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

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Authors' contributions

This work was carried out in collaboration among all authors. Authors AB, MN and SD designed the study and wrote the first draft of the manuscript. Authors AB, MN and SD managed the literature searches. All authors read and approved the final manuscript.

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Review Article

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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. Dragon fruit can be propagated by various ways both sexually via seeds as well as asexually via stem cuttings, grafting and also via micropropagation. In this review various methods of propagation of dragon fruit are described along with some propagation aspects related to dragon

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fruit on which very limited information is available. Potential areas on dragon fruit propagation that require further research to generate more data in order to improve the techniques are also discussed.

Keywords: Dragon fruit; propagation; sexual; asexual; micropropagation.

1. INTRODUCTION

Dragon fruit (*Hylocereus* spp.) is one of the most sought after delicious fruit packed with both nutritional and medicinal elements whose importance is being recognised very recently. It is a crop belonging to the Cactaceae family. The origin of the fruit is traced back at tropical and sub tropical forest regions of Central and South America. Only a few crops of the Cactaceae family are edible and dragon fruit is one of them. It also have an ornamental aspect due to having very beautiful night blooming flowers which may be the reason for its local names such as 'night blooming cereus', 'belle of the night' and 'queen of the night'. It is very popularly known as 'Pitaya' or 'Pitayaha' [1]. Some other nicknames include 'noble woman', 'conderella plant' and 'jesus in the cradle'. Dragonfruit is also well known wordwide due to its antioxidant and medicinal properties such as antidiabetic [2,3], antibacterial [4,5,6], antiproliferative [7], antimicrobial [8,9], anticancer [7], wound healing property [10,11] etc. The wide acceptability of the fruit across the globe is not only because of it's delicious fruits enriched with medicinal components but also for its water use efficiency and early yielding ability *i.e*. within 2 years after planting [12,13]. It also minimises its transpiration loss through Crassulacean Acid Metabolism (CAM), where the cactus and succulents close their stomata during the hot days and carry on its CO_2 fixation during the day time and opens its stomata during the cool night and carry on its CO_2 assimilation [14,15]. The estimated area under its cultivation in India is merely 400 ha. It is well suited for cultivation in the dry and frost free areas of North Eastern, South Eastern and Western India.

As dragon fruit is gaining popularity, it has become necessary to generate planting materials in large scale. The previous studies show that dragon fruit can be propagated in a number of ways both sexually and asexually. Research works pertaining to sexual and asexual methods of propagation, micropropagation and other factors related to dragon fruit multiplication have been reviewed in this paper with certain objectives which are as follows:

a) To review various propagation methods either sexual or asexual
b) To deduce the best propagation method for mass production

2. MULTIPLICATION OF DRAGON FRUIT

2.1 Sexual Propagation

Sexual propagation in dragon fruit is carried out through seeds. The dragon fruit seeds show 83% viability [14]. But, seed propagation is very rare in dragon fruit as seedlings raised from seeds require a long period to yield and also the seedlings are less vigorous and also not true to type when compared to the vegetatively propagated seedlings [16]. 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.

The literatures available on seed propagation in dragon fruit are very limited. The seeds are minute in size 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. [16] stated that the seedlings do not become ready to be transplanted in the main field even one year after germination.

Dragon fruit seed germination is affected by several factors such as growing media, temperature and light intensity received by the seeds. Ahmed et al. [14] 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 16^{0} C. With the increase of 2000 lux light intensity from 12 hrs/day to 24 hrs/day, there was a drop of 19% germination.

2.2 Asexual Propagation

Every plant cell has the capacity to develop into a new plant [17]. This fact simplifies the use of several plant parts such as the leaves, nodes and internodes, buds, scion, cuttings, bulb, corm etc. in plant propagation [18]. Stem cutting is the most common technique for propagation of dragon fruit. However, very limited works have been conducted in case of grafting of dragon fruit.

