 25 ± 2°C. Culture observations were recorded on the shoot proliferation and root formation. Proliferation trials continued by being subcultured every four weeks in a fresh culture medium. Micropropagation was evaluated at the end of 5th subcultures and the rooting studies was evaluated at the end of 6 months.

Acclimatization of the Tissue Culture Plantlets

After 6 months of incubation in the culture room, plantlets were transferred from the bottle, and the agar was gently removed from the roots. The *ex vitro* plantlets were maintained in plastic bags containing sand, biofertilizer, soil (1:1:2). The plantlets were cultured on the terrace under field conditions. For the adaptation to the environment, the *in vitro* plantlets were transplanted to a tray with sterile potting mixture After 2 months, the *in vitro* plantlets were fully exposed to external environmental conditions and hydrated once per week

Statistical Analysis

In this context, all three varieties and micropropagation media were evaluated. In shooting studies, number of shoot and shoot length (cm) were evaluated. For rooting, only number of root was recorded. A fully randomized design was used to duplicate each treatment five times for the *in vitro* experiment. It was recorded how many additional shoots sprouted from each original shoot. Also noted was the quantity of roots that sprouted throughout the experiment. Using the statistical program Minitab 13.1, one-way ANOVA was used to analyze the collected data and P=0.05 was used to measure deviations between means. To evaluate the treatment means, least significant differences (LSD) were computed at the 5% level of significance.

RESULTS AND DISCUSSIONS

The present study was conducted for micropropaga-tion of three different pitaya varieties in the basal MS media containing different concentrations of plant growth hormone BAP. There is no culture showed signs of contamination, and satisfactory outcomes were noted. Investigations have been done into the production of pitaya plants using *in vitro* tissue

culture [32]. Three distinct pitaya type in the *in vitro* micropropagation, shoot multiplication, and rooting on MS medium supplemented with various concentrations of BAP were assessed. Our research revealed that several pitaya species reacted differently to various media. Consequently, it is crucial to create procedures for each kind, and this research provides the data to do so.

In this study, the highest number of shoot average per explant (11.00 ± 2.65) was obtained from MS medium containing 0.5 mg/l BAP and the least (2.667 ± 0.577) was found without using BAP in Yellow pitaya (Table 1). The highest shoot development in average number of shoot per explant (11.33±3.06) was observed with MS medium containing 0.4 mg/l BAP and the lowest (2.667 ± 0.577) also found with control treatment in Red pitaya variety (Table 2). The use of MS medium supplemented with 0.4 mg/l BAP also gave highest multiplication rate of White pitaya shoot with (4.000 ± 1.000) found in MS medium supplemented with 0.4 mg/l BAP and the least amount of shoot (2.000 ± 1.000) was also found in control treatment but lower than Red pitaya (Table 3). Similar to this, Khalafalla et al. [33] reported that Opuntia ficus-indica shoot development is positively impacted by MS media supplemented with plant growth regulators (benzyladenine (BA), kinetin (Kin), and naphthalene acetic acid (NAA)). MS medium supplemented with 5 mg/l BAP had the most shoot multiplication, according to their research. When the plant components were cultivated on multiplication media containing various quantities and combinations of plant growth regulators, shoot multiplication occurred. There have been previous reports of multiple shoot induction from various cactus explants in relation to the response to utilizing different cytokinin hormones, particularly BAP. (Dahanayake et al., 2011) [34] [35].

Considering the number of root, MS supplemented with 0.4mg/l BAP (5.67±2.08) is the hormone concentration that gives the best results according to the average root number in Yellow pitaya (Table 1). Although there was no significant difference between BAP 0.1 mg/l and BAP 0.2 mg/l, it was determined that the two hormone concentrations were successful in terms of root development for this pitaya species. But there was found that hormone free medium gave the least root formation in Yellow pitaya. Controversially, in both Red and White pitaya, the best root formation (5.000 ± 1.000) and (4.333 ± 1.528) were observed in hormone free MS medium respectively (Table 2 and 3). Accordingly, it was observed that the hormone-free MS medium might be sufficient for the lengthening of the roots. Similarly, Clayton et al. found that 11 rare or endangered cactus species provide successful rooting in hormone-free MS medium [36].

It was also determined that well-rooted plantlets had higher survival rates after transplanting into soil [37]. It has been determined that the plants cling to life and continue to grow during the acclimatization of the *in vitro* propagated pitaya cultivars to the external environment [38]. It was determined that all the pitaya varieties, which gave successful results in *in vitro* rooting, was also successful in acclimatization (Figure 8). On the other hand, it was observed that the growth progressed slowly in all cultivars.

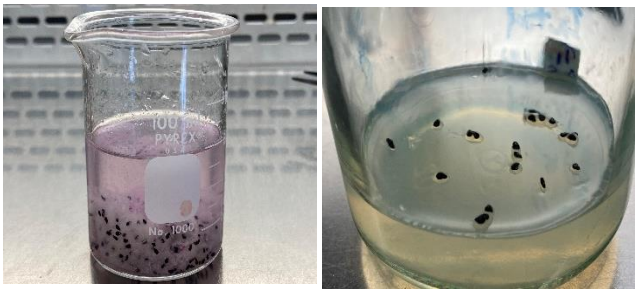

Figure 1: Surface sterilization and seed culture initiation of Pitaya

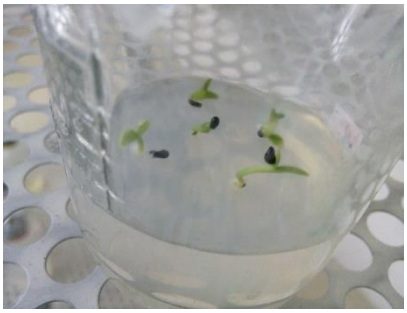

Figure 2: Seed germination of Pitaya on MS basal medium

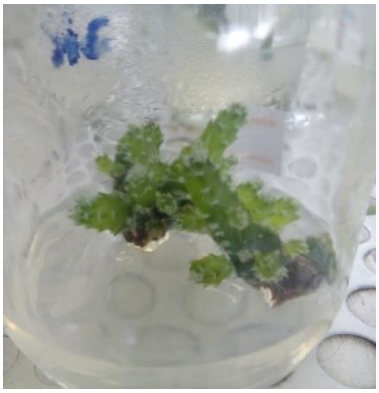

Figure 3: Shoot initiation on MS media supplemented with BAP 0.3 mg/l of Yellow pitaya

Figure 4: Shoot multiplication on MS media supplemented with Different BAP concentrations of Yellow pitaya

Figure 5: Shoot multiplication on MS media supplemented with Different BAP concentrations of Red pitaya

Figure 6: Shoot multiplication on MS media supplemented with Different BAP concentrations of White pitaya

Figure 7: Root formation of red pitaya in MS medium without plant growth regulator

Figure 8: Hardening of *in vitro* propagated Pitaya plantlet

Table 1: Effect of different BAP concentrations on shoot development and root formation of Yellow Pitaya

BAP (mg/l)	number of shoot	shoot length (cm)	number of root
0	2.333 ± 0.577	2.933 ± 0.379	2.667 ± 1.528
0.1	10.00 ± 2.00	5.07 ± 2.05	5.333 ± 0.577
0.2	10.333 ± 1.528	4.367 ± 1.415	5.33 ± 2.52
0.3	8.000 ± 1.000	4.833 ± 1.258	4.667 ± 1.528
0.4	10.667 ± 1.155	4.833 ± 1.258	5.67 ± 2.08
0.5	11.00 ± 2.65	3.200 ± 0.854	4.000 ± 1.000

Data presented are means ± standard deviation

Table 2: Effect of different BAP concentrations on shoot development and root formation of Red Pitaya

BAP (mg/l)	number of shoot	shoot length (cm)	number of root
0	2.667 ± 0.577	3.567 ± 0.603	5.000 ± 1.000
0.1	3.667 ± 1.528	3.433 ± 0.493	3.667 ± 1.528
0.2	4.667 ± 1.528	3.300 ± 0.361	1.333 ± 0.577
0.3	4.333 ± 1.528	3.267 ± 0.586	1.667 ± 0.577
0.4	11.33 ± 3.06	3.1667 ± 0.1528	1.333 ± 0.577
0.5	4.67 ± 2.08	4.567 ± 1.193	1.000 ± 0.000

Data presented are means ± standard deviation

Table 3: Effect of different BAP concentrations on shoot development and root formation of White Pitaya

BAP (mg/l)	number of shoot	shoot length(cm)	number of root
0	2.000 ± 1.000	3.567 ± 0.603	4.333 ± 1.528
0.1	2.667 ± 0.577	3.433 ± 0.493	3.000 ± 1.000
0.2	3.333 ± 1.528	3.300 ± 0.361	3.67 ± 2.08
0.3	2.667 ± 1.155	3.267 ± 0.586	2.000 ± 1.000
0.4	4.000 ± 1.000	3.1667 ± 0.1528	1.667 ± 0.577
0.5	3.333 ± 1.528	4.567 ± 1.193	1.667 ± 0.577

Data presented are means ± standard deviation

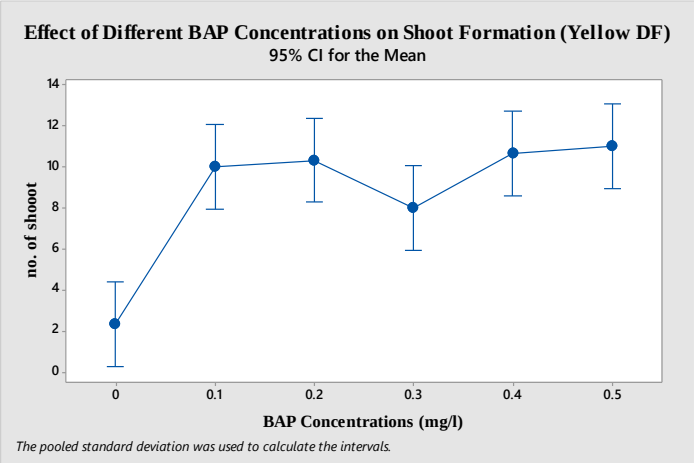

Figure 9: Effect of Different BAP Concentrations on Number of Shoot Formation (Yellow Pitaya)

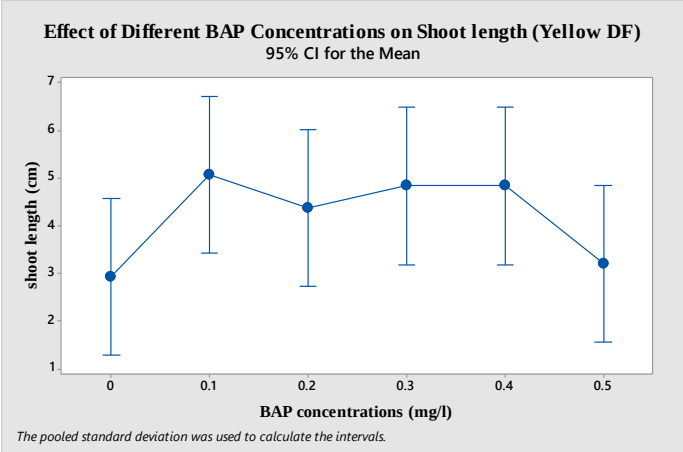

Figure 10: Effect of Different BAP Concentrations on Shoot length (Yellow Pitaya)

