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Optimization of low-cost potting mixture for hardening of *in vitro* raised plants of dragon fruit

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Abstract

Dragon fruit cultivation is surging massively because of its high nutritional and medicinal value and profit to the farmers. Micropropagation offers healthy, vigorous and uniform planting material to meet the soaring demand. However, the high cost of tissue-cultured planting material prevents farmers from buying it. Commonly used potting media, peat, perlite, and vermiculite, contribute significantly to the higher cost of micropropagules. In this study, low-cost potting media such as soil, sand and vermicompost were evaluated as alternative potting media for hardening tissue-cultured plants of dragon fruit. Well-rooted micro shoots were transplanted into Protrays containing different proportions of soil, sand and vermicompost and the plant survivability, the number of shoots and roots and their respective length and chlorophyll content were recorded. Sand and vermicompost (1:1 v/v) were found to be the best potting media exhibiting 100% survivability (15 days of hardening of the plants), producing the highest number of shoots (7.66 shoots/plant) with a mean length of shoots (10.61 cm) and 11.27 roots per plant with a mean length of 10.38 cm root length (at 120 days of hardening). Cent per cent survivability was noticed even at 120 days after hardening. Sand and vermicompost, with less than 10% of the cost of peat, perlite and vermiculite may be used as low-cost potting media for the hardening of tissue-cultured plants.

Key words: Dragon fruit, micropropagation, hardening, low-cost potting media, sand and vermicompost

Introduction

Pitaya (*Hylocereus undatus* (Haw.) Britton and Rose)), commonly known as the dragon fruit, is a nutritionally rich and medicinally important fruit crop. It is a climbing epiphytic perennial cactus belonging to Cactaceae. It is a climbing plant with aerial roots that bear a glabrous and scaly berry (Fournet, 2002). The cultivated dragon fruits are diploid (2n = 22). Most of the *Hylocereus* spp. are native to Latin America (Mexico and Columbia), and some ornamental species have originated from the West Indies (Le Bellec *et al.*, 2006). However, they are distributed to most tropical and subtropical regions, including the arid coastal plains of the Pacific Coast and in more humid, cloud forest conditions at higher elevations (Trivellini *et al.,* 2020).

Dragon fruits are low-calorie fruits, rich in vitamins (C, B_1, B_2, B_3), high soluble fibre, and minerals (Ca, Fe, P). Soft edible seeds are rich in essential fatty acids, linoleic acid and linolenic acid- a necessity in human metabolism. Still, they cannot be synthesized from other food components by the human body (Vaillant *et al.*, 2005; Nerd *et al.*, 1999; Stintzing *et al.*, 2002). Dragon fruit is a promising source of alternative medicine (Hitendraprasad *et al.*, 2020) as it has enormous health benefits such as anti-microbial, antioxidant, constipation, anti-cancer, immune system, anti-diabetic, maintaining cholesterol level, promoting healthy hair and skin, preventing anaemia, improve appetite, vision and brain function (Sharma *et al.,* 2020).

Dragon fruit is a non-facultative CAM-type plant with high water use efficiency and maintains photosynthesis under stress conditions (Kanno *et al.*, 2009). Dragon fruit exhibits drought tolerance and adaptation to dry and hot environments due to their diurnal closure and nocturnal stomata opening to save water (Wang *et al.*, 2019). Due to its drought tolerance and high profit with minimal inputs, dragon fruit cultivation has been spread to several countries like the Philippines, Myanmar, Malaysia, Mexico, Guatemala, Nicaragua, northern Australia, Okinawa (Japan), Sri Lanka, southern China, southern Florida, Taiwan, Thailand, Vietnam, Hawaii, Indonesia, Israel and the West Indies (Mercado-Silva, 2018). Dragon fruit was introduced to India during the late 1990s (Arivalgan *et al.*, 2019). The area under cultivation in India gradually increased from 4 ha to over 400 ha in different states from 2005 to 2017. Its fruit production increased drastically to more than 12,000 tons over 3,000-4,000 ha in 2020 (Wakchaure *et al.,* 2020).

With increasing cultivation worldwide, the availability of quality planting material is a major constraint. Dragon fruit is conventionally propagated either vegetatively through stem cuttings or stem fractions or sexually through seeds (Kakade *et al.,* 2021). However, conventional propagation methods are not efficient for producing a large number of true-to-type plants within a short period. Production of disease-free, clonal plantlets through micropropagation is gaining momentum to fulfil the increasing demand for this potent fruit crop. Multiple *in vitro*

direct regeneration techniques have been developed (Dahanayake and Ranawake, 2011; Vinas *et al.*, 2012; Fan *et al.,* 2013; Nunez *et al.*, 2014; Roman *et al.*, 2014; Hua *et al.,* 2015; Javier *et al.*, 2015; Thinesh and Seran, 2015; Qin *et al.*, 2017; Ng *et al.*, 2019; Pedda Kasim *et al.,* 2019; Bozkurt *et al.*, 2020; Mustafa and Saad, 2020). Most methods are efficient in the clonal production of large planting material.

