

Original Research Article

Economic analysis of micropropagation of dragon fruit (*Hylocereus undatus* (Haw.) Britton and Rose)

ABSTRACT

Cultivation of dragon fruit is rising in many tropical and sub-tropical countries because of its high nutritional and medicinal values and is highly remunerative for the farmers. However, the supply of planting material is a bottleneck in meeting the increasing demand. The upsurged demand for planting material demands tissue cultured planting material, which ensures the crop's clonal fidelity and availability of planting material throughout the year. Considering the higher cost of micropropagation, an economic analysis of *in vitro* regeneration for thousand plants was calculated and compared with the conventional stem cuttings. Cost of tissue cultured plantlets (Rs 20.02) /plant) was found to be higher than the conventional method (Rs. 14.93 per plant). The major cost involved in micropropagation is attributed to the cost of skilled labours required for multiple sub-cultures, followed by the hardening media and tissue culture growth media. Nevertheless, the cost of tissue cultured plantlets is far lesser than the market price (Rs. 40/plant). Hence, considering the clonality, uniformity and disease freeness of tissue cultured plantlets, micropropagation of dragon fruit may be considered feasible for both the producers and farmers. A further cost of production may be reduced with training skilled labours to improve their efficiency and other cheap sources of potting media may be explored.

Keywords: *micropropagation, dragon fruit, economics, in vitro, stem cutting*

1.0 INTRODUCTION

Dragon fruit (*Hylocereus* spp.), also known as pitaya or pitahaya, is an emerging and highly remunerative fruit crop in tropical regions worldwide. It is one of the most important tropical fruit crops of Cactaceae. It is a perennial, epiphytic, climbing cactus with triangular, fleshy, jointed green stems. The fruits are round to slightly oval, with numerous tiny black seeds embedded in the fruit pulp with different peel and pulp colours depending on the species [10]. Fruits are consumed either as fresh fruits and salads or in the processed form as jam, jelly, ice cream, juice, wine, face-packs etc. Various coloured fruits are famous for their rich nutritional and medicinal values. Low in calories, dragon fruit is rich in vitamin-c, phosphorus, calcium, iron, pectin and dietary fibre. Antioxidant-enriched fruits prevent chronic illness by protecting the body from free radicals and decelerates ageing. Fruits or fruit extracts control cancer and diabetes, lowering cholesterol levels and blood pressure [11].

Dragon fruit is primarily grown in Vietnam, Thailand, Colombia, Mexico, Costa Rica, and Nicaragua [18]. But its cultivation is rapidly spreading to other subtropical regions of the world, including India, because of their nutritional and medicinal values. Dragon fruit was introduced to India during the late

1990s [2]. India's estimated area of dragon fruit cultivation is 3085 ha, with a production of 12113.0 MT and 10.7 MT/ ha productivity [19]. Impulsive increased dragon fruit cultivation leads to increased demand for the planting material.

Dragon fruit is conventionally propagated either vegetatively through stem cuttings or stem fractions or sexually through seeds [9]. However, dragon fruit being a cross-pollinated crop, plants produced through seeds are often not true to type and propagation through stem cuttings is time-consuming as the cactus is slow in growth. On the other hand, micropropagation provides an avenue for more excellent production of new material in a shorter period. It creates large, disease-free, uniform plants with identical selected genetic traits [12]. However, the increased cost of tissue cultured planting material due to the involvement of skilled labour and sophisticated laboratory is a severe concern. This study compares the two methods of propagation of *H. undatus*, considering the cost involved in producing a thousand plantlets.

