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STREAMLINED *IN VITRO* PROPAGATION PROTOCOL FOR RAPID MASS MULTIPLICATION OF DRAGON FRUIT (*HYLOCEREUS* spp.)

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ABSTRACT

The present investigation entitled "Streamlined in vitro Propagation Protocol for Rapid Mass Multiplication of Dragon Fruit (*Hylocereus spp.*)" were carried out in the biotechnology laboratory and experimental field of Horticulture, faculty of agriculture, Guru Kashi University, Talwandi Sabo. For In vitro shooting the lowest number of days taken for shoot initiation, highest number of shoots/explants, maximum length of shoot (2.75cm) were obtained from treatment that contained MS Media+ BAP 2.0mg/l+ IBA 0.2mg/l. MS Media+ IBA 1.5mg/l + NAA 0.1mg/l produced the maximum number of roots and maximum length of root. For hardening maximum survival percentage were observed in treatment number H_2 (Sand: Soil: Vermicompost 1:1:1) (91.00%).
Keywords **:** Dragon Fruit, In-Vitro, Explant, MS Media, Hardening

Introduction

Hylocereus spp., also known as dragon fruit or pitaya, is one of the most important tropical fruit crops belonging to family Cactaceae. Dragon fruit is expected to be grown on 3085 hectares of land in India. India produces roughly 4,200 metric tons of dragon fruit, which is a tiny amount (0.20%) of global output (*Wakchaure et al.*, 2020). Dragon fruit has been regarded as the most beautiful species in the cactus family because of its eye-catching colours and sweet, juicy, humorous flavour. The pharmaceutical and culinary sectors both employ pitaya as an additive. Dragon fruit's nutritional qualities and organoleptic traits have greatly captivated customers. As a fruit and decorative crop, it has a lot of potential. and it is in high demand both domestically and abroad. The flowers have delicate, fragrant, creamy white petals with green tepals and several yellow stamens that bloom at night. They are roughly 25 to 35 cm long and 30 cm wide. Various species have various peel and pulp colours, and the fruit is round to slightly oval with many tiny black seeds embedded in the fruit meat (Kanchana *et al*., 2019).

Dragon fruit is often propagated either sexually through seeds or vegetatively through stem cuttings or stem fractions. In order to ensure the genetic integrity of plants grown in vitro and satisfy the increasing demand for its culture, an effective micropropagation technology must be developed (Roman *et al.,* 2014). *H. polyrhizus* explants cultured on MS medium supplemented with 5.5 mg/I. BAP and 0.1 m/l NAA produced maximum average shoot number (6.4) per explant and maximum average plantlet height (2.8 cm) within 30 days (Qin *et al.* 2017).

The present studies were focused to develop an efficient protocol for micropropagation under Punjab Sub-tropics with main objective for standardization in vitro shoot proliferation, rooting and hardening.

Material and Methods

The present investigations on "Streamlined in vitro Propagation Protocol for Rapid Mass Multiplication of Dragon Fruit (*Hylocereus spp.*)" were carried out at the Biotechnology Laboratory & fruit research farm, Faculty of Agriculture, Guru Kashi University, Talwandi Sabo.

Experimental Conditions*:* Healthy and disease-free plant material (apical meristems) of dragon fruit were collected. All the tools like glassware's and culture media were sterilized by autoclaving at 15 psi pressure

and 121°C temperature for 20-30 minutes. Then these were dried in hot air oven at 80-100°C for 2-4 hours.

Surface Sterilization Explants: Surface sterilization is the important step in preparation of explant because controlling fungal and bacterial contamination of plant from field sources is very difficult. Explants were rinsed off properly with tap water to remove soil and washed with 2-3 drops of labolene for 7-10 minutes. Then Bavistin (0.4%), streptomycin (0.1%) for 30 minutes and mercuric chloride (0.1%) for 3 minutes were used during experiment. The explants were inoculated on Murashige sand Skoog (MS) media with pH of the media was kept at 5.7 and the segments of sterilized apical meristem were removed by scalpel and forceps under laminar air flow chamber.

Inoculation and Incubation: The surface sterilized explants were inoculated on MS media supplemented with various concentrations of BA, BAP, KIN, IBA and NAA under the hood of laminar air flow cabinet. The brown cut ends of explants were removed before inoculation to avoid toxic effects of sterilant and browning. After inoculation, the culture jars and test tubes were incubated in incubation room at 25±2°C temperature, to 75% relative humidity, with photoperiod 16 hours of light and 8 hours of light with 3000 lux light intensity using white fluorescent light.

Explants were given different treatments at different concentration for shoot initiation and multiplication, rooting and hardening. In order to standardize best combination of growth hormones, different growth regulators BAP, IBA, NAA were used. The details of treatment are as:

Treatment Details

Table 1: Standardization of shoot proliferation

Treatment no.	Treatment (mg/l)
T_1	MS Media (no growth regulator)
T_2	MS Media + BAP 1.0
T_3	MS Media + BAP 2.0
T_4	MS Media + BAP 1.0 + IBA 0.2
T_5	MS Media + BAP 1.5 + IBA 0.2
T_6	MS Media + BAP 2.0 + IBA 0.2
T_7	MS Media + BAP 2.5 + IBA 0.5
T_8	MS Media + BAP 3.0 + IBA 0.5

Table 2: Standardization of Rooting

Treatment no.	Treatment (mg/l)
G_1	MS Media (no growth regulator)
G_2	MS Media + IBA 0.5
G_3	MS Media + IBA 1.0
G_4	MS Media + IBA 1.5
G_5	MS Media + IBA 0.5 + NAA 0.1
G_6	MS Media + IBA 1.0 + NAA 0.1
G_7	MS Media + IBA 1.5 + NAA 0.1
G_8	MS Media + IBA 2.0 + NAA 0.1

Table 3: Influence of different hardening treatments for dragon fruit var. Siam red

Treatment No.	Treatment
H_1	Soil + vermicompost (2:1)
H_2	Sand + Soil + vermicompost (1:1:1)
H_3	Cocopeat + vermicompost (2:1)
H_4	Sand + Cocopeat + vermicompost (1:1:1)

Statistical Analysis

The collected raw data during experiment trial was transferred on excel sheet in Microsoft Excel. The experimental design used was CRD (completely randomized design) with four replications of each treatment. The data was analysed using the software OPSTAT developed by CCSHAU, Hisar.