However, high mortality is observed upon transfer of micro shoots to *ex vitro* conditions as the cultured plants have non-functional stomata, weak root systems and poorly developed cuticles (Mathur *et al.*, 2008). In dragon fruit, 96.40 and 92.90 % survivability in perlite + peat moss (2:1) and vermiculite + peat moss (1:1), respectively, was observed (Vinas *et al.*, 2012). Similarly, Mustafa and Saad (2020) obtained 80 % survival when well-developed *in vitro* regenerated plantlets were transferred in plastic pots containing a mixture of sand, perlite, and peat (1:1:2 v/v) within two months. However, the high cost of perlite, peat moss and vermiculite increases the cost of production of micropropagated planting material. Hence, less expensive potting media, sand, soil and vermicompost in different combinations were evaluated in this study to decrease the cost of production.

Material and methods

***In vitro* regeneration of dragon fruit:** Stem pieces excised from the six months old healthy mother plants grown under the greenhouse were used as explants. Stems were surface sterilized by treating with bavistin @ 0.2 % for 30 minutes and streptomycin sulphate @ 0.05% along with Folicur @ 100 ppm. Surface sterilized stems were cut into 3-4 cm and, treated with mercuric chloride (0.05%) for 30 seconds and washed thoroughly with sterile water inside the laminar airflow chamber. Both the ends of shoot fragments were excised to remove dead cells and cut into 1 cm explants.

The explants were inoculated in MS media supplemented with 1.5 mg/L BAP for 30 days for adventitious shoot initiation. Multiple shoots observed at 30-45 days of culture were separated into individual micro shoots and subcultured on MS media supplemented 1.5 mg/L BAP for further shoot multiplication. The well-developed micro shoots were rooted on MS media supplemented with 0.1 mg/L IBA for 30-45 days. Plantlets with well-developed shoots (>2 cm) and roots (>1 cm) were used for hardening.

Hardening: Well-developed plantlets were carefully removed from the culture bottles without damaging the shoots and roots. Media attached to the roots were thoroughly washed with sterile water and blotted on a sterile blotting paper. Subsequently, plantlets were treated with 0.5% bavistin and 0.05% streptomycin sulphate for 10 min and thoroughly washed with sterile water and transplanted for different pre-sterilized potting media.

Potting media: *in vitro* regenerated plantlets were transplanted into Protrays containing different potting media; Sand alone, red laterite soil alone, vermicompost alone, sand + vermicompost (1:1) (v/v), soil + vermicompost (1:1) (v/v), sand + soil (1:1) (v/v) and sand + soil + vermicompost (1:1:1) (v/v/v). Protrays were kept in the growth room for thirty days and later transferred to the 50% shade house. Protrays were moistened with half-strength liquid MS media through a portable hand sprayer twice a week for one month and later regularly watered at weekly intervals.

Observations recorded: The survivability of the plants on different potting media was recorded 15 days after hardening. The number of shoots/ plantlet, shoot length (cm), the number of roots per plantlet, root length (cm) and chlorophyll content (mg/g fresh weight of tissue) were recorded on 120th day of hardening.

Quantification of chlorophyll content: Chlorophyll content in the leaves was estimated at 120th days after hardening. It was determined as per DMSO (Dimethyl sulfoxide) method of Hiscox and Israelstam (1979). In brief, 100 mg of the freshly harvested shoot was washed with sterile water and cut into pieces. Stems were homogenized in 7 mL of DMSO and incubated at 65 ^oC for three hours. The volume of the crude extract was made up to 10 mL with DMSO, and the optical density of the extract was measured at 645 and 663 nm using DMSO as blank. The amount of chlorophyll content in the extract was calculated in mg chlorophyll per gram fresh weight of tissue using the following equations by Arnon (1949):

Chlorophyll- a = $12.7\ (A_{663}) - 2.69\ (A_{645}) \times V/(1000 \times W \times a)$ (mg/g fresh weight)

Chlorophyll- b = $22.9\ (A_{645}) - 4.68\ (A_{663}) \times V/(1000 \times W \times a)$ (mg/g fresh weight)

