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RESEARCH ARTICLE :

Response of dragon fruit (*Hylocereus undatus*) explants on MS media with growth regulators under *in vitro* for mass multiplication

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SUMMARY : *Hylocereus undatus* (Dragon fruit) was micro propagated *in vitro* on MS (Murashige and Skoog, 1962) basal medium supplemented with growth regulators like BAP, Kinetin, 2-4 D, NAA and the explants response was observed. Explants were regenerated less number of shoot (1.0±0.20) on MS basal medium without growth hormones and it was acted as the control, but explants regenerated maximum number of shoots (12±0.5) on MS media supplemented with 3 mg/L BAP + 1 mg/L KIN. Explants were regenerated less number (1.0±0.22) and length (0.24±0.02 cm) of roots on MS medium with 3mg/L BAP+1mg/L KIN without NAA and it acted as control. Explants were regenerated maximum number (8.0±0.50) and length (3.6±0.06 cm) of roots on MS basal media with 3 mg/L BAP +1 mg/L KIN + 0.2 mg/L NAA. The minimum size (0.12±0.01 cm) of the somatic embryos was observed on MS media without 2,4-D and its acted as control. The maximum size (1.04±0.02 cm) of the somatic embryos formation was observed on the MS basal media with 2 mg/L of 2,4-D. The maximum number (16 ± 0.82) of shoots and length (3.3 ± 0.17 cm) of the shoots were observed by explants on the MS media + 3 mg/L BAP + 1 mg/L KIN + 40 gm/L sucrose. After shoots and roots formation, the plantlets were transferred into green house and then to soil.

KEY WORDS :
Explants, Callus, Somatic embryos, Acclimatization

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BACKGROUND AND OBJECTIVES

H. undatus, a climbing cactus with aerial roots belong to Cactaceae family and blooms only at night, called moon flower or queen of the night (Barthlott and Hunt, 1993; Britton and Rose, 1963; Fournet *et al*. 2002). The important part in this plant is fruit, commonly known as dragon fruit. The fruit has 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 flavor (Gao-Xi and Wan, 2004). The juicy flesh can use in marmalades, jellies, ice creams and soft drinks (Xiang & Rui, 2004) Dragon fruits do not contain cholesterol,

saturated fat. So, regular consumption will help manage blood pressure and control cholesterol levels (Zhijian and Xin, 2003). The seeds have high in poly unsaturated fatty acids (omega-3 and omega-6 fatty acids), reduce triglycerides and lower the risk of cardiovascular disorder (Wichienchot *et al.,* 2010; Dasaesamoh *et al*., 2016; Vaillant *et al.,* 2005; Le Bellec, 2003). Fruits are high in fiber (regular consumption can help avoid constipation, improve digestive health and help to reduce weight), rich in vitamin C, B (B1, B2, and B3), calcium, iron, lycopene and antioxidants helps in human health (Stintzing *et al.* 2003). The fruit as a food substitute for rice and as source of dietary fiber. Fruits contain phytoalbumins, which have antioxidant properties that help prevent the formation of cancer cells (Ruzainah *et al.* 2009). The flower buds of dragon fruit are used to make soups or mixed with salads and the red pulp of dragon. The multiplication of *H. undatus* is easily by cutting off the stems, *in vitro* multiplication of younger shoots of mature plant and sowing of seeds (Le Bellec and La, 2003; Yassen, 2002). The plants take 3 years for grown from seed. So, the multiplication of dragon fruit under *in vitro* condition is superlative method.

RESOURCES AND METHODS

The present investigation was conducted at tissue culture lab of AG Bioteck Laboratories (I) Ltd., with green house for acclimatization of plantlets located in Hyderabad. All the glassware's were obtained from Borosil India Ltd., Mumbai and molecular grade chemicals were obtained from LOBA Chemicals, Sigma, Hi-Media, E-Merck and Qualigens. MS medium (1962), consisted of salts, sucrose 3% (w/v), agar 0.8% (w/v) and different growth regulators (NAA (Naphthylacetic acid), 2,4-D (2,4-Dichlorophenoxyacetic acid), KIN (Kinetine) and BAP (Benzylaminopurine)) at different concentrations either alone or in combinations were added to the medium. The pH was adjusted to 5.7 with 1N NaOH (v/v) and 1N HCL (v/v) before autoclaving at 121^{o}C for 20 min. The medium was then dispensed into culture vessels, *i.e.* glass bottles (baby jars of 250 ml capacity) at the rate of 40 ml to each bottle. These vessels were plugged with polypropylene caps and then autoclaved along with other instruments required for transfer operation, at 121 ^{0}C, at a pressure of 15 lbs for 20 min.