Result and Discussion

Standardization of shoot proliferation

Out of eight treatments the minimum number of days (32days) for root initiation, Highest number of shoots/explants (4.75) and maximum length of shoot/explant (2.75cm) were obtained from treatment that contained MS Media+ BAP 2.0mg/l+ IBA 0.2mg/l. Maximum number of days (85 days), minimum number of shoots/explants (1.00) and minimum length of shoot (0.25cm) were obtained in treatment that contained MS basal media.

Similar results were observed by Dahanayake and Ranawake (2011) and Vinas *et al.,* (2012). They stated that in vitro shoots of dragon fruit cultured on MS media supplemented with 2.0mg/l produced highest number of shoots. Higher concentration of BAP can reduce total number of shoots (Lema Ruminska and Licznerska, 2004).

Standardization of rooting

Maximum number of roots (3.75%) and maximum length of root (5.75cm) were obtained from treatment fortified with MS Media+ IBA 1.5mg/l+ NAA 0.1mg/l. Minimum number of roots per plantlet (0.25%) and minimum length of root (0.25cm) by MS basal media.

The similar findings were observed by El Finti *et al.* (2012) that a combination of auxins results in highest number of roots. In his study for higher number of roots combination of 0.5ppm IBA with NAA were used.

Standardization of protocol Hardening of regenerated plant

Out of the treatments the maximum survival percentage was observed in treatment number H_2 (Sand: Soil: Vermicompost) (91.00%) and minimum survival percentage was observed in treatment H_1 (Soil: Vermicompost) (60.50%).

Similar high success rates, have been reported in several other cactus species (Quiala *et al.,* 2009).

The success of *in vitro* regeneration protocol largely depends on survival and growth performance of propagated plantlets *ex vitro* (Josho and Dhar, 2003).

Conclusion

The In vitro shoot regeneration of dragon fruit was optimum when explants were cultured on MS media fortified with BAP 2.0mg/l and IBA 0.2mg/l. For rooting, maximum rooting were obtained when regenerated shoots were cultured on MS media supplemented with IBA 1.5mg/l and NAA 0.1mg/l. Potting mixture consisting of sand, soil, vermicompost (1:1:1) exhibited the highest survival rate.

Table 4: Effect of growth hormones on shoot proliferation

Treatment No.	Treatment (mg/l)	Days taken for shoot initiation	Total no. of shoots/ explant	Average length of shoot /explant (cm)
T_1	MS Media (no growth regulator)	85.00	1.00	0.25
T_2	MS Media +BAP 1.0	72.25	1.25	0.50
T_3	MS Media +BAP 2.0	65.00	1.50	0.50
T_4	MS Media +BAP 1.0 + IBA 0.2	57.25	1.75	0.75
T_5	MS Media + BAP 1.5 + IBA 0.2	40.75	3.25	1.75
T_6	MS Media + BAP 2.0 + IBA 0.2	32.00	4.75	2.75
T_7	MS Media + BAP 2.5 + IBA 0.5	54.50	2.50	1.50
T_8	MS Media + BAP 3.0 + IBA 0.5	52.25	2.00	1.00
	CD (0.05)	0.59	0.67	0.73
	SE(m)±	0.20	0.22	0.25
	SE(d)	0.28	0.32	0.35
	C.V(%)	0.71	20.28	44.44

Table 5: Effect of growth hormones on in vitro rooting

Treatment no.	Treatment (mg/l)	Total no. of roots /plantlet	Average length of roots/plantlet (cm)
G_1	MS Media (no growth regulator)	0.25	0.25
G_2	MS Media + IBA 0.5	2.00	1.25
G_3	MS Media + IBA 1.0	2.25	1.25
G_4	MS Media + IBA 1.5	2.50	1.50
G_5	MS Media + IBA 0.5 + NAA 0.1	2.75	2.25
G_6	MS Media + IBA 1.0 + NAA 0.1	3.00	4.50
G_7	MS Media + IBA 1.5 + NAA 0.1	3.75	5.75
G_8	MS Media + IBA 2.0 + NAA 0.1	2.25	1.75
	C.D (0.05)	0.65	0.76
	SE(m)±	0.22	0.26
	SE(d)	0.31	0.36
	C.V(%)	18.98	22.50

Table 6: Influence of different potting mixture on survival rate

Treatment no.	Treatment	Survival (%)
H_1	Soil + vermicompost (2:1)	60.50
H_2	Sand + Soil + vermicompost (1:1:1)	91.00
H_3	Cocopeat + vermicompost (2:1)	78.50
H_4	Sand + Cocopeat + vermicompost (1:1:1)	83.25
	C.D (0.05)	0.74
	SE(m)±	0.23
	SE(d)	0.33
	C.V(%)	0.61

(a) Shoot proliferation

(b) *In vitro* rooting

(c) Hardening

Fig. 1: (a) Shoot proliferation, **(b)** *In vitro* rooting and **(c)** Hardening of Dragon Fruit

Scope for the Future

To increase the rate of multiplication for upcoming breeding initiatives, more research utilising various explants is required.

Author Disclaimer Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of this manuscript.

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