Total chlorophyll = $20.2\ (A_{645}) + 8.02\ (A_{663}) \times V/(1000 \times W \times a)$ (mg/g fresh weight)

Where,

A= Absorbance at specific wavelength (645 and 663 nm)
V = Final volume of the chlorophyll extract (10 mL)
W = Fresh weight of the sample (0.10 g)
A = Path length of light (1 cm)

Statistical analysis: The experiment was conducted in a complete randomized design (CRD) consisting of seven treatments replicated thrice. The level of significance used for the F test and critical difference (CD) was calculated at P=0.01. Values in percentages were subjected to arc sine transformation to ensure homogeneity. Wherever values were 0 per cent or 100 per cent, arcsin (1/4n) and arcsin (100-1/4n) were taken, where n is the number of observations that make up the percentage, were substituted, respectively (Zar, 1984).

Results

Hardening of micropropagated plants is a crucial task in increasing the survivability and vigour of the plants. Dragon fruit, being suited for humid sandy loam soils, different potting media were tested for hardening of micropropagated plants. The plantlets with well-developed shoots and roots were acclimatized in room condition for one month and inside the greenhouse for three months on different potting mixtures (Fig. 1). Observations on different phenotypic characters such as per cent survival (%), number of shoots/ plant, shoot length (cm), number of roots/ plant, root length (cm) were recorded and presented in Table 1 (Fig. 2). To estimate the photosynthetic efficiency, chlorophyll content in the hardened plants were estimated and statistically analyzed.

Survival of plantlets (%): There was a significant effect of different potting media on the per cent survivability of tissue-cultured derived plantlets of dragon fruit. A hundred per cent survival was recorded in T_3 (vermicompost), T_4 (sand + vermicompost (1:1), T_5 (soil + vermicompost (1:1) and T_7 (sand + soil + vermicompost (1:1:1), which were on par with potting medium containing only sand (88.89 %). T_2 (soil) recorded the

Fig. 1. Acclimatization of tissue culture regenerated plants of dragon fruit (a) Initial hardening of tissue cultured dragon fruit plant, (b) Hardening of tissue cultured dragon fruit plant in greenhouse after four months, (c) Tissue cultured dragon fruit plants transferred in poly bags

