Review Article

Review on Propagation Techniques in *Hylocereus spp.* (Dragon Fruit)

Abstract

Dragon fruit (*Hylocereus spp*.) is a recently introduced super fruit in India, gaining popularity both in the rural and urban areas because of its attractive colour, delicious taste, high nutritive and medicinal values. It is adaptable to humid as well as semi-arid tropical and subtropical conditions. The growing acceptability of the fruit along with its immense antioxidants and medicinal properties has led to high demand of its cultivation followed quality planting materials in desired quantity. Propagation of Dragon fruit can be done by both sexual via seeds and asexual methods via stem cuttings, grafting and also micro propagation. The current review highlights several propagation methods of dragon fruit along with some related propagation aspects to the crop on which very limited information is available. The review also focuses on the potential areas of the Dragon fruit propagation which requires any further research to generate more data so as to improve the propagation techniques related to the crop.

Key words: Dragon Fruit**,** PGR, propagation, rooting media, rooting

Introduction

Dragon fruit (*Hylocereus undatus*) is rich in several vitamins, minerals and essential fatty acids (**Morton 1987**) and a good source of betalins (**Vinas *et al*. 2012**). The fruit belongs to family Cactaceae and thrives best in a dry, tropical or subtropical climate with annual rainfall ranging between 20-50" per year (**Dahanayake and Ranawake 2011**). The crop is being taken up in cultivation worldwide because of its high industrial, medicinal and economic potential. (**Ortiz-Hernandez and Carrillo Salazar 2012**). In India, it has been recently introduced and cultivated in different regions. Due to its hardy nature, less water and fertilizer requirement, it can be taken up in poor soils and harsh climatic conditions with little care. Stem cuttings are commonly used as propagation materials as the seed viability of stored dragon fruit is quite low. In stem cuttings, entire segment of cladode or cuttings varies from 10-60 cm length are used (**Zee *et al*. 2004**). Length of cuttings theatres a significant role in rooting and is a vital variable determining the rooting success (**Leakey 2004**). Also, the optimization of length of the cutting is crucial as longer cuttings results in propagation material wastage whereas the smaller ones might lead to poor rooting and establishment. Further, rooting and length of cutting used for propagation are correlated and species

dependent. For example; **Aminah *et al*. in 2015** reported a longer cutting length resulted into greater success in *Tinospora crispa*. Whereas, shorter cuttings (10 cm) produced higher rooting (15 and 20 cm) in *Larix kaempferi* (**Wang *et al*. 1997**). However, intermediate cuttings also caused better rooting (**Naidu and Jones 2017**). No significant effect of cutting length was reported on the rooting in *Allanblackia floribunda* (**Atangana and Khasa 2008**). Micro propagation is another method of propagation for large scale plantlet production but very little information is available on protocols for production of good quality planting material via tissue culture. The current review aims at highlighting various propagation methods which can be used as an effective means for producing healthy and quality Dragon fruit plants.

Sexual Propagation

Sexual propagation of dragon fruit is carried out through seeds. The dragon fruit seeds show 83% viability **(Ahmed, 2006)**. However, sexual propagation is rare in this crop as a long time period is required for the seedlings to yield. Also, the seedlings produced are less vigorous and not true to type when compared to the vegetatively propagated seedlings **(Tripathi *et al*, 2014)**. But, in genetic studies seed propagation is an indispensable tool as it provides genetic variability, prolonged lifespan and disease and pest resistance to its seedlings up to a certain level.

There is a very limited literature available on seed propagation of dragon fruit. The seeds of the fruit are very tiny and black in colour. A higher germination percentage is obtained when sown soon after extraction. Seeds are sown in polybags or trays and later the two month old seedlings are transplanted to pots and kept there until they become ready to be transplanted in the main field. **Tripathi *et al*. (2014)** stated that the seedlings do not become ready to be transplanted in the main field even one year after germination.

Seed germination of the fruit is affected by numerous factors such as growing media, temperature and light intensity. **Ahmed, (2006)** reported the highest germination percentage (82%) in peat moss+ sand mixture (1:1) at 24 ^{o} C and the least time (18 days) was required for germination in peat moss. The germination was fastest at 160 C. With the increase of 2000 lux light intensity from 12 hrs/day to 24 hrs/day, there was a drop of 19% germination.

Asexual Propagation

Every plant cell has the capacity to develop into a new plant **(Hartmann *et al*, 2011)**. For this reason, several plant parts like cuttings, buds, nodes and internodes, leaves, scion, bulb, corm

etc. has been used in plant propagation **(Poethig, 2013)**. Propagation through stem cuttings is the most common technique in dragon fruit. Conversely, extremely limited work has been done related to propagation through grafting.

Stem cutting

The most commercial method of propagation of dragon fruit is through stem cutting as it yields true to type fruits in the shortest time. This propagation technique's success depends on the cutting length, age or maturity of the cuttings, time of taking cuttings, stem portion used for cutting, media used for the rooting of the cuttings, application of PGRs, fresh weight of the cuttings and environmental conditions for raising environmental conditions.