2.2.1 Stem cutting technique

Stem cutting technique is the commercial method of dragon fruit propagation as it yields true to type fruits in the shortest time. The success of the stem cutting technique depends on the factors such as the size of the cuttings, maturity or age of the cuttings, time of taking cuttings, portion of the stem used for cutting preparation, media used for the rooting of the cuttings, application of PGRs, fresh weight of the cuttings and the environmental conditions under which cuttings are raised.

2.2.1.1 Size of the cuttings

Size of the cuttings is one of the most important factors for the rooting or shoots initiation of the dragon fruit cuttings as it is proved that the higher carbohydrate content and higher rate of photosynthesis in the larger cuttings leads to early root and shoot initiation with better quality. Mickymaray [19] found that 15 cm size cuttings gave better root and shoot development in dragon fruit even without the application of IBA. Kakade et al. [20] recommended cuttings of 35-45 cm size for better growth and development. Cuttings of 5 cm size were found to be an efficient size when treated with 10 mM IBA solution [14].

2.2.1.2 Maturity or age of the 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 [21] recommended 1 to 2 years old stems for better survival and growth of the cuttings.

2.2.1.3 Time of taking cuttings

Cuttings should be taken at a particular time of the year due to the fact that the levels of endogenous plant growth regulators, rooting cofactors and carbohydrates in the mother plant varies in different parts of the year [17]. The seasonal variation for the success of the cuttings may be attributed to the changes in the levels of phenolic compounds in the mother plant. The seasonal variation in the success of cuttings also depends on the levels of shoot RNA, whose levels are high during the season having highest number of successful cuttings [22]. 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 [23,22]. It was also reported that there is higher cambial activity in the season showing highest rooting percentage which is attributed to the fact that there is higher levels of endogenous auxins during this season which enhances the cambial activity [15,24]. Nandi, Tarai and Ghosh [25] reported highest survival percentage of dragon fruit cuttings in the months of November, December, January, February and March and lowest survival percentage in the months of September and October.

2.2.1.4 Effect of Plant Growth Regulators (PGRs)

Plant growth regulators, specially auxins and cytokinins are responsible for the rooting of cuttings. The auxins promote rooting whereas the cytokinins inhibit rooting. Although, there are higher levels of endogenous auxins in the peak period for cutting but the importance of its exogenous application arises in order to carry out year round production of the cuttings and also to enhance rooting of smaller size cuttings. 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. Ahmed [14] reported that at 10mM IBA concentration good quality cuttings of even 5cm length can be obtained. Ahmed [14] and Siddiqua, Thippesha [26] recommended 100 ppm, 7000 ppm and 6000 ppm IBA solution respectively for better establishment of the cuttings. [19] also reported that at 250 ppm IBA concentration best levels of nitrogen and protein in the shoots obtained.

2.2.1.5 Effect of portion of the stem used for cutting

Various experiments were carried out to determine whether the proximal,central or distal portions of the stem gives better quality root and

shoot characteristics. Fumuro [21] reported highest rooting percentage in the basal or proximal portion of the stem. Whereas, Nandi, Tarai and Ghosh [25] reported maximum live cuttings, maximum length of roots and maximum root numbers when cuttings are prepared from the central portion of the stem.

2.2.1.6 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 [14] 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. [16].

2.2.1.7 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 [21] reported highest rooting percentage and root fresh weight when the fresh weight of the cuttings was 6 -7 g per cm of cutting length.

2.2.1.8 Effect of environmental conditions under which cuttings are raised

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 [17]. Therefore, an optimum shade should be provided to the cuttings. Lone et al. [27] 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.

2.2.2 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 (Fig. 2). A tender shoot is taken as scion and a wedge shape is provided to 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 [28,4].

