The Pharma Innovation Journal 2022; 11(8): 1849-1852

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(8): 1849-1852

www.thepharmajournal.com
Received: 07-05-2022
Accepted: 17-06-2022

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Standardization of acclimatization and field establishment of *in vitro* regenerated Dragon fruit (*Hylocereus undatus*)

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Abstract
Dragon fruit (*Hylocereus undatus*) belongs to the family *Cactaceae* and are climbing vines that have received worldwide attention recently. The seed viability of stored dragon fruit is very low, stem cuttings are used as planting material in dragon fruit but the plant dose not exhibit a sufficient number of buds to regenerate plants on a large scale. Therefore, *in vitro* propagation techniques can meet the high demand of plants throughout the year. When the successfully *in vitro* raised plantlets of Dragon fruit are transplanted from the culture room to greenhouse conditions, they may desiccate or wilt rapidly and can die because of changes in the environment, unless substantial precautions are taken to adapt plantlets to a new environment. To improve the survival percentage under hardening Soil, sand, vermicompost and cocopeat in (1:1:1:1) combination was proved to be the best for maximum success in *ex vitro* survival of dragon fruit plantlets.

Keywords: Acclimatization, training, survival, cocopeat, *in vitro*

Introduction
Dragon Fruit belongs to the family Cactaceae and grows best in dry, tropical, or subtropical climates where annual rainfall ranges from 20-50" per year. The important part of this plant is the fruit, commonly known as dragon fruit. The fruit is red-skinned covered with large and long scales, red and green at the tips with white flesh and black seeds. 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 flavour (Gao-Xi and Wan, 2004) [3]. The juicy flesh can be used in marmalade, jellies, ice creams and soft drinks (Xiang *et al.* 2004) [18]. Dragon fruits do not contain cholesterol, saturated fat. Therefore, regular consumption will help manage blood pressure and control cholesterol levels (Zhijian and Xin, 2003) [20]. 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 (Wichienchot, 2010; Dasaesamoh *et al*., 2016; Vaillant *et al.*, 2005; Le Bellec, 2003) [17, 1, 16, 6]. 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 (Stintzing *et al.* 2003). 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) [12]. The flower buds of dragon fruit are used to make soups or mixed with salads and the red pulp of the dragon. The multiplication of *H. undatus* is easily by cutting off the stems, *in vitro* multiplication of younger shoots of mature plants, and sowing of seeds (Le Bellec and La, 2003; Yassen, 2002) [6, 19]. The plants take 3 years to grow from seed. Thus, the multiplication of dragon fruits under *in vitro* conditions is a superlative method. Tissue culture provides an alternative solution for producing many genetically similar, phytosanitarily and physiologically high-quality plantlets within a limited period. In this process, a high survival percentage, associated with a high standard of acclimatized plantlets, is desirable in commercial labs and companies involved with dragon fruit micropropagation. Tissue culture-raised plants often require extensive-hardening treatments to prevent high mortality after transfer to *ex vitro* conditions (Pospíšilová *et al.*, 1999; Ziv and Chen, 2008) [9, 21].

Plantlets produced *in vitro* are unable to develop resistance to minor and major microbial pathogens or other biotic and abiotic stresses caused by *in vitro* controlled conditions, which are characterized by an aseptic environment with small variations in temperature, high relative air humidity, high availability of nutrients, low light intensity and a low carbon dioxide (CO_2) concentration (Teixeira da Silva *et al*., 2015) [15]. These conditions result in photo mixotrophic development and the need for a carbohydrate source in the culture medium. Their subsequent survival, after transfer to greenhouse or field conditions, is adversely affected by physiological and anatomical deficiencies.

Materials and Methods

1. Acclimatization of *in vitro* derived Plantlets

In vitro rooted plantlets having at least 2 roots of 2 to 4-cm length were washed carefully with water to remove traces of agar and then transferred to the pots containing different potting mixtures *viz*. 1. Soil + Vermicompost (1:1), 2. Soil + sand + vermicompost (1:1:1), 3. Soil + sand + vermicompost + Cocopeat (1:1:1:1). Cocopeat thoroughly drenched with 0.1% Bavistin for 10 min before mixing with the other substrates. The pots were covered with tight plastic covers to prevent desiccation and to avoid rapid changes in the environment, and were acclimatized in the modified polyhouse 20–30 °C temperature and 14 h illumination. During the hardening procedure, plastic covers were gradually perforated after 15 days, and after 1 month they were removed and the plants were maintained in the shade net house conditions for 15 more days.

2. Field planting and training of plantlets

Successfully acclimatized *in vitro-grown* plants of dragon fruit were planted at the Horticulture Research Centre of Sardar Vallabhai Patel University of Agriculture and Technology. Cemented pols with overhead mounted cemented rings were established for training the plantlets. The dragon fruit block was laid out in a square system in which poles were placed at a spacing of 3 m × 3 m between the rows and 3m × 3 m between the plants. 2 plants on each pole in east-west direction and 20cm away from the pole was planted.

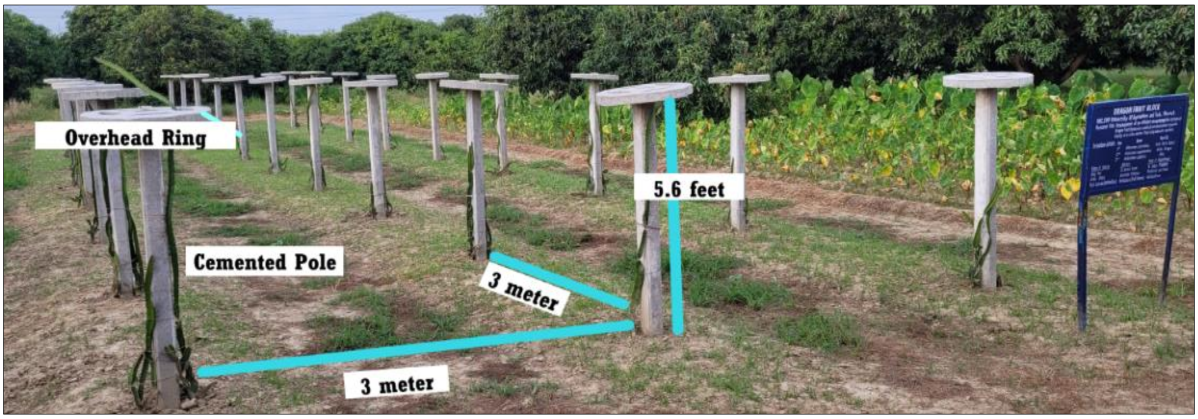

Fig 1: Dragon fruit block

Experimental findings

Sudden environmental changes during transfer from *in vitro* to *ex vitro* conditions are a stress for plantlets. Plantlets must repair structural and functional abnormalities caused by *in vitro* environments (Pospíšilová *et al*., 1999; Ziv and Chen, 2008) [9, 21] and must adapt to a new environment. Chemical and physical factors of both the *in vitro* and the *ex vitro* environments may affect the success of this transition, i.e., the success of acclimatization. Therefore, there are two opportunities to increase the rate of survival and enhance the growth of plants during acclimatization: one is by harnessing and using the long-term or post-effects of *in vitro* conditions (Teixeira da Silva *et al*., 2015a) [15], and the other is the appropriate choice of conditions for acclimatization.

Ex vitro conditions, such as environmental factors and the substrate used for acclimatization, as applied to Dragon fruit, are listed in Table1 and Figure1. The *in vitro* derived plantlets of Dragon fruit were planted in different combinations of substrates to evaluate the survivability during acclimatization. The highest survival percentage was observed under TM3 (Soil + Sand + Vermicompost + Cocopeat (1:1:1:1), which gives 91.33±1.15% success, followed by TM2 (Soil + Sand + Vermicompost (1:1:1). However, lowest survivability 76.55±2.87% were observed under TM1 (Soil + Vermicompost (1:1).