Length of cutting

Length of the cuttings plays a significant role in rooting and shoots initiation of the dragon fruit as it is a proven fact that the longer cuttings resulted in early root and shoot initiation of higher quality as a result of higher rate of photosynthesis causing higher carbohydrate content in the tissues. **Mickymaray (2019)** in his study reported that a cutting size of 15 cm gave better rooting and shoot development even without IBA application in this fruit. Another study was conducted by **Kakade *et al*. (2019)** who recommended cuttings of 35- 45 cm length for better growth and development whereas, cutting length of 5 cm was found to be of an efficient size on treatment with 10 mM IBA solution by **Ahmed (2006)**.

Another study which was conducted by **Kakade *et al*. in 2019** indicated that length and dry weight of shoots and fresh weight of roots were higher when cuttings of length 35–40 cm were used for multiplication at 30 days of planting (DAP). On the other hand, increase in fresh weight of shoots, number of 1st order roots and length of longest 1st order root were highest on taking 20–25 cm long cuttings. Nevertheless, the mean new shoots length (33.06 cm), mean shoot dry weight (32.56 g), mean number of 1st order roots (7.25) and mean longest 1st order root length (32.37 cm), roots fresh and dry weight (12.52 g and 4.75 g respectively) was observed highest in longer cuttings, i.e., above 30–35 cm cutting length. Conversely, fresh weight of shoots was highest when cutting length of 25–30 cm was taken while least values were observed in smaller cuttings at 60 DAP. Consequently, the study proves that although dragon fruit is propagated with ranges of cutting lengths, larger cuttings (40±5 cm) are acclaimed for better growth and development.

Age of Cuttings

While selecting the stem of the mother plant to be used for the preparation of the cuttings, the age or maturity of the selected stem is an important factor which must be taken into consideration. **Fumuro (2011)** recommended 1 to 2 years old stems for better survival and growth of the cuttings.

Time of Cuttings

A specific time of the year should be followed for taking cuttings for propagation owing to the fact that the endogenous plant growth regulators level, rooting cofactors and carbohydrate levels in the mother plant differs in different parts of the year **(Hartman *et al*. 2011)**. The seasonal variation for the success of the cuttings may be ascribed to the variations in the levels of phenolic compounds in the mother plant. The seasonal variation in the cuttings success also rests on the shoot RNA levels, which are high during the season having maximum number of successful cuttings **(Davies, 1984)**. It was identified that for the initiation of the cell division of the root initials a certain level of protein and DNA synthesis is required and the gene regulation for protein and DNA synthesis is regulated by increased shoot RNA activity (**Molnar, 1972**, **Davies, 1984**). Various studies like **Cabahug *et al* (2018), Wodzicki (1978)**; **Nandi, Tarai and Ghosh (2019)** have observed a higher cambial activity in the season showing highest rooting percentage which is possibly due to the presence of higher levels of endogenous auxins during November-March, thereby, enhancing the cambial activity. Also, highest survival percentage of the fruit was observed between the same time period whereas, the months of September and October witnessed the lowest survival percentage.

Effect of Plant Growth Regulators

Plant growth regulators, especially auxins and cytokinins are responsible for the rooting of cuttings. The auxins promote rooting whereas the cytokinins inhibit rooting. Though, higher concentrations of endogenous auxins are present in the peak period for cutting but the prominence of its exogenous application arises so as to carry out the production of the cuttings throughout the year and also to augment rooting in cuttings which are smaller in size. Extensive studies have been carried out in order to determine the appropriate levels of Indole Butyric Acid (IBA) and Indole-3-Acetic Acid (IAA) for the enhancement of the rooting of the cuttings. A report given by **Ahmed (2006)** reveals that a concentration of 10mM IBA produces good quality cuttings even if cutting as small as 5cm length is taken. Also, a recommendation of 100 ppm, 7000 ppm and 6000 ppm IBA solution respectively was given

by **Ahmed (2006)** and **Siddiqua, Thippesha (2018)** for better establishment of cuttings. Another study was conducted by **Malsawmkimi *et al*. (2019)** who reported that best levels of nitrogen and protein was obtained in the shoots at 250 ppm IBA concentration.

Effect of portion of the stem used for cutting

Several studies have been carried out to determine which portion of the stem (viz. proximal, central or distal) gives better quality root and shoot characteristics. **Fumuro (2011)** reported highest percentage of rooting in the basal or proximal portion of the stem. Whereas, **Nandi, Tarai and Ghosh (2019)** reported maximum live cuttings, maximum length of roots and maximum root numbers when cuttings are prepared from the central portion of the stem.

Effect of the media used for the rooting of the cuttings

The rooting media plays a crucial role in the rooting of the cuttings. The media should have proper water holding capacity combined with a proper drainage capacity in order to provide sufficient water to the cuttings by avoiding stagnation of water in the medium. **Ahmed (2006)** reported highest root number (43) in peat moss and highest root length in sand (8.2 cm). Soil, farmyard manure and sand mixture in the ratio of 1:1:2 for the stem cuttings of dragon fruit was recommended by **Tripathi *et al*. (2014)**.