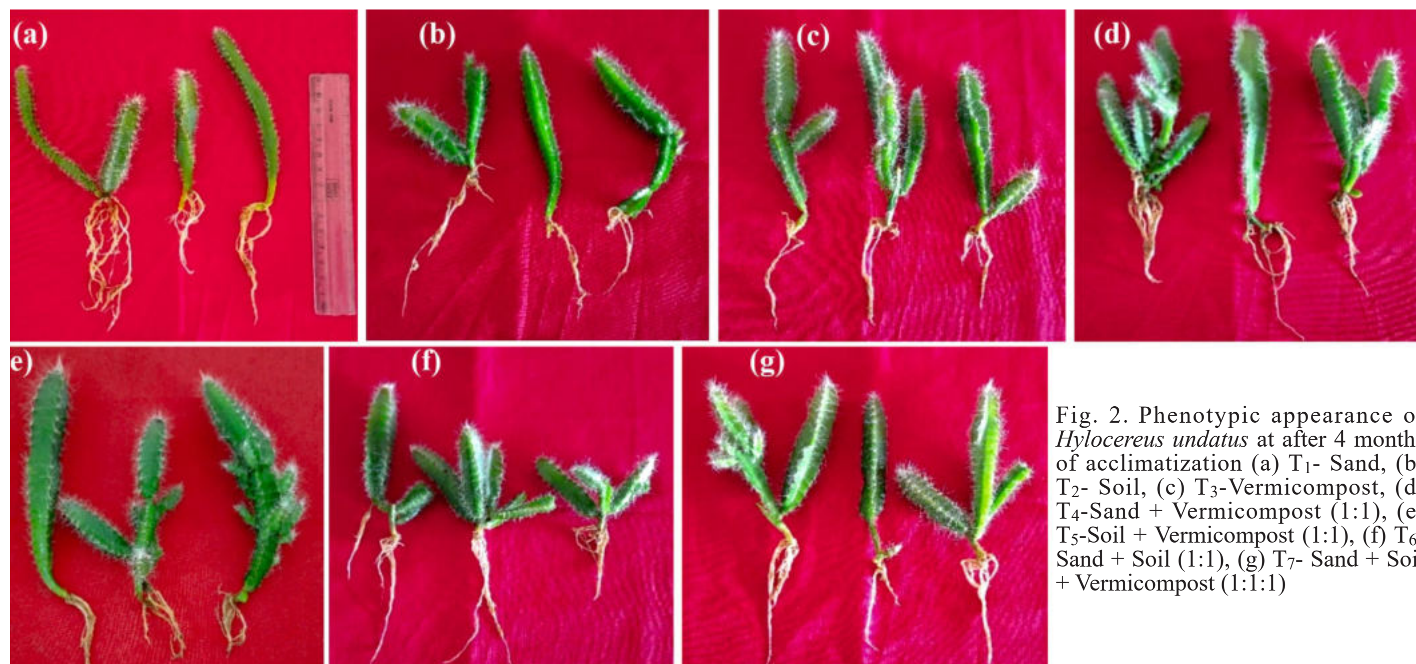

Fig. 2. Phenotypic appearance of *Hylocereus undatus* at after 4 months of acclimatization (a) T_1- Sand, (b) T_2- Soil, (c) T_3-Vermicompost, (d) T_4-Sand + Vermicompost (1:1), (e) T_5-Soil + Vermicompost (1:1), (f) T_6-Sand + Soil (1:1), (g) T_7- Sand + Soil + Vermicompost (1:1:1)

Table 1. Effect of potting media on acclimatization of *in vitro* regenerated plantlets of dragon fruit

Treatment	Per cent survival	No. of shoots/ plantlet	Length of shoots/ plantlet (cm)	No. of roots/ plantlet	Length of roots/ plantlet (cm)	Chlorophyll a (mg/g fresh weight)	Chlorophyll b (mg/g fresh weight)	Total Chlorophyll (mg/g fresh weight)
T_1: Sand	88.89 (73.94)*	2.00	8.61	9.11	9.61	0.264	0.184	0.448
T_2: Soil	77.77 (62.18)	1.91	7.42	4.63	5.42	0.324	0.211	0.535
T_3: Vermicompost	100.00 (90.00)	5.11	8.83	6.20	7.41	0.330	0.193	0.523
T_4: Sand + Vermicompost (1:1) (v/v)	100.00 (90.00)	7.66	10.61	11.27	10.38	0.277	0.193	0.470
T_5: Soil + Vermicompost (1:1) (v/v)	100.00 (90.00)	2.77	8.00	6.93	4.65	0.284	0.206	0.489
T_6: Sand + Soil (1:1) (v/v)	83.33 (65.90)	4.17	6.42	8.60	8.30	0.253	0.185	0.438
T_7: Sand + Soil + Vermicompost (1:1:1) (v/v)	100.00 (90.00)	4.66	8.33	8.37	6.92	0.291	0.176	0.467
S. Em ±	3.35	0.46	0.77	0.23	0.43	0.032	0.016	0.043
CD at 1%	14.09	1.95	NS	0.96	1.82	NS	NS	NS

*The values given in parentheses are arc sine transformed values

lowest survival per cent (77.77 %) followed by T_6; potting mix containing sand + soil (1:1), recording 83.33 per cent.

Number of shoots per plant: Significantly highest number of shoots (7.66) per plant was noticed in sand + vermicompost (1:1) (T_4) followed by T_3 (vermicompost), recording 5.11 shoots per plant and statistically on par with T_7 (sand + soil + vermicompost) and T_6 (sand + soil) that recorded 4.66 and 4.17 shoots per plant, respectively. A minimum number of shoots (1.91) per plant was recorded in T_2 (soil).

Length of shoots per plant: The effect of potting media on the mean length of shoots didn't differ significantly, but the mean length of the shoot was found to be better in treatment T_4 (sand + vermicompost) (10.61 cm) followed by T_3 and T_1. The shortest mean shoot length was observed in T_6 (6.42 cm).

Number of roots per plant: The number of roots per plant was significantly different among the different potting mixtures used. Treatment T_4 (sand + vermicompost) recorded significantly the highest number of roots (11.27), which was followed by T_1 (sand), producing 9.11 roots per plant and on par with T_6 (sand + soil) and T_7 (sand + soil + vermicompost) recording 8.60 and 8.37 roots per plant, respectively. A significantly low number of roots per plant (4.63) was noticed in T_2 (soil).

Length of roots per plant: The length of roots was also influenced by potting media with significant differences. The longest root (10.38 cm) was observed in T_4 (sand + vermicompost), which was statistically on par with T_1 (sand) (9.61 cm). The shortest length of root (4.65 cm) per plant was recorded in T_5 (soil + vermicompost).