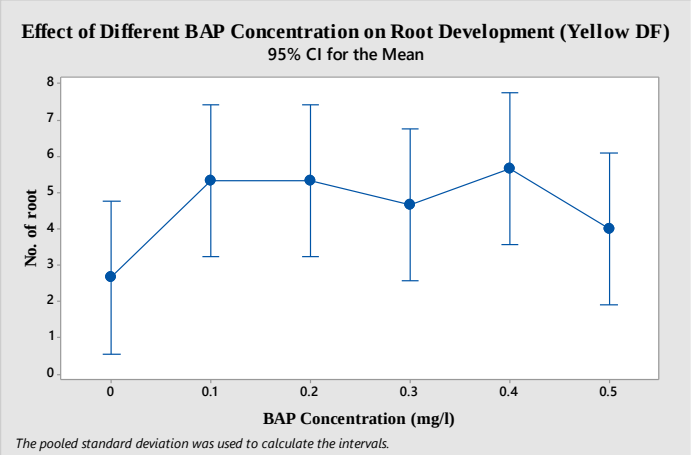

Figure 11: Effect of Different BAP Concentrations on Number of root (Yellow Pitaya)

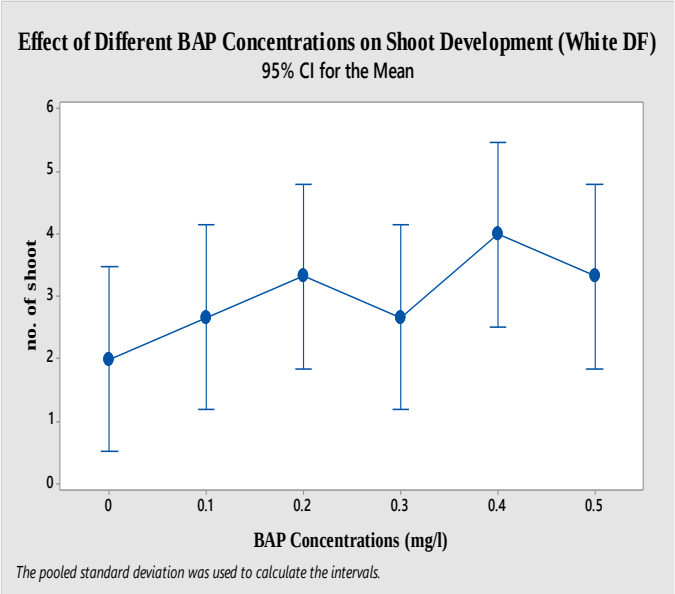

Figure 12: Effect of Different BAP Concentrations on Number of Shoot Formation (White Pitaya)

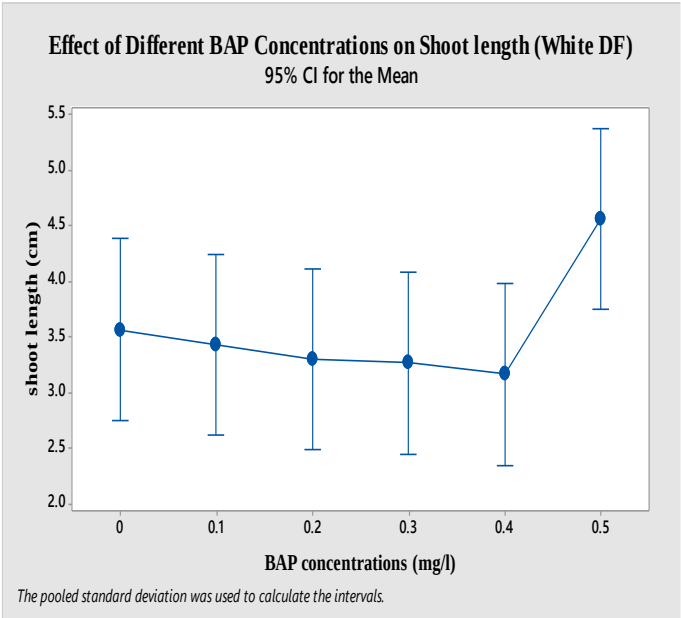

Figure 13: Effect of Different BAP Concentrations on Shoot length (White Pitaya)