The healthy explants were selected from the green house of AG Bioteck Laboratories (I) Ltd., Hyderabad. The leaves were cut just above shoots with inter-nodal regions. The shoots were washed in the running tap water followed by a fungicide containing one drop of tween-20 for 15 min. with intermittent shaking. Then with surface disinfectant $HgCl_2$ (0.1% w/v for 2 min) after repeated washes in double distilled water, the sterilized segments were then washed thoroughly with sterilized distilled water. After completion of washing, dry the explants properly by using blotting paper.

After sterilization, explants were transferred to culture bottles containing MS medium or MS basal media with growth enhancers aseptically. After inoculation of explants, the mouth of bottle was quick flamed and bottles were tightly capped and sealed with parafilm to avoid contaminations. After proper labeling, bottles were transferred to growth/culture room. The culture room at the temperature of 25±2°C, 60- 70% relative humidity with photo period 16hrs light and 8hrs dark cycle with 3000 lux light intensity using fluorescent lights (Philips India Ltd.,). The photoperiod and temperature was maintained.

OBSERVATIONS AND ANALYSIS

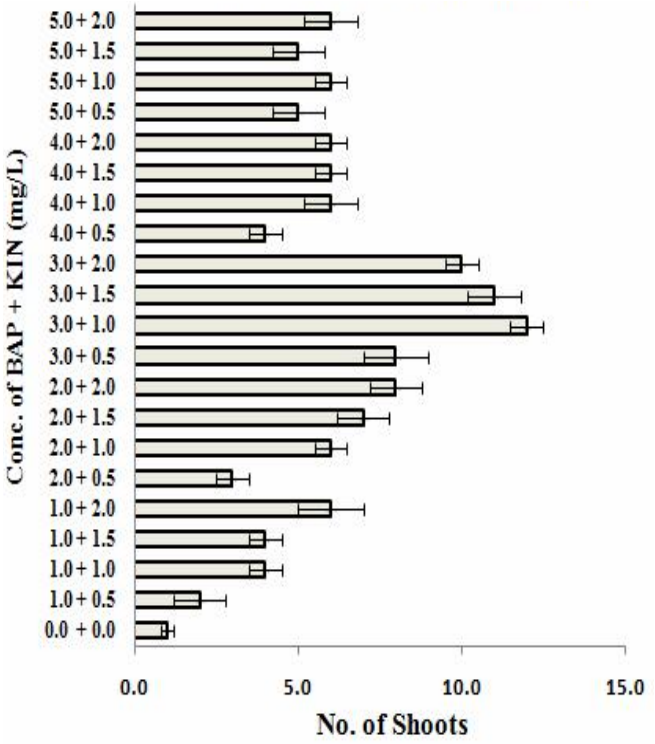
Usually the micro propagation was a sensitive method and used chemicals like growth hormones were very expensive. In this protocol, only less number of growth hormones were taken and observed the growth of the *H. undatus* for the large scale production of plantlets. So, the plantlets were produced commercially in a large scale as cheap as possible in micro propagation.

Shoot initiation:

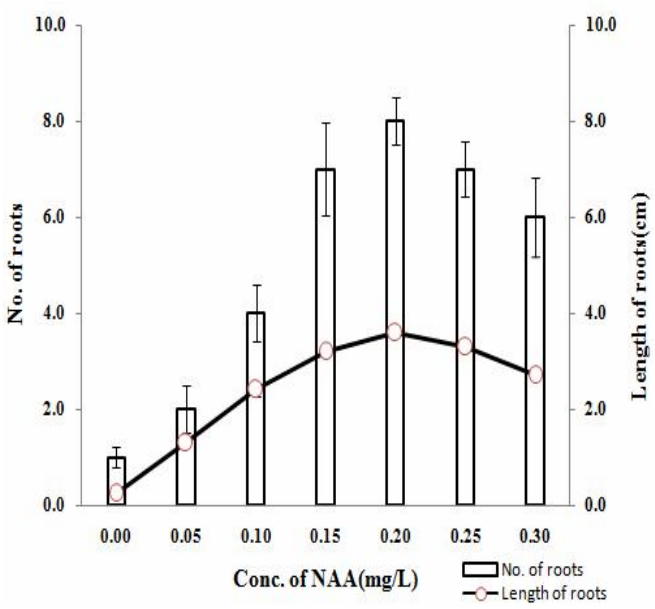
Initiation was the starting procedure for plant establishment. The formation of *H. undatus* shoots was carried out by the use of different concentration of growth hormones (BAP, from 1 to 3 mg/L and KIN, from 0.5 to 1 mg/L) in MS basal medium**.** Explants were regenerated less number of shoot (1.0 ± 0.20) on MS basal medium without growth hormones and it was acted as the control. Explants were regenerated maximum number of shoots (12 ± 0.5) on MS media supplemented with 3 mg/L BAP + 1 mg/L KIN (Graph 1).