2.0 MATERIAL AND METHODS

2.1 *In vitro* regeneration:

The healthy and newly sprouted stems were collected from the mother plant under greenhouse conditions. They were washed under running tap water for about 10 mins, and later dipped in fungicide solution Bavistin @ 0.2 % along with a few drops of Tween 20 for about 30 minutes. They were subsequently washed with distilled water 4-5 times and dipped again in a solution of Streptomycin sulphate @ 0.05% along with Folicur @ 100 ppm and incubated on a rotary shaker for 30 minutes retaining at 50 rpm. Sterilised stems were washed with sterile water and cut into 3-4 cm lengths under a laminar airflow chamber. Cut stems were subjected to Mercuric chloride treatment @ 0.05% for about 30 seconds in a closed container and repeatedly washed with sterile water inside the laminar airflow chamber. Both the end of the mercury chloride treated stems were cut with clean blades to remove any dead tissues. The stems were fragmented into 1 cm explants and inoculated in MS media supplemented with 1.5 mg/L BAP for 30 days. Newly generated multiple shoots were separated into individual nodes, and sub-cultured on MS media supplemented 1.5 mg/L BAP. Plantlets were sub-cultured for a maximum of five sub-cultures in 30 days intervals. The well-developed shoots were transferred to MS media supplemented with 0.1 mg/L IBA and incubated for 30 days. Plantlets with shoot length of > 2 cm and root length of > 1 cm were carefully removed from the culture bottles and washed with sterile water and transplanted into portrays containing sand + vermicompost (1:1) (v/v). Portrays were moistened with half-strength liquid MS media for one month and later regularly watered at weekly intervals.

2.2 Estimation of cost of production:

This study was conducted at the College of Horticulture, University of Horticultural Sciences, Bagalkot, India, from 2019 to 2021. Hence, the cost of production in Indian rupees is calculated based on fixed and variable costs that prevailed during 2019-20 in the region, including taxes.

2.2.1 Tissue cultured plantlets

The cost of production for thousand plantlets of *Hylocereus undatus* through *in vitro* micropropagation was worked out by considering the cost of growth media, hardening cost, fixed capital cost and labour cost etc.

Cost of media

The cost of media (chemicals and growth regulators) for one litre was calculated by considering the best treatments during regeneration.

Cost of media (1L) = (Cost/unit chemical x reqd. quantity for 1 L media)/ Quantity per unit chemical

Cost of growth media for thousand plants

The cost of chemicals required to prepare one-litre media was first calculated. Similarly, the required volume of media for one plantlet right from the initiation of culture till the rooting stage was calculated and multiplied by thousand plants. Consequently, the cost of media for a thousand plants was calculated.

Cost of media for 1000 plants = (Vol. of media reqd. for 1000 plants x cost of 1L media)

Fixed capital cost

The prices of fixed resources prevailing at their use were considered to work out the cost of fixed capital with depreciation calculated at 15 per cent.

The total cost of production (1000 plants)

The total cost of production was calculated by adding the cost involved in media for thousand plants, hardening cost, labour cost, overhead cost and miscellaneous cost.

2.2.2 Conventional stem cuttings

The cost of production of planting materials through the conventional method of stem cutting was worked out in consultation with the nursery, University of Horticultural Sciences, Bagalkot. The costs of cuttings, polybags, media, labour costs and maintenance of the nursery and rooting of cuttings for each unit were estimated. Subsequently, a total cost of production for thousand plants was worked out.

2.3 Cost comparison:

The cost saving was calculated by considering the cost of production per plant of dragon fruit through *in vitro* micropropagation and compared with the cost of production of conventional stem cuttings propagation method.

3.0 RESULTS:

The *in vitro* regeneration of dragon fruit was optimum when *in vivo* stem explant was cultured on MS media supplemented with 1.5 ppm BAP with 100.0 % response (personal communication). Hundred per cent of rooting, early root initiation, and optimum root length were obtained when *in vitro* regenerated shoots were cultured on MS media supplemented with 0.1 ppm IBA. During acclimatisation, the potting mixtures consisting of sand and vermicompost (1:1) exhibit 100.0 % survival, the highest number of shoots and roots per plant, longest shoot and root length.

The production economics was estimated by considering the cost of the best treatment standardised during shoot multiplication, *in vitro* rooting and hardening mentioned above. The total cost of production of planting material through micropropagation has been worked out roughly by considering the cost of media for producing thousand plants, capital involved, labour cost, and hardening, etc., which are discussed in detail.