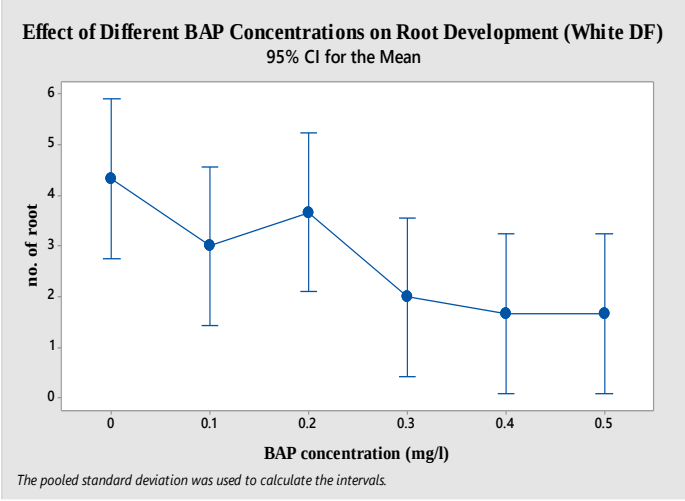

Figure 14: Effect of Different BAP Concentrations on Number of root (White Pitaya)

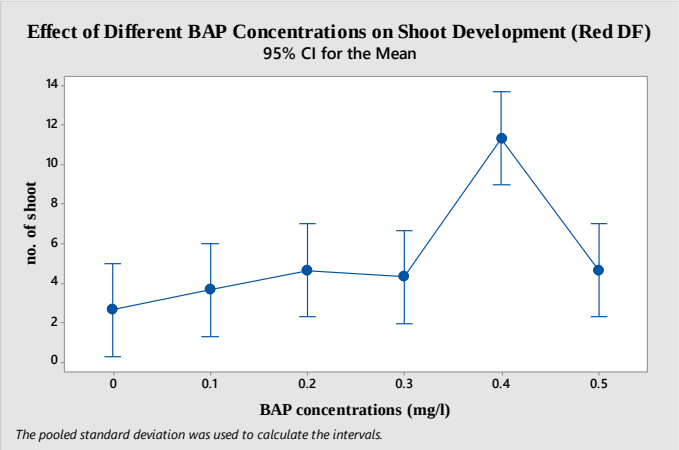

Figure 15: Effect of Different BAP Concentrations on Number of Shoot Formation (White Pitaya)

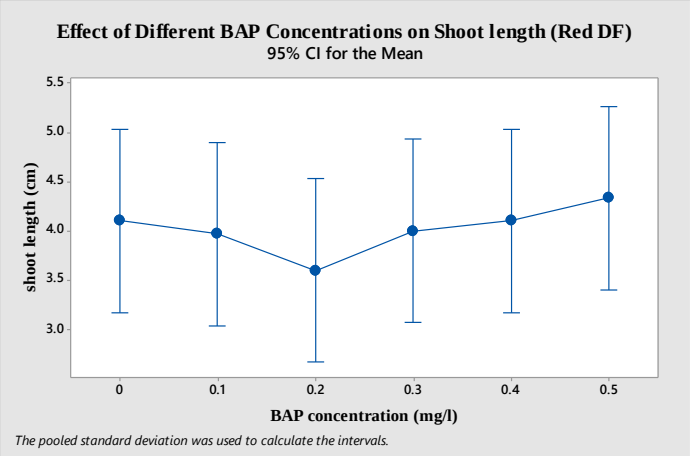

Figure 16: Effect of Different BAP Concentrations on Shoot length (Red Pitaya)