Root initiation :

The formation of the root was also important in micro propagation. NAA was a root inducing hormone and it



Graph 1: Effect of BAP and KIN on shoot regeneration from explants on MS medium



Graph 2: Effect of NAA on root regeneration from explants on MS media.

was developed the rooting system in plants (Skoog and Miller, 1957; Hussey and Stacey, 1984). For these, the media (MS basal media with 3 mg/L BAP + 1 mg/L KIN with different concentration of NAA, from 0.05 to 0.30 mg/L) was prepared. The explants were inoculated in this media and responses of root were observed. In this media, shoots were also regenerated, but only roots numbers and length of roots was measured for observation of root development from explants.

The explants were regenerated less number (1.0±0.22) and length (0.24±0.02 cm) of roots on MS medium with 3mg/L BAP+1mg/L KIN without NAA and it acted as control. Explants were showed superior response and regenerated maximum number (8.0±0.50) and length (3.6±0.06 cm) of roots on MS basal media with 3 mg/L BAP +1 mg/L KIN + 0.2 mg/L NAA. The explants were correspondingly regenerated same number and length of roots on MS media supplemented with 0.15 mg/L and 0.25 mg/L of NAA, respectively (Graph 2).

The explants were regenerated maximum number of shoots and not the roots on MS media with 3 mg/L BAP +1 mg/L KIN (Fig. 1). Maximum numbers of shoots, maximum number and length of roots were regenerated by explants on MS media with 3 mg/L BAP +1 mg/L KIN+ 0.2 mg/L NAA (Fig. 2). Similar types of results were reported by in *H. undatus* (Dahanayake and Ranawake, 2011), in soybean (Franklin *et al.* 2004), in rice (Pádua *et al.*, 1998) and in wheat (Anwaar *et al.*, 2002).

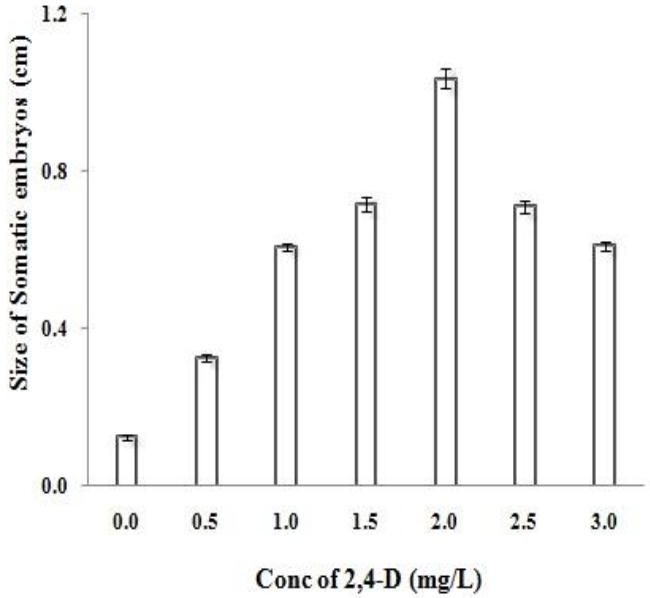
Somatic embryos:

The explants were inoculated on the MS basal media with different concentration of 2,4-D from 0.5 to 3.0 mg/L. The explants were regenerated the somatic embryos within 25-30 days. The minimum size (0.12±0.01 cm) of the somatic embryos was observed on MS media without 2,4-D and its acted as control. The maximum size (1.04±0.02 cm) of the somatic embryos formation was observed on the MS basal media with 2 mg/L of 2,4-D (Fig. 3) and next size was observed on MS media with concentration of 1.5 mg/L and 2.5 mg/L of 2,4-D, respectively (Graph 3). The somatic embryos were kept on other media, it's were regenerated only shoots on MS media + 3 mg/L BAP + 1 mg/L KIN, Fig 4(a) and both shoots and roots were regenerated on MS media + 3 mg/L BAP + 1 mg/L KIN + 0.2 mg/L NAA, Fig 4(b).

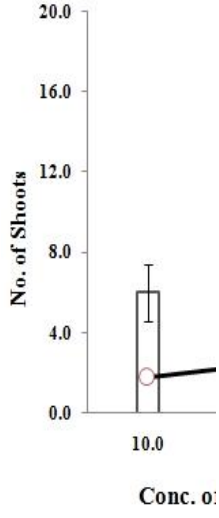
Mass multiplication :

Once the explants were established on the initiation media, the callus or explants or somatic embryos were further transformed multiplication media for large scale

production. Explants were responded and regenerated shoot on MS media with 3mg/L BAP+1 mg/L KIN. Different concentration of sucrose (from 10-40 gm/L) was used in MS media + 3mg/L BAP + 1 mg/L KIN to get superior response of explants for formation of shoots. The maximum number (16 ± 0.82) of shoots and length (3.3 ± 0.17 cm) of the shoots were observed on the MS media + 3 mg/L BAP + 1 mg/L KIN and 40 gm/L sucrose within 25 days. Moderate numbers of multiple shoots with identical numbers were observed in 20 gm/L and 30 gm/L of sucrose (Graph 4).



Graph 3: Effect of 2, 4-D on Somatic embryos regeneration from explants on MS media



Graph 4: Effect of sucrose on shoot regeneration from explants on MS media with 3mg BAP and 1mg KIN

Acclimatization of plantlets :

After *in vitro* method, the plantlets must be transferred into *ex vitro* condition for the further development of plantlet. The Plantlets were transferred to pots containing pre-autoclaved mixture of vermiculite and coco peat in the ratio of 1:1 at diffused light conditions at the green glass house. The transferred plantlets were covered with polythene covers for maintaining the humidity. After 4-6 days, the polythene covers were removed. The regenerated plants were transferred into natural environmental condition (*ex vitro*) at 20-25 days old (Fig. 5). The survival rate was 85-90 percentages.

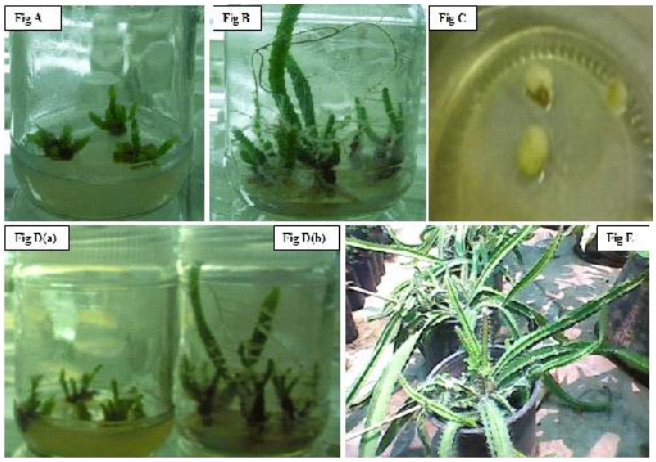


Fig. 1-5: (A) Explants regenerated maximum number of shoots on MS media with 3mg/L BAP and 1mg/L KIN. (B) Maximum shoots and maximum number and length of roots were regenerated on MS media with 3mg/L BAP+1mg/L KIN+0.2mg/L NAA by explants. (C) Explants was formatted max size of the somatic embryos on MS basal media with 2mg/L of 2,4-D. (D(a)): Somatic embryos were regenerated shoots on MS media+3mg/L BAP+1mg/L KIN. (D(b): Somatic embryos were regenerated shoots and roots on MS media+3mg/L BAP+1mg/L KIN+0.2 mg/L NAA. (E:) Acclimatized plants

Conclusion :

H. undatus (Dragon fruit) was micro propagated under *in vitro* on MS medium supplemented with growth regulators like BAP, Kinetin, 2-4 D, NAA and explants response were observed. Explants were regenerated maximum number of shoots (12±0.5) on MS media supplemented with 3 mg/L BAP + 1 mg/L KIN. Explants were showed superior response and regenerated maximum number (8.0±0.50) and length (3.6±0.06 cm) of roots on MS basal media with 3 mg/L BAP +1 mg/L KIN + 0.2 mg/L NAA. The maximum size (1.04±0.02 cm) of the somatic embryos formation was observed on the MS basal media with 2 mg/L of 2,4-D. The maximum

number (16 ± 0.82) of shoots and length (3.3 ± 0.17 cm) of the shoots were observed by explants on the MS media + 3 mg/L BAP + 1 mg/L KIN + 40 gm/L sucrose. The Plantlets were transferred into pots, then transferred into natural environmental conditional area and survival rate was 80-90 percentages. This protocol can be used for the mass production of Dragon fruit in *in vitro* method.

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