3.1 Cost of production of tissue cultured plantlets

3.1.1 Cost of chemicals for MS media (1L)

The cost of chemical involved in the preparation of 1L MS media (Stock-A, Stock-B, Stock-C and Stock-D) was determined by estimating the required quantity of chemical (mg) per litre. For growth regulators, the best-standardized concentration in the study (1.5 ppm BAP for shoots and 0.1 ppm IBA for roots) was taken for calculation for the same volume (Table 01). It was observed that the cost of macronutrients, micronutrients, and organic nutrients, including vitamins required for preparing 1L MS media, was Rs. 93.61 and Rs. 0.05 for growth regulators negligible. Thus, Rs. 93.67 was invested in producing one-litre press combined with the best growth regulators.

3.1.2 Cost of growth medium for thousand plants

The media required for producing a thousand plants was worked out by considering the number of

Table 01. Cost of chemicals for 1L MS media used in micropropagation of dragon fruit

SI No.	Chemicals	Quantity (g/unit)	Quantity (mg/unit)	Cost/unit (Rs.)	Required quantity for 1L (mg/L)	Cost/L media (Rs.)
1.	Stock A					
	NH ₄ NO ₃	500	500000	145	1650	0.479
	KNO ₃	500	500000	419	1900	1.592
	CaCl ₂ 2H ₂ O	1000	1000000	2614	440	1.150
	MgSO ₄ 7H ₂ O	1000	1000000	718	370	0.266
	KH ₂ PO ₄	500	500000	686	170	0.233
2.	Stock B					
	H ₃ BO ₃	500	500000	504	6.2	0.006
	MnSO ₄ 4H ₂ O	500	500000	448	22.3	0.020

	ZnSO ₄ 7H ₂ O	500	500000	434	8.6	0.007
	Na ₂ Mo ₄ 2H ₂ O	500	500000	3220	0.25	0.002
	KI	250	250000	2408	0.83	0.008
	CuSO ₄ 5H ₂ O	500	500000	896	0.025	0.000
	CoCl ₂ 6H ₂ O	500	500000	4340	0.025	0.000
3.	Stock C					
	FeSO ₄ 7H ₂ O	500	500000	420	27.8	0.023
	Na ₂ EDTA2H ₂ O	500	500000	3304	37.3	0.246
4.	Stock D					
	Inositol	100	100000	1120	100	1.120
	Nicotinic Acid	100	100000	463	0.5	0.002
	Pyridoxine HCl	25	25000	315	0.5	0.006
	Thiamine HCl	25	25000	514	0.1	0.002
	Glycine	500	500000	1050	2	0.004
	Sucrose	500	500000	506	30000	30.360
5.	Plant growth regulators					
	6-Benzyladenine	25	25000	890	1.5	0.05
	Indole Butyric Acid	25	25000	2030	0.1	0.00
	Total					93.67

necessary sub-cultures, including rooting, the volume of media needed per culture bottle, and the number of plants accommodated per bottle. Approximately 30 mL of MS media, including growth regulators, was used, accommodating an average of five plants requiring four subculturing (3 for shoots and 1 for roots). Thereby a single plant would require about 24 mL right from initiation to completion of rooting, and thousand plants would require about 24000 mL of MS media for complete regeneration. Since the total cost of MS media plus growth regulators for 1000 mL was estimated to be Rs. 93.67, the production of thousand plants of dragon fruit through micropropagation would require approximately Rs. 2,248.08 (Table 02).