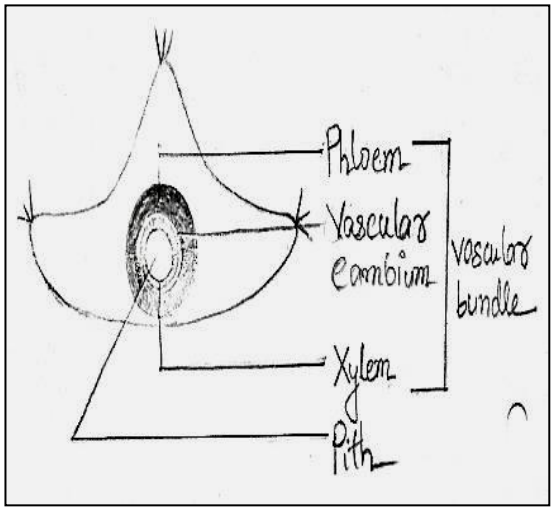

Fig. 1. Cross-section of dragon fruit stem

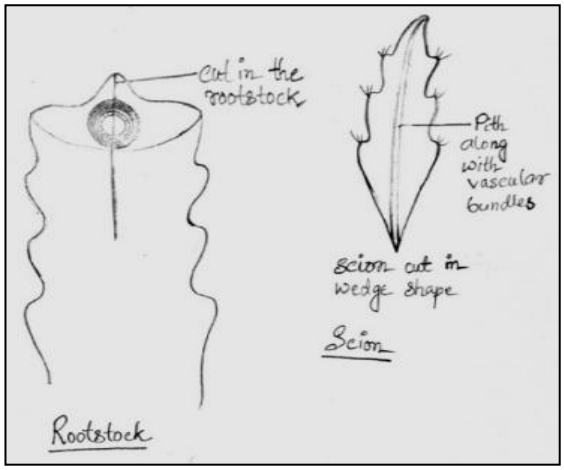

Fig. 2. Grafting technique [29]

2.2.3 Micropropagation or *in-vitro* propagation in dragon fruit

Micropropagation refers to the process of vegetative growth and multiplication by providing special growth media to the plant tissues or seeds in aseptic and favourable conditions. The micropropagation technique of dragon fruit is affected by the factors such as the explant used, media used, shoot culturing, sub culturing, in-vitro rooting and acclimatization of the in-vitro propagated plantlets. Disease free mother plant is selected to prevent contamination. Generally, the mother plant to be used as explants source is kept under check for a considerable period of time before extracting the explants from them. The explants are to be disinfected and cultured in aseptic and sterile nutrient medium.

2.2.3.1 Type of explants to be used

In dragon fruit, stem segments, areoles with thorns, young in-vitro germinated seedlings of about 4 weeks old and 40 days old cotyledonary leaves of the previously germinated seeds are generally used as explants. Vinas and Brenes [30] reported more number of shoots from the areoles taken from the central portion of the stem than the distal and proximal portions. In-vitro germinated seedlings have a little advantage because although the seedlings are not true to type with the mother plant but as the seeds are directly extracted from the fruits, a basic idea of the characteristics of the fruit like the colour of the pulp can be determined which is not possible when the other two types of explants are used because their fruit characteristics can be determined only after fruiting. Kari, Lukman, Zainuddin and Ja'afar [31] reported highest germination percentage of dragon fruit seeds when treated with 0.5 ppm IBA + 1 ppm kinetin along with Chinese A as basal media. Sheng, Sundarasekar, Sathasivam and Subramaniam [32] reported maximum germination percentage (93.33%) in semi solid MS medium supplemented with 1 ppm BAP and 0 ppm IBA. Kasim and Basri [33] reported maximum germination percentage in half strength MS medium.

2.2.3.2 Disinfection of the explants

The explants that are taken from the mother plant are disinfected by following specific methods before they are introduced into the culture medium. Kasim and Basri [33] recommended rinsing the seeds with 15% Bayclin for 15 minutes after removing seed pulp followed by washing in sterile distilled water three times for seed sterilization. For the sterilization of stem explants, first the explants are washed in running tap water then followed by washing the explant by a fungicide solution containing one drop of tween-20 for 15 mins with intermittent shaking. After that the explants are treated with a surface disinfectant $HgCl_2$ (0.1% w/v for 2 mins) followed by repeated washes in double distilled water. At last the sterilized segments are washed thoroughly with sterilized distilled water. After completion of washing, the explants are dried properly by using blotting paper [34].

2.2.3.3 Culturing explants in aseptic and sterile medium

In this phase new shoots, callus and somatic embryos are induced from the explants.

Shoot induction: Although the nutrient medium composition of shoot induction in the culture stage and multiplication stage is almost similar but the nutrient medium of culture stage have comparatively lower levels of nutrients than the multiplication phase. Hua et al. [35] reported maximum number of vigorous shoots in MS medium with 3 µM zeatin and 0.5 micro molar IBA. Suman, Rani and Reddy [34] reported maximum number of shoots (7-17) on MS media supplemented with 3 mg/L BAP + 1 mg/l KIN. Thiha [36] recommended 10 micro molar BAP containing medium for shoot induction. Kasim and Basri [33] reported maximum shoot induction in MS basal medium supplemented with 3 mg/l BAP and 0.5 mg/l NAA.

Callus induction: Callus is an undifferentiated or unorganised cell mass which is induced in in-vitro conditions by the combination of two plant growth promoting hormones, auxin and cytokinin. Sheng, Sundarasekar, Sathasivam and Subramaniam [32] reported highest callus induction in dragon fruit when the medium is supplemented with 3.6 ppm 2, 4-D + 1.8 ppm BAP. Kasim and Basri [33] reported highest callus formation from the stem explants in MS medium containing 3mg/l BAP and 0.5 mg /l NAA.

Somatic embryos: Somatic embryogenesis is the artificial induction of embryo from somatic cell or tissues. Suman, Rani and Reddy [34] reported maximum size of the somatic embryos in dragon fruit in MS basal medium supplemented with 2 mg/l of 2, 4-D.

Multiplication: Once the explants get established on the initial culture medium, the callus or explants or somatic embryos were transferred to the multiplication medium for large scale production. Multiplication in tissue culture can be done through: 1) Callus mediated multiplication (2) Adventitious shoots mediated multiplication (3) By apical or axillary shoots and (4) direct embryogenesis. Suman, Rani and Reddy [34] reported MS media supplemented with 3 mg/l BAP + 1mg/l KIN and 40 mg/l sucrose as the best multiplication media which gives maximum number and length of shoots.

Root induction: The survival of in-vitro regenerated shoots depends on their rooting efficiency. Although in-vitro generated roots are not fully functional roots and die during planting out but they help the in-vitro plantlets to resist the transplantation shock. Thiha [36] recommended 0.3µM NAA containing media for root induction and also stated that NAA level higher than 0.3 µM NAA tends to induce abnormal shoots and callus. Kasim and Basri [33] reported maximum root induction in medium containing 3 mg/l BAP and 0.5 mg /l NAA. Suman, Rani and Reddy [34] reported maximum number and length of shoots when MS media is supplemented with 3mg/l BAP + 1mg/l KIN + 0.2 mg /l NAA.

Acclimatization: Acclimatization is an important factor that determines the survival of the in-vitro generated plantlets. Here the in-vitro generated plantlets are transferred to ex-vitro conditions for further development of the plantlets. The plantlets are generally transferred to pots containing autoclaved mixture of vermiculite and coco peat in the ratio of 1:1 at diffused light conditions at green glass house. The transferred plantlets are covered with polythene covers for maintaining the humidity. After 4-6 days, the polythene covers are removed. The regenerated plants are then transferred into natural environment condition (ex-vitro) at 20-25 days. Suman, Rani and Reddy [34] reported 85-95% survival percentage in dragon fruit plantlets when the above acclimatization method is followed.

3. CONCLUSION

Dragon fruit is a highly remunerative fruit having excellent export potential. This fruit can be propagated easily in the tropical and subtropical conditions by sexual as well as asexual methods. For mass supply of dragon fruit plantlets, micropropagation is the best way. 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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