Chlorophyll content: The chlorophyll a and b and total chlorophyll content of *in vitro* derived hardened plants didn't significantly differ among the treatments. Chlorophyll-a content was highest in T_3 (vermicompost) (0.33 mg/g of fresh weight) and the lowest (0.25 mg/g of fresh weight) in T_6 (sand + soil). The maximum chlorophyll b content of 0.211 mg/ g of fresh weight was noticed in T_2 (soil) and the minimum (0.176 mg/ g net weight) in T_7 (sand + soil + vermicompost). The total chlorophyll content as influenced by potting media was also non-significant, but it was found to be better in treatment T_2 (soil) (0.535 mg/ g of fresh weight), and the least (0.438 mg/ g of fresh weight) was recorded in treatment T_6 (sand + soil).

Discussion

The success of any *in vitro* regeneration protocol largely depends on the survival and growth performance of propagated plantlets *ex vitro* (Joshi and Dhar, 2003). Acclimatization is the most crucial process of micro-propagation as the *in vitro* raised plantlets are not readily adapted for *in vivo* conditions (Vasane, and Kothari, 2006). A heterotrophic mode of nutrition and poor mechanism for control of water loss further renders high mortality. The physiological and anatomical characteristics of micro-propagated plantlets necessitate that they are gradually acclimatized to the environment of the greenhouse or field (Hazarika, 2003). Thus, different potting mixes (soil, sand, vermicompost) in the different ratios were evaluated in the present study for the successful acclimatization of *in vitro* regenerated plantlets of *H. undatus*.

The per cent survival after three months of hardening ranged from 77.77 % (T_2: soil) to 100.00 % (T_3: vermicompost, T_4: sand + vermicompost (1:1), T_5: soil + vermicompost (1:1) and T_7: sand + soil + vermicompost (1:1:1)). Per cent survivability of plantlets was similar to the reports of Mustafa and Saad (2020) with 80.0 % survival in a potting mix of sand, perlite and peat (1:1:2) within two months in *H. undatus.* Similarly, survivability of tissue-cultured planting material was >90 % in perlite + peat moss (2:1) and vermiculite + peat moss (1:1).

The number and length of shoots were better in T_4 where sand and soil were used in equal proportion (7.66 shoots/ plant and 10.61 cm, respectively) followed by vermicompost alone. However, shorter shoot length (6.42 cm) and lower shoot number (1.91) were recorded in hardening media where soil + sand or soil alone were used, respectively. Similarly, the number and length of roots were also better in treatment T_4 (sand + vermicompost) (11.27 and 10.38 cm, respectively), followed by sand (9.11 shoots/ plant and 9.61 cm). However, the number and length of roots were inferior where soil alone or soil + vermicompost were used. The chlorophyll a, b, and total chlorophyll content didn't show any significant difference and were found to be similar in all the treatments, but better chlorophyll contents were observed when the soil was used as a hardening medium.

The better result found with T_4 (sand + vermicompost) may be due to the presence of vermicompost, which is the source of organic nutrients that the plantlets can absorb, and the porosity or aeration provided by the sand. Kansara *et al.* (2013) reported that better hardening in vermicompost might be due to rich organic matter sources providing strength and essential nutrients for the survival of the *in vitro-raised* plants. Unlike vermicompost and sand, the soil doesn't have enough aeration and mineral nutrients that are essential to establish the plants. This may be attributed to the compact nature of the clay soil restricting the growth and development of new roots for nutrient absorption to sustain the plants; thus, high mortality and inferior growth were observed. El Finti *et al.* (2012) and Khalafalla *et al.* (2007) successfully acclimatized *Opuntia ficus-indica* with 100.0 % survival in autoclaved soil and autoclaved soil + sand, respectively. Vinas *et al.* (2012) successfully acclimatized purple pitaya with 96.4 % and 92.9 % in perlite + peat moss (2:1) and vermiculite + peat moss (1:1), respectively, in the greenhouse. Parkhe *et al.* (2018) reported 100.0 % survival of plantlets with maximum height (30.86 cm), pseudo stem girth (2.15 cm), number of leaves (7.15), root length (28.2 cm) after 45 days of hardening in poly bag containing garden soil and FYM (3:1) and chlorophyll content of 1.405 mg/g after 120 days of planting in tissue cultured Grand Naine banana.

In vitro regenerated dragon fruit platelets hardened on the sand + vermicompost (1:1 v/v) performed as the best potting mix with maximum survival percentage and best vegetative and root growth. The cost of both media is less expensive than peat, perlite and vermiculite, thus decreasing the cost of production. Though the vegetative parameters were inferior in the sand, the root numbers and length were satisfactory. However, soil alone was found to be the most unfavourable for hardening plantlets of *H. undatus*. The physical, chemical and biological properties of the potting mixture are important for the establishment of *in vitro* produced plantlets.

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