Table 02. Cost of growth medium used in micropropagation of dragon fruit

SI No.	Particulars		Cost of media for 1000 plants (Rs.)
1.	Volume of media required/ sub culture (mL)	30	2,248.08 (24.0 L X Rs. 93.67)
2.	Number of subcultures including rooting	4	

3.	Average no. of plants accommodated per culture bottle	5	
4.	Total media required for 5 plants (mL)	120	
5.	Total media required/ plant (mL)	24	
6.	Total media required for 1000 plants (mL)	24000	

3.1.3 Fixed capital cost

The cost of equipment used for culturing, cost involved in erection of polyhouse (100 m²), the cost for laboratory construction and other miscellaneous cost was approximately estimated to be Rs. 69.12 lakh and depreciation of Rs. 10.37 lakh when calculated at 15 % per cent (Table 03). Based on industrial standard, a labour can culture thousand plants a day, and approximately five lakhs' plants can be produced in a year by depreciation of Rs. 2074 was worked out and added for further calculations.

3.1.4 The total cost of production for thousand plants

The total cost of production for thousand plants involves the cost of media for culturing a thousand plants (Rs. 2,248.08), the labour cost of Rs. 6000 (Rs. 1000/day) when estimated that six labour can produce thousand plants (1 day for media preparation, four days for culturing and one day for hardening as per information obtained from Rohini Biotech, Mahalingpur, Karnataka), cost of materials for hardening (Rs. 6,200), depreciation cost of Rs. 2074 (calculated @ 15 %) and another miscellaneous cost of Rs. 500. Thus, a total cost of production for thousand plants was worked out to be Rs. 20,022.08 as shown in Table 04.

Table 03. Cost of fixed capital incurred in micropropagation of dragon fruit

SI No.	Equipment	Cost (in Lakhs Rs.)
1.	Autoclave (3.5 x 2 units)	7.50 L
2.	Laminar Air Flow (1.5 x 2 units)	3.00 L
3.	Growth rack	0.15 L
4.	Air Conditioner	0.35 L
5.	Culture Bottles & Glass wares	0.30 L
6.	pH meter	0.20 L
7.	Weighing Balance	0.50 L
8.	Distillation unit	0.25 L
9.	Magnetic stirrer	0.02 L

10.	Refrigerator	0.15 L
11.	Utilities (Electrical fitting and water supply)	0.10 L
12.	Building	55.00 L
13.	Medium cost Greenhouse (100 m ²)	1.50 L
14.	Miscellaneous	0.10 L
15.	Total cost	69.12 L
16.	Depreciation @ 15 %	10.37 L

Table 04. Total cost of production of dragon fruit plantlets through micropropagation

SI No.	Particulars	Cost (Rs.)	Percent
1.	Growth media	2,248.08	11.23
2.	Hardening a) Potting media b) protrays and polybags c) Agrochemicals	200.00 3000.00 1000.00	20.97
3.	Labour a) Media preparation (1 day) b) Culturing (4 days) c) Hardening (1 day) d) Spraying and watering (2 days) e) Total labour cost (1000 x 8 days)	8000.00	39.97
4.	Utilities (Power and water)	3000.00	14.98
5.	Depreciation @ 15 % of fixed cost	2074.00	10.36
6.	Miscellaneous	500.00	2.50
7.	Total cost of production	20,022.08	100

3.2 Cost of production through conventional stem cuttings

The major cost of conventional propagation through stem cuttings is the potting media and initial stem cuttings, which account for Rs 10 per plant, which is >67% of the total production (Table 05). The maintenance of the nursery requires regular watering in weekly intervals and weeding. Hence, labour and other maintenance costs are very minimal for convention propagation. However, 5-15% of plant deaths are noticed during maintenance.

Table 05. Cost of production of dragon fruit planting materials through stem cuttings

SI No.	Particulars	Cost/unit (Rs.)	Cost/ 1000 cuttings (Rs.)	% contribution
1.	Stem cuttings	5.00	5000.00	0.34

2.	Polybags	1.00	1000.00	0.07
3.	Media	5.00	5000.00	0.34
4.	Labour cost for planting of 1000 cuttings (2 labour)	275.00	550.00	0.04
5.	Labourer required for maintenance of nursery for two months (5 labour)	275.00	1375.00	0.1
6.	Miscellaneous		2000.00	0.14
	Total cost of production		14,925.00	

3.3 Cost Comparison

The cost of production through conventional stem cuttings is lower (Rs. 14.92 per plant) compared to the tissue cultured plantlets (Rs 20.02/plant). However, the current market price of the planting materials is Rs. 40/ plant (information obtained from local nurseries). Hence, the cost of production of tissue cultured plantlets is almost 100% lower than the market price.

4.0 DISCUSSION

The production of planting materials through micropropagation requires high investment, technical know-how and skilled workforce. Favourably, the cost of tissue culture planting material of *Hylocereus undatus* worked out to be economical compared to the market price of planting material raised through stem cuttings with a cost-saving of Rs. 19.98. The cost of hardening accounted for 30.97 % of the total production cost, followed by skilled labour (29.97 %). Mostly, the cost of micropropagated plants will be higher than the conventional method of propagation, as in *Coffea canephora* [1]. The price of tissue culture planting material was as high as US\$ 0.23/ plant against US\$ 0.12 under the conventional method of rooting cuttings. The overall cost of production can further be reduced by scaling up production, enhancing the efficiency of the labour and adaption low-cost *in vitro* techniques such as the use of alternate sources of media, indigenously built equipment over high-cost equipment, and *ex-vitro* rooting instead of *in vitro* rooting. In the present research, the cost of skilled workforce was calculated at the expense of one labour producing 1000 plants in 05 days with five sub-cultures for each plant (60000 plantlets per year). At the commercial level, ten workers (5 days a week, working eight h/day) can produce around 1.0–1.2 million plants per year (100,000–120,000 plantlets/worker/year) during micropropagation of Anthurium, gerbera, and orchid plantlets on agar culture media [4]. The cost of production may be further reduced by 50 % by scaling up the show to 1.5 to 2.0 million plantlets per labour per year [5]. The MS nutrient media may be replaced with Hoagland's solution and liquid fertilisers [13, 14]. Agar and sucrose can be replaced with cheaper alternatives such as Isubgol husk (*Plantago ovata*) and refined sugar/ jaggery, respectively, which can still reduce the overall cost of production [15]. Evidence for the potentiality of psyllium husk as a low-cost gelling agent was reported by Babbar and Jain [3] Razzaq et al. [17] in the *in vitro* wheat culture and use of guar gum was reported by Jain et al. [8] in fungi and bacterial culture. Used of corn starch,

potato starch, rice flour and cassava flour as agar alternatives were also reported by Daud et al. [7] in *Celosia* sp. The hi-tech greenhouse for hardening can also be replaced by a low-cost shade net or plastic tunnels, which will reduce the overall input cost. The cost of production of tissue culture raised culture-raised plants was significantly reduced by *ex-vitro* rooting rather than *in vitro* rooting [16].

5.0 CONCLUSION

Micropropagation offers the advantage of rapid production of disease-free planting material. In dragon fruit, reduced cost of production is an added advantage for the commercial laboratories and the farmers. This study can also help entrepreneurs and farmers decide on the plant production system best suited to their needs and qualifications.

REFERENCES

1. Alves dos Santos MR, Augusto de Souza C, Felix da Rocha J, Ventura de Araujo L, Curitiba Espindula M. Comparison of economic efficiency between *in vitro* and field methods for vegetative propagation of *Coffea canephora*. *Aust. J. Basic Appl. Sci.* 2015; **9**(20): 1-7.
2. Arivalagan M, Karunakaran G, Roy TK, Dinsha M, Sindhu BC, Shilpashree VM, Satisha GC. and Shivashankara KS. Biochemical and nutritional characterization of dragon fruit (*Hylocereus* species). *Food Chem.* 2021; **353**: 1-11.
3. Babbar SB, Jain N. 'Isubgol' as an alternative gelling agent in plant tissue culture media. *Plant Cell Rep.* 1998; **17**(4): 318-322.
4. Cardoso JC, Sheng Gerald LT, Teixeira da Silva JA. Micropropagation in the Twenty-First Century. In: Loyola-Vargas V., Ochoa-Alejo N. (eds) Plant Cell Culture Protocols. *Methods in Molecular Biology*, vol 1815. Humana Press, New York. 2018; pp. 17-46.
5. Chen C. Cost analysis of micropropagation of *Phalaenopsis*. *Plant Cell Tissue Organ Cult.* 2016; **126**:167-175.
6. Choffe JMR, Victor SJ, Murch, Saxena PK. *In vitro* regeneration of Echinaceae pur-purea L: Direct somatic embryogenesis and indirect shoot organogenesis in petiole culture. *In Vitro Cell. Dev. Biol. Plant.* 2000; **36**(1): 30-36.
7. Daud N, Taha RM, Noor NNM, Alimon H. Potential of alternative gelling agents in media for the *in vitro* micropropagation of *Celosia* sp. *Int. J. Bot.* 2011; **7**(2): 183-188.
8. Jain R, Anjaiah V, Babbar SB. Guar gum: a cheap substitute for agar in microbial culture media. *Lett. Appl. Microbiol.* 2005; **41**: 345-349.
9. Kakade V, Jinger D, Dinesh G, Singh G, Bhatnagar PR, Pande VC, Jat RA, Singh AK. Nursery and propagation techniques of dragon fruit. Technical report; ICAR-Indian Institute of Soil and Water Conservation, Research Centre, Anand, Gujarat. 2021; pp. 1-2.
10. Kanchana P, Santha ML, Devi SK, Latha PP, Spruthi N. A review on *Hylocereus undatus*. *Intl. J. Pharm. Technol.* 2019; **11**(1): 6831-6854.

11. Luu TTH, Le TL, Huynh N, Quintela-Alonso P. Dragon fruit: A review of health benefits and nutrients and its sustainable development under climate changes in Vietnam. *Czech J. Food Sci.* 2021; **39**: 71–94.
12. Maximova SN, Alemanno L, Young A, Ferriere N, Traore A, Guiltinan MJ. Efficiency, Genotypic Variability and Cellular Origin of Primary and Secondary Somatic Embryogenesis of the *Theobroma cacao* L. *In vitro Cell. Dev. Biol. Plant.* 2002; **38**: 252-259.
13. Orego KO, Mburugu GN, Mwangi M, Ngugi MM, Ombori O. *In vitro* micropropagation of Cassava through low-cost tissue culture. *Asian J. Agric. Sci.* 2012a; **4**(3): 205-209.
14. Orego KO, Mburugu GN, Mwangi M, Ngugi MM, Ombori O. Low-cost tissue culture technology in the regeneration of sweet potato (*Ipomoea batatas* Lam). *Res. J. Biol.* 2012b; **2**(2): 71-78.
15. Pant M, Mehta R. The economics of plant tissue culture: an Indian perspective. *Asian J. Pharm. Clin. Res.* 2016; **9**(3): 1-2.
16. Ranaweera KK, Gunasekara MTK, Eeswara JP. *Ex vitro* rooting: A low cost micropropagation technique for Tea (*Camellia sinensis* (L.) O. Kuntz) hybrids. *Scientia Hortic.* 2013; **155**: 8-14.
17. Razzaq A, Arshad M, Ashraf S, Akram A, Qayyum A, Mahmood I. Evaluation of Psyllium husk (*Plantago ovata*) as a low-cost gelling agent for callus formation and regeneration in wheat (*Triticum aestivum* L.) cultivar GA-2002. *Wulfenia J.* 2013; **20**(7): 153-161.
18. Vinas M, Fernandez BM, Azofeifa A, Jimenez VM. *In vitro* propagation of purple pitahaya (*Hylocereus costaricensis* [F.A.C. Weber] Britton & Rose) cv. Cebra. *In vitro Cell. Dev. Biol. Plant.* 2012; **48**: 469-477.
19. Wakchaure GC, Kumar S, Meena KK, Rane J, Pathak H. Dragon fruit cultivation in India: Scope, Marketing, Constraints and Policy issues. Technical Bulletin, Pune, Maharashtra, India. 2020; pp. 54.