

Development of high frequency multiple shoots in the yellow cactus, *Selenicereus megalanthus*

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Abstract

An efficient micropropagation system via multiple shoot induction directly from seedlings of *S. megalanthus* was developed. Seeds were germinated on Murashige and Skoog (MS) basal medium supplemented with 6-Benzyl amino purine (BAP). The shoot tips of the germinating seedlings started callusing when left on the same medium. After two weeks, the entire shoot apex portion of epicotyl gave rise to a friable callus, portions of which started becoming green and morphogenetic. The callus gave rise to multiple shoots. The highest number of shoots *i.e.* 18.6 was produced on MS medium supplemented with 1.5 mgL⁻¹ BAP. The shoots, 3-4 cm in length, when transferred to MS basal medium supplemented with 1.0 mgL⁻¹ IBA, rooted within one week.

Key words: *Selenicereus megalanthus*, *in vitro*, yellow pitaya, multiple shoots, MS-Murashige and Skoog

Introduction

Selenicereus megalanthus is a cactus species native to northern South America. The species is grown commercially for its fruit, but is also an impressive ornamental vine with large yellow flowers. According to Anderson (2001) these are the largest flowers within the cactus family. Because of its yellow flowers it is known as yellow pitaya. It is a climbing or procumbent cactus, the fruit is a medium sized oblong berry with a yellow peel bearing tubercles and thorns that are shed during ripening. The pulp is white, lacking distinct aroma and contains numerous small edible black seeds. In recent years yellow pitaya has been seen as a marketable fruit crop with an increasing demand.

The earliest reported work on shoot proliferation and somatic embryogenesis in yellow pitaya is that of Infante (1992), who used epicotyls as explants in a culture medium containing various combinations of naphthaleneacetic acid (NAA) and 6-benzyladenine (BAP). Pelah *et al.* (2002) reported the organogenesis in the yellow pