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Propagation and Preservation of *Hylocereus undatus via* Tissue Culture Technique

Sabha S. S. Mustafa, Mohamed M. A. Saad

Tissue Culture Unit, Genetic Resources Department, Desert Research Center, Egypt

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ABSTRACT

The dragon fruit (*Hylocereus undatus*) is a beautiful plant in the family Cactaceae. It is a climbing vine which has received worldwide attention, first, as an ornamental plant and then as a fruit crop. For rapid propagation, stem nodal segments were obtained from fruiting plants. For in vitro establishment, on Murashige and Skoog (MS) medium, supplemented with Thidiazuron (TDZ) or 6-benzyl adenine (BA) at two concentrations (2.27, 4.55 or 2.22 and 4.48 µM). The shoots obtained from an establishment stage were transferred to multiplication on MS medium with different concentrations of TDZ (2.27, 4.55, 6.82 and 9.1 µM). For rooting shoots were cultured on MS medium supplemented with different concentrations (0.0, 2.69, 5.38 and 8.07 µM) of α-naphthaleneacetic acid (NAA). The highest concentration of TDZ that gave the maximum mean number of shoots of 6.75 and length of 6.36 shoots was 4.55 µM TDZ. The same concentration gave the maximum multiplication of shoot. The best rooting (100%) was observed at the concentration of 2.69 µM NAA, it gave 6 roots with 10 cm length. For acclimatization, the percentage of survival of about 80 % after four months. The protocol can be utilized for large-scale micropropagation of dragon fruit to meet the demand of this valuable plant.
KEYWORDS: dragon fruit, micropropagation, in vitro, pitaya.

INTRODUCTION

Hylocereus undatus Britton and Rose, is called pitaya or dragon fruit and is a perennial, epiphytic, climbing cactus native to southern Mexico, Guatemala and Costa Rica **(Zee et al., 2004)**. Its botanical name, *Hylocereus*, refers to both its habitat "hulos" means forest in Greek and the way in which it resists drought the wax or "cereus" covering the stems. The weight of fruit depends on pollination as well as the variety. When ripe, the dragon fruit is most often consumed fresh. In some regions of South America, the pulp is used in drinks. **(Le Bellec et al., 2006).**

The dragon fruit is a beautiful plant in the family Cactaceae. It is a climbing vine, which has received worldwide attention, first, as an ornamental plant and then as a fruit crop (**Dahanayake et al., 2010)**. Dragon fruit is considered as a fruit crop to be grown commercially in dry regions **(Vaillant et al., 2005 and Temak et al., 2018)**. Dragon fruit plants start to produce significant crops two to three years after planting and reach complete production after five years **(Raveh et al., 1997 and Jacobs, 1999)**. There are limitations for large-scale propagation of dragon fruit **(Viñas et al., 2012)**. It is mainly propagated by cuttings, while seed viability of preserved dragon fruit is very low (**Dahanayake et al., 2010)**. The cutting is cured by storing in a dry place for a week before potting into a free raining mix **(Merten, 2003 and Luders and Mahon, 2006)**.

Recently, dragon fruit produces super fruit because of its attractive purple fruit color and mouth watering pulp with edible black seeds imbedded inside the pulp as reported by **Perween et al., (2018)**. The skin is covered with bracts or scales. The small seeds are consumed with the fruit **(Vaillant et al., 2005 and Temak et al., 2018)**. It has nutraceuticals value, excellent export potential and high yield as reported by **Perween et al., (2018)**. There is a potential source of betalains for the food industry **(Viñas et al., 2012)**. Besides the nutrient-dense fruit, dragon fruit is high in fiber, low in protein and calories and carbohydrates. It provides a good amount of several vitamins and gives a high amount of magnesium and iron. Dragon fruits are also full in antioxidants **(Vaillant et al., 2005 and Temak et al., 2018)**. They are consumed either as fresh fruits or in salads, for the production of jam, jelly, ice-cream, juice, pastries, vinegar and face-packs. Dragon fruit seed oils are a good source of essential fatty acids and tocopherols, with a high oxidative stability. Fruits are rich in various nutrients, vitamins and minerals and accordingly owing high medicinal values, it is believed to able to lower cholesterol concentration, to balance blood sugar concentration, to prevent colon cancer, to strengthen

Corresponding Author: Sabha S. S. Mustafa, Tissue Culture Unit, Genetic Resources Department, Desert Research Center, Egypt.

kidney function and bone, to strengthen the brain workings and even used as cosmetic ingredients **(Liaotrakoon et al., 2013 and Padakatti and Meti, 2020)**.

Tissue culture technology could help to produce high quality and large scale plantlets. The perusal of literature regarding tissue culture in dragon fruit has revealed very few recently researches (**Castillo et al., 2003 and Le Bellec, 2004)**. According to **Dahanayake et al., (2010**) they examined the potential of direct regeneration of dragon fruit explants using leaf and stem cuttings in different concentrations of BA in MS basal regeneration media. Also **Abdul Razak, (2017)** developed protocol for in vitro regeneration and mass propagation. Stem explant cultured on MS medium supplemented with BAP, NAA and Kin different concentrations. **Trivellini et al., (2020)** discussed various aspects to support the establishment of dragon fruit cultivation, for micropropagation.

Seedlings are slow growing and unreliable for fruit production making them unfavourable for propagation and take four years before their first bloom. The other disadvantage of seed germination variation affecting fruit quality and production. Healthy, green cuttings are preferred for rapid propagation, but they should be obtained from proven fruiting plants and must be about 30 cm in length for successful propagation. The objectives of this study were the propagation of this plant by tissue culture technique instead of traditional propagation.

MATERIALS AND METHODS

This study was conducted in the Tissue Culture Unit, Genetic Resources Department, Desert Research Center, El-Matarya, Cairo, Egypt.

Sterilization of explants Stem nodal segments of dragon fruit obtained from adult plants were cleaned thoroughly under running tap water with two drops of soup, then surface sterilized by soaking in commercial bleach containing sodium hypochlorite (5.25%) under laminar airflow cabinet (Holten Lamin Air HVR 2448, USA), at concentration (1.0% sodium hypochlorite solution). After 15 min, the explants were taken out and thoroughly washed thrice with sterilized distilled water.

Establishment stage Stem nodal segment explants were transferred to Murashige and Skoog (1962) (Caisson, USA) medium MS medium supplemented with 3% (w/v) sucrose and 0.25% (w/v) phytagel and different concentrations of 6-benzyl adenine (BA) of 2.22, and 4.48 µM or Thidiazuron (TDZ) of 2.27 and 4. 54 µM (Sigma Cell Culture, min. 90%, St. Louis, USA). MS medium without Plant Growth Regulators (PGRs) served as a control. The pH of the medium was adjusted to 5.7 ± 0.1 and autoclaved at a pressure of 1.06 kg/cm and 121 °C for 15 min. Cultures were incubated at 25 ± 1 °C at a photoperiod of 16/8 h light/darkness under cool white fluorescent tubes of 2500-3000 lux. The mean number and length (cm) of shoots/explant were recorded after two months of culture.

Multiplication stage The in vitro produced shoots were transferred to MS medium supplemented with 3% (w/v) sucrose and 0.25% (w/v) phytagel and different concentrations of cytokinin (Sigma Cell Culture, min. 90%, St. Louis, USA) TDZ; 2.27, 4.55, 6.82 and 9.1 µM), individually. MS medium without (PGRs) served as a control. The pH of the medium was adjusted, autoclaved, and cultures were incubated as mentioned in the shoot induction stage. The number and length (cm) of shoots/explant were recorded after 3 months of culture. Subculturing was done every four weeks.

Rooting and acclimatization stages The multiplied shoot clusters were transferred for rooting on MS medium supplemented with different concentrations of α-naphthalene acetic acid (NAA Sigma Cell Culture, min. 90%, St. Louis, USA) NAA at 2.69, 5.38 and 8.07 µM. MS medium without PGRs served as a control for rooting induction. The pH was adjusted, media autoclaved, and cultures were incubated as mentioned in the previous stages. The percentage, mean number and length (cm) of roots/explant were scored after 16 weeks of culture on the rooting medium. The rooted plantlets were removed from the nutrient medium and washed thoroughly with distilled water to eliminate the residues of the medium, then were transferred to plastic pots in a mixture of sand, perlite, and peat (1:1: 2 v/v) (Peat moos, PROMIX®). The pots were covered by translucent polythene plastic bags to maintain high humidity and prevent the dissection of the newly transferred plantlets. Transplants were irrigated and the plastic bags were pored (one pore/10 days for one months) to decrease the humidity and acclimatize the plants to the external atmosphere gradually. After one month, the plastic bags were removed completely and the plants allowed to grow under open conditions.

Experimental design and statistical analysis Experiments were subjected to the completely randomized design. At least ten replicates were cultured for each treatment and the experiments were repeated twice. One-way analysis of variance (ANOVA) using Costat statistical package software. Data was used to evaluate significant differences between the mean values of different treatments, using Duncan's Multiple Range Tests Duncan (1995). The differences between means were compared at $p < 0.05$.

RESULTS AND DISCUSSION

Production of plants by in vitro tissue culture method has been investigated. In vitro micropropagation and rooting on MS medium supplemented with different concentrations of PGRs were evaluated. As a result of our studies, it is especially important to develop protocols for in vitro propagation and this research has the data to provide this.

Establishment stage the disinfected explants were cultured on establishment media to initiation explant growth. The media were supplemented with BA and (Fig.1a)

Data in Table (1) show the effect of two cytokinins BA and TDZ on explant growth. The number of shoots per explant was the highest on the medium supplemented with 4. 54 µM TDZ, it was 6. 5 shoots/explant. The mean shoot length was also the highest in the same medium which was 6.25 cm/shoot this medium followed by that supplemented with 2.27 µM TDZ. BA gave the lowest value mean of number and length of shoots. it is observed from data in Table (1) that TDZ was more effective than BA on shoots formation and shoot length for dragon fruit stem nodal segments. These results disagree with the results of **Dahanayake and Ranawake (2011)** they found that buds were initiated on explants on MS basal medium supplemented with 11.1 µM BA and 0.053 µM NAA and buds took nearly 60 days to elongate to 1.5 cm on the same medium. Also, **Abdul Razak, (2017)** who used stem explants of dragon fruit and found that the highest number of shoots was observed on MS medium supplemented with 0.88 µM BAP, while the longest shoots were observed on MS medium supplemented with 0.93 µM Kinetin when cultured horizontally. The increase in shoot lengths due to the growth regulator may be attributed to its role in increasing cellular division in apical meristem and adding new cells to the plant **(Guo et al., 2011).**

Table (1) The effect of cytokinins on stem nodal segment explants of dragon fruit in the establishment stage

Concentrations (µM)		Mean number of shoots / explant	Mean length of shoot (cm)
0.0	0.0	0.00	**0.00**
BA	2.22	2.75±0.85 ^{d}	**2.44± 0.62^{d}**
	4.48	3.25± 0.25^{c}	**3.60±0.44^{c}**
TDZ	2.27	5. 50 ±0.87^{b}	**4.00±0.89^{b}**
	4. 54	6. 50±0.96^{a}	**6.25±1.65^{a}**

Data are expressed as mean ±SE within columns by the Duncan's multiple range test (DMRT) and different lowercase letters indicate significant difference at a 5% level

Multiplication stage After the establishment stage, explants were transferred to the multiplication media with different concentrations of TDZ (Fig.1b).

Data in Table (2) show that the mean shoots number decreased by increasing TDZ concentration more than 4.55 µM and the shoot length was also decreased with the same trend. The best concentration of TDZ was 4.55 µM then 2.27 µM, the concentration of 4.55 µM, gave the maximum mean shoot number and the maximum mean shoot length 6.75 and 6.36 cm respectively. The results disagree with **Dahanayake and Ranawake (2011)** who found that the highest number of shoots on MS medium supplemented with 2.5mg/l BA and 0.01 NAA mg/l compare to that of the other hormone combinations. Also, **Fan et al., (2015)** found that the best medium for *in vitro* multiplication of dragon fruit was obtained on MS medium supplemented with 8.88 µM (BA) and 2.68 µM (NAA). The highest proliferation ratio and the most vigorous shoots were attained in one month on this medium. While, **Bozkurt et al., (2020)** found that the highest value of the multiplication of dragon fruit culture on MS medium supplemented with 8.88 µM BA. On the other hand, **Hua et al., (2015)** found that MS medium with 13.68 µM Zeatin and 2.45 µM IBA was suitable for shoot propagation of diverse dragon fruit varieties and selections. It was reported that the concentrations higher than 1µM, TDZ can stimulate the formation of adventitious shoots. In woody plant species, TDZ has successfully been utilized in micropropagation systems for *Semecarpus anacardium* L. (**Panda and Hazra, 2012**) and sandal wood (*Santalum album* L.) (**Singh et al., 2013**). The effect of TDZ was strong compared with the BA effect in shoots multiplication. TDZ enhanced the multiplying of shoots and their lengths. Cytokinins are responsible for the stimulation of cell divisions, initiate bud development, growth and proliferation (**Van Staden et al., 2008**). The mechanism for multiple shoot formations can be due to the suppression of apical dominance which is a general role of cytokinins (**Hwang et al., 2012**). TDZ is a cytokinin-like urea derivative that has been found to facilitate the efficient micropropagation of many recalcitrant woody species as reported by **Huetteman and Preece (1993).**

Table(2) The effect of TDZ on multiplication stage of dragon fruit after 90 days

Concentrations TDZ (µM)	Mean number of shoots / explant	Mean length of shoot (cm)
2.27	5.75 ± 0.75^{ab}	**4.25 ± 1.09^{ab}**
4.55	6.75 ±0.95 ^{a}	**6.36± 1.71 ^{a}**
6.82	3.50 ±0.50 ^{ab}	**3.13± 0.13 ^{ab}**
9.1	2.75 ±0.75 ^{b}	**2.93± 0.87 ^{b}**

Data are expressed as mean ±SE within columns by the Duncan's multiple range test (DMRT) and different lowercase letters indicate significant differences at a 5% level.

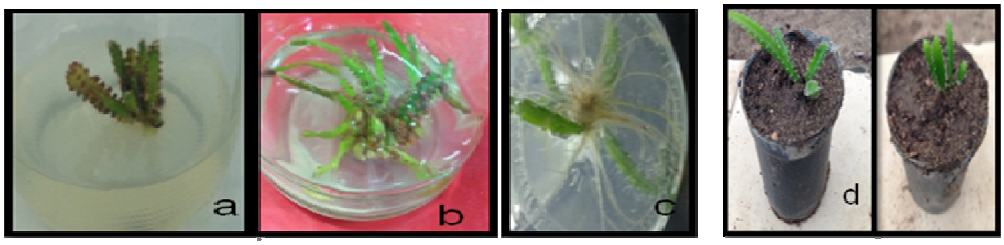

Fig. 1 In vitro propagation of dragon fruit; (a) in vitro establishment of stem nodal segment, (b) multiplication of shoot (c) rooted plantlet, and (d) acclimatization of transplants in the greenhouse

Rooting stage. Multiple shoots were separated into two shoots were cultured on the rooting media (Fig.1c).

Data in Table (3) show that there is a significant effect of NAA treatments on the rooting measurements. The rooting percentage was 100% in the presence of NAA at 2.69 µM and 5.38 µM, while no rooting occurred with control and 8.07 µM NAA treatments. This phenomenon indicates that it requires auxin for rooting at two concentrations. The root number/ shoot was 6.0 roots and the mean length was 10.0 cm root at the concentration of 2.69 µM NAA with a higher effect than the concentration of 5.38 µM NAA. The results revealed that both concentrations of 2.69 µM and 5.38 µM NAA affect the rooting process of dragon fruit shoot, but the concentration of 2.69 µM was more effective than 5.38 µM NAA. These results are in agreement with the results obtained by **Fan et al., (2015)** who observed that the rooting percentage of a dragon fruit reached 100%, and approx. six-to-ten white roots, each 5 – 8 cm in length, were obtained from each shoot in 3 weeks. **Bozkurt et al., (2020)** found that root formation was detected between 10-95% when the medium and dragon fruit varieties were compared. While the best medium for rooting was MS medium supplemented with 1 4.90 µM IBA, they have been reported that MS medium without (PGRs) can also be used for rooting of dragon fruit.

Table(3) The effect of MS medium with NAA on shoot in the rooting stage of dragon fruit

Concentrations NAA (µM)	Percentage of formation root (%)	Mean number of roots / explant	Mean length of root (cm)
0.0	0	0 .00±0.0 ^{c}	**0.00±0.0 ^{a}**
2.69	100	6.00±1.15 ^{a}	**10.00±6.0 ^{a}**
5.38	100	3.34±0.67 ^{b}	**4.83± 0.73 ^{a}**
8.07	0	0.00±0.0 ^{c}	**0.00±0.0 ^{a}**

Data are expressed as mean ±SE within columns by the Duncan's multiple range test (DMRT) and different lowercase letters indicate significant differences at a 5% level

Acclimatization stage. Healthy rooted shoot were successfully transferred to a mixture of 1:1:2 v/v sand: perlite: peat for 2 weeks in a growth chamber, then gradually acclimatized in the greenhouse with a percentage of survival of about 80% after two months (Fig.1d).

CONCLUSION

For the first time propagated the dragon fruit in Egypt by tissue culture technique. Stem nodal segment cultures on MS medium. The interactive role of cytokinins and auixn proves to be quite promising for multiplication and rooting of shoots. Our results also highlight a fact that such shoots have excellent potential to be exploited as a starting material for the development of in vitro cultures technique in this plant. The use of forced of dragon fruit shoots resulted not only in the establishment of in vitro cultures, but also in their further successful maintenance, rooting and establishment, thus providing a good protocol for in vitro of dragon fruit.

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