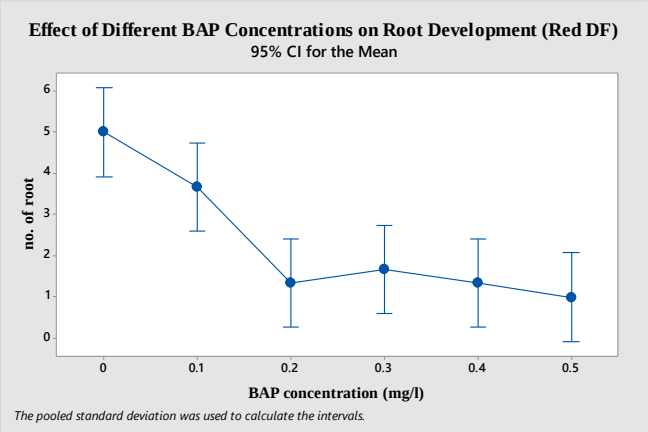

Figure 17: Effect of Different BAP Concentrations on Number of root (Red Pitaya)

CONCLUSIONS

The best alternative approach for producing large amounts of disease-free, true-to-type planting material in the pitaya industry is *in vitro* propagation [39]. This study provides a successful protocol for micropropagation of three different pitaya varieties (Yellow, Red and White). On the micropropagation of various pitaya types, the effects of the media made with various BAP concentrations were assessed. The development of shoots, production of roots, and length of shoots were examined in media formulations with various concentrations of BAP and pitaya types. The inclusion of plant growth regulators such as BAP (0.4

mg/l and 0.5 mg/l) in culture media has been able to boost the elongation of the shoots, which may lead to the conclusion that the technique of micropropagation of pitaya types can be improved. Both of Red and White pitaya varieties were shown to be able to regenerate a substantial amount of roots on hormone-free medium, whereas Yellow pitaya kinds were found to develop roots well on the medium containing MS with 0.4 mg/l BAP. It is anticipated that several investigations would produce fruitful outcomes [40]. The outcomes will offer very effective reference methods for pitaya micropropagation.

Acknowledgment

Grants from the Ministry of Science and Technology of Myanmar helped to fund this effort. For her approval and support throughout this research project, the author additionally acknowledges Dr. Aye Aye Khai, Deputy Director General, Department of Biotechnology Research, Kyaukse Township, Mandalay Region, Myanmar. For their generosity and kind assistance during this research, I would especially want to thank the members of our lab team at the Department of Biotechnology Research Plant Tissue Culture Laboratory.

Conflict of Interest

None declared.

Financial Support

None declared.

REFERENCES

1. Caetano DG, Escobar R, Caetano CM, Vaca JC. Standardization of A regeneration protocol in yellow pitahaya (*Selenicereus megalanthus* (K. Schum. ex Vaupel) Moran). Acta Agronomica 2014;63(1):31-41.
2. Bozkurt T, Inan S, Dundar I, Kozak S. Effect of different plant growth regulators on micropropagation of some pitaya varieties. Journal of Tropical Life Science 2022;12(2):183-190.
3. Bozkurt T, Inan S, Dundar I. Micropropagation of different pitaya varieties. International Journal of Agricultural and Natural Sciences. 2020;13(1):39-46.
4. Kari R, Lukman AL, Zainuddin R. Basal media for *in vitro* germination of red-purple dragon fruit (*Hylocereus polyrhizus*). Journal of Agrobiotechnology. 2010;1:87-93.
5. Kasim DP, Kishore NS, Suneetha P, Rao KB, Kumar MN, Krishna MSR. Multiple shoot regeneration in seed-derived immature leaflet explants of red dragon fruit (*Hylocereus costaricensis*). Research Journal of Pharmacy and Technology. 2019;12(4):1491-1494.
6. Le Bellec F, Vaillant F, Imbert E. Pitahaya (*Hylocereus* spp.): a new fruit crop, a market with a future. Fruits. 2016;61(4):237-250.
7. Drew RA, Azimi M. Micropropagation of Red Pitaya (*Hylocereus undatus*). Acta Hort. 2002;5(75):93-98.
8. Esquivel P, Stintzing FC, Carle R. Comparison of morphological and chemical fruit traits from different pitaya genotypes (Hylocereus sp.) grown in Costa Rica. J Appl Bot Food Qual. 2007;81(1):7-14.
9. Fan QJ, Zheng SC, Yan FX, Zhang BX, Qiao G, Wen XP. Efficient regeneration of dragon fruit (*Hylocereus undatus*) and an assessment of the genetic fidelity of *in vitro*: derived plants using ISSR markers. J Hortic Sci Biotechnol. 2013;88(5):631-637.
10. Harivaindaran KV, Rebecca OPS, Chandran S. Study of optimal temperature, pH and stability of dragon fruit (*Hylocereus polyrhizus*) peel for use as potential natural colorant. Pak J Biol Sci. 2008;11(18):2259-2263.
11. Infante R. *In vitro* axillary shoot proliferation and somatic embryogenesis of yellow pitaya Mediocactus coccineus (Salm-Dyck). Plant Cell Tissue Org Cult. 1992;3(1):155-159.
12. Le Bellec F, Vaillant F, Imbert E. Pitahaya (Hylocereus spp.): a new fruit crop, a market with a future. Fruits. 2006;61(4):237-250.
13. Liaotrakoon W, De Clercq N, Van Hoed V, Dewettinck K. Dragon fruit (Hylocereus spp.) seed oils: their characterization and stability under storage conditions. J Am Oil Chem Soc. 2013;90(2):207-215.
14. Harivaindaran KV, Rebecca OP, Chandran S. 2008. Study of optimal temperature, pH and stability of dragon fruit (*Hylocereus polyrhizus*) peel for use as potential natural colorant. Pak. J. Biol. Sci. 2008;11:2259-2263.
15. Tel-Zur N, Abbo S, Bar-Zvi D, Mizrahi Y. Clone identification and genetic relationship among vine cacti from the genera Hylocereus and Selenicereus based on RAPD analysis, ci. Hortic. 2004;100:279-289.
16. Yassen MY. Micropropagation of pitaya (*Hylocereus undatus* Britton & Rose) *In Vitro* Cell. Dev. Biol.-Plant. 2002;38:427-429.
17. Suh DH, Lee DY, Heo YS, Kim SK, Cho S, Lee CH. Metabolite profiling of red and white pitayas (*Hylocereus polyrhizus* and *Hylocereus undatus*) for comparing betalain biosynthesis and antioxidant activity. J Agric. Food Chem. 2014;62:8764-877.
18. Wang YS, Zhou MH, Kang LF, Cao DM, Zhang C, Duan JJ. Tissue culture and rapid propagation of Hylocereus undatus stem. J. Shanxi Agric. Sci. 2012;40:455-458
19. Shen SH, Peng ZJ, Xie P, Wang SS, Ma YH. Effect of different culture condition on seed germination of *Hylocereus undatus*. South China Fruits. 2014;43:99-100
20. Tenore GC, Novellino E, Basile A. Nutraceutical potential and antioxidant benefits of red pitaya (*Hylocereus polyrhizus*) extracts. Journal of Functional Foods. 2012;4(1):129-136.
21. Zhou YZ, Liu Z, Ren FY. Study on research status and development trend of pitaya in China based on bibliometric. J. Anhui Agric. Sci. 2011;39:22162-22163.
22. Zainoldin KH, Baba AS. The effect of *Hylocereus polyrhizus* and *Hylocereus undatus* on physicochemical, proteolysis, and antioxidant activity in yogurt. World Academy of Science, Engineering and Technology. 2009;60:361-366. doi: 10.5281/zenodo.1078639.
23. Ruzainah AJ, Ahmad Ridhwan, Bin Abdul Rahman, Nor Zaini Che Mahmod, Vasudevan R. Proximate analysis of dragon fruit (*Hylecereus polyhizus*). American J Appl. Sci. 2009;6(7):1341-1346.
24. Stintzing FC, Schieber A, Carle R. Evaluation of colour properties and chemical quality parameters of cactus juices, Eur. Food Res. Technol. 2003;216:303-311.
25. Thiha S. Effects of Explants and Growth Regula-tors on In vitro Regeneration of Dragon Fruit (*Hylocereus undatus* Haworth) (Doctoral dissertation). 2019
26. Jaafar RA, Rahman ARBA, Mahmod NZC, Vasudevan R. Proximate analysis of dragon fruit (Hylecereus polyhizus). American Journal of Applied Sciences. 2009;6(7):1341-1346. Doi: 10.3844/ajassp.2009.1341.1346
27. Suman K, Rani AR, Reddy PV. Response of dragon fruit (*Hylocereus undatus*) explants on MS media with growth regulators under in vitro for mass multiplication. Agric. Update 12 (TECHSEAR-9). 2017;1-8. Doi: 10.15740/HAS/AU/12.TECHSEAR(9)2017/1-8
28. Dano A, Tupas R, Rallos LE. Effect of Commer-cial plant growth regulator on the growth of dragon fruit (*Hyolocereus* sp.) cuttings under greenhouse condition. Journal of Engineering, Environment and Agriculture Research. 2020;2:29-36. doi: 10.34002/jeear.v2i0.43
29. De Feria M, Rojas D, Reyna M, Quiala E, Solìs J, Zurita F. *In vitro* propagation of Hylocereus purpusii Britton & Rose, a mexican species in danger of extinction. Biotecnol. Veg. 2012;12:77-83.
30. Murashige T, Skoog F. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol. Plant. 1962;15:473-497.

31. Ahmad H, Mirana AS, Mahbuba S, Tareq SM, Uddin AJ. Performance of IBA concentrations for rooting of dragon fruit (*Hylocereus undatus*) stem cuttings. Interna-tional Journal of business, social and scientific research. 2016;4(4):231-234.
32. Fan QJ, Zheng SC, Yan FX, Zhang BX, Qiao G, Wen XP. Efficient regeneration of dragon fruit (*Hylocereus undatus*) and an assessment of the genetic fidelity of *in vitro*: derived plants using ISSR markers. The Journal of Horticultural Science and Biotechnology. 2013;88(5):631-637.
33. Khalafalla MM, Abdellatef E, Mohameed Ahmed MM, Osman MG. Micropropagation of cactus (Opuntia ficus-indica) as strategic tool to combat desertification in arid and semiarid regions. International Journal of Sustainable Crop Production. 2007;2(4):1-8.
34. Dahanayake N, Ranawake AL. Regeneration of dragon fruit (*Hylecereus undatus*) plantlets from leaf and stem explants. Trop. Agric. Res. Ext. 2011;14:85-89.
35. Liaotrakoon W, De Clercq N, Van Hoed V, Dewettinck K. Dragon fruit (Hylocereus spp.) seed oils: their characterization and stability under storage conditions. Journal of the American Oil Chemists' Society. 2013;90(2):207-215.
36. Clayton PW, Hubstenberger JF, Phillips GC, Butler-Nance SA. Micropropagation of members of the Cactaceae subtribe Cactinae. J. Am. Soc. Hortic. Sci. 1990;115:337-343.
37. Kasim DP, Kishore NS, Suneetha P, Rao KB, Kumar MN, Krishna MSR. Multiple shoot regeneration in seed-derived immature leaflet explants of red dragon fruit (*Hylocereus costaricensis*). Research Journal of Pharmacy and Technology. 2019;12(4):1491-1494.
38. Nurul SR, Asmah R. Variability in nutritional composition and phytochemical properties of red pitaya (*Hylocereus polyrhizus*) from Malaysia and Australia. International Food Research Journal 2014;21(4).
39. Gunasena HPM, Pushpakurama D, Kariyawasam M. Dragon Fruit-*Hylocereus undatus* (Haw.) Britton and Rose: Field Manual for Extension Workers. Sri Lanka Council for Agricultural Policy 114, Colombo. 2006.
40. Nerd A, Sitrit Y, Kaushik RA, Mizrahi Y. High summer temperatures inhibit flowering in vine pitaya crops (*Hylocereu*s spp.). Scientia Horticulturae. 2002;96(1-4):343-350. Doi: 10.1016/S0304-4238(02)00093-6