Table 1: Survival percentage of *in vitro* derived plants during hardening in different media

Treatments	Substrate	Survival
TM1	Soil + Vermicompost (1:1)	76.55±2.87a
TM2	Soil + Sand + Vermicompost (1:1:1)	82.94±1.47b
TM3	Soil + Sand + Vermicompost + Cocopeat (1:1:1:1)	91.33±1.15c
SE(m)		2.21

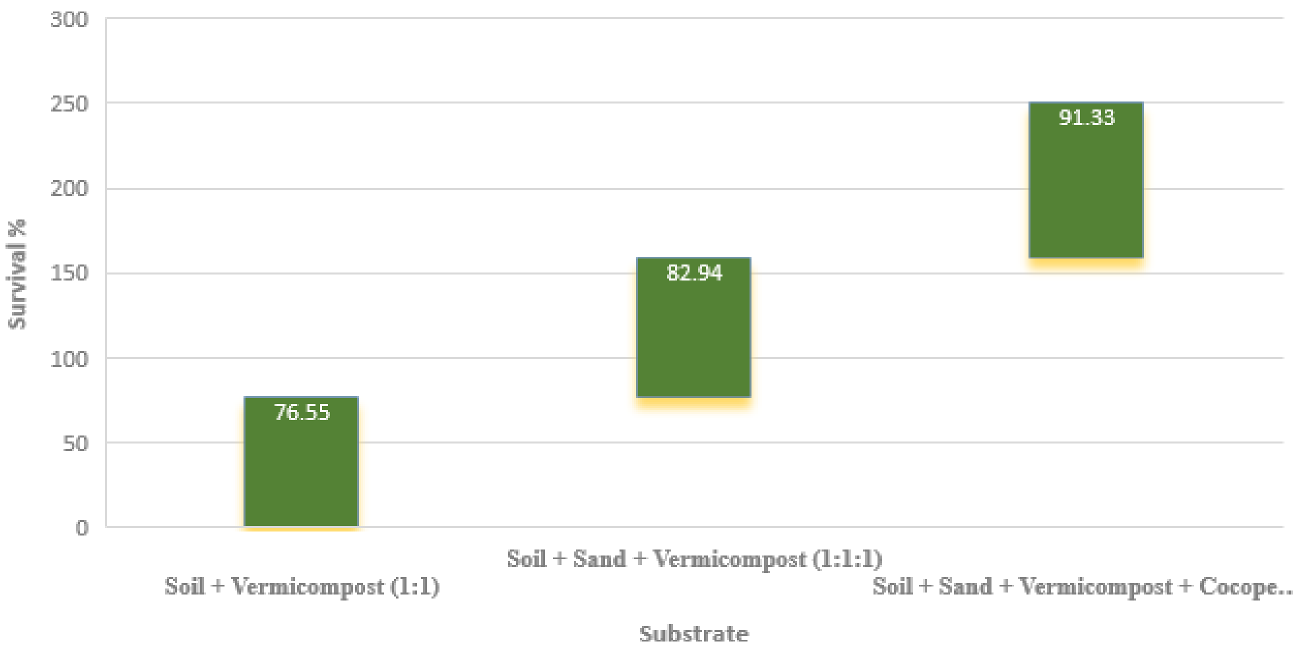

Fig 1: Survival percentage of *in vitro* derived plants

Discussion

Hardening of *in vitro*-derived plants is easy in cactus plants (Ramirez-Malagon *et al.*, 2007; Estrada-Luna *et al.*, 2008) [11, 2]. According to Malda *et al.*, (1999) [7] plants with succulent stems and crassulacean acid metabolism (CAM) are positive characteristics that minimize water stress during acclimatization. In this study, more than 90% survival was observed when Soil + Sand + Vermicompost + Cocopeat (1:1:1:1) was used as the substrate. Similar high success rates, have been reported in several other cactus species (Giusti *et al.* 2002; Sriskandarajah and Serek 2004; Orea and Medrano 2004; Quiala *et al.* 2009) [5, 13, 8, 10].

Acknowledgements

The authors acknowledge the Vice-Chancellor of the University and College of Horticulture, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut 250110, Uttar Pradesh.

Conflicts of Interest

All authors declare that they have no conflict of interest.

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