Effect of fresh weight of the cuttings

As the stored food materials in the cuttings helps in the rooting of dragon fruit therefore, the fresh weight of the cuttings is an important factor for the success of the cuttings. **Fumuro (2011)** reported highest percentage of rooting and root fresh weight when the fresh weight of the cuttings was 6 -7 g per cm of cutting length.

Effect of environmental conditions

Generally, the environmental conditions such as sunlight percentage, atmospheric temperature and relative humidity play an important role in rooting of cuttings of dragon fruit. The cuttings should receive an optimum level of sunlight so that the cuttings can carry on the photosynthesis process and also have minimum degradation of endogenous auxins stored in the cuttings, as it was found that light cause degradation of the pre-existing auxins (**Hartman *et al*, 2011**). Therefore, an optimum shade should be provided to the cuttings. **Lone *et al*. (2018)** recommended partial shading of 23% - 42% for the better growth of roots and shoots in dragon fruit cuttings. However, no published works on atmospheric temperature and relative humidity requirement for dragon fruit cuttings are available up to date.

Grafting technique in dragon fruit propagation

Grafting is an important asexual propagation technique in dragon fruit. It enables a species of dragon fruit more suitable in a particular soil and climatic conditions to be used as rootstock and a species of dragon fruit with desired qualities but less adaptable in the concerned soil and climatic conditions to be used as scion. Wang patented a grafting technique in dragon fruit which may be described as follows. For scion a tender shoot selected with a wedge shape provided at its base. A rootstock is selected and a transverse cut is made in the stem to be used as rootstock and removed the above portion. A longitudinal cut is made at the centre of the surface of the transverse cut. The scion is immediately put into the longitudinal cut made at the rootstock. The thorns in the rootstock are removed. The union is tied tightly with a plastic tape and covered with a plastic bag. It was claimed that it provides more than 95 % survival percentage. A few other scientists have also worked and patented grafting techniques of dragon fruit (**Ziyou, 2015 and Zain *et al*, 2019**).

Micropropagation

The propagation technique of Dragon fruit through *in vitro* tissue culture technique is one of the most ideal methods for producing fast and disease-free plants. Although various studies have been conducted on different propagation methods for the fruit; very little information is present on protocols for high quality planting material production via tissue culture. In a study conducted by **Bozkurt *et al*, 2020**, culturing of young shoots of different Dragon fruit cultivars was done with Murashige and Skoog (MS) basal medium which was supplemented with different PGRs like 6-benzylaminopurine (BAP), gibberellic acid (GA3), Indole-3-butyric (IBA). It was observed that highest rate of multiplication rate was observed in variety Halley's Comet variety cultured in MS medium supplemented with 2 ppm BAP whereas Bloody Mary cultivar recorded lowest value was in MS medium supplemented with 2 ppm BAP. Best rooting was however observed when MS medium was supplemented with 1 ppm IBA.

Another study was reported by **Dahanayake and Ranawake (2011)** on direct shoot regeneration of Dragon fruit explants using leaf and stem cuttings. Results revealed that the regeneration ability of shoot buds was greatly influenced by the explants type. Stem explants displayed a higher regeneration capacity (18 buds/explant) as compared to the explants taken from leaves (3 buds/ explant) without vitrification. However, the regeneration of maximum number of shoots from stem and leaf explants was witnessed in MS medium supplemented

with 2.5 ppm BA and 0.01 ppm NAA ppm as compared to other hormonal combinations. Highest rooting was witnessed in regenerated mature shoots when MS medium was supplemented with 0.01 ppm NAA.

A study conducted by **Lee and Chang (2022)** aimed at establishing an improved micropropagation protocol for the Dragon fruit 'Da Hong' cultivar. It was observed that an increase in NAA concentration led to increased root number but reduction in root length. The addition of activated charcoal (AC) resulted in increased shoot length and prohibited the regeneration of clustered, dried-out and abnormal shoots. Maximum shoot number was observed in the plantlets on treatment of 200 mg/L AC and 0.10 mg/L NAA. A strong correlation was observed between the weight and shoot surface area of plantlets.

Another micropropagation study was described by **Yasseen (2002)** by using explants excised from young joints of mature plants and were cultured on MS containing 0.5 μ*M* NAA and 0.5 μ*M* TDZ. Shoots which emerged from these primary explants were either decapitated or cut by longitudinal division into three parts for producing secondary explants were further cultured on MS supplemented with 0.5 μ*M* NAA and either 0.01, 0.09, 0.5, or 0.9 μ*M* TDZ. It was observed that decapitated explants produced higher number of shoots at higher frequency that the longitudinal explants. For both types of secondary explants, most shoots were developed from the distal parts.

Conclusion

From the above review it can be concluded that Dragon fruit is a highly remunerative fruit having excellent export potential. This fruit can be easily propagated in the tropical and subtropical conditions by sexual and asexual methods. Micropropagation or *in vitro* tissue culture is considered one of the best methods for fast and disease-free production of plants. Several protocols which can be used for the mass production of Dragon fruit in *in vitro* method has been discussed. However, in most cases, it is not affordable due to high cost involvement. Stem cutting technique may be considered as the most preferred technique for dragon fruit. It necessitates further study on comparative field performance of dragon fruit propagated by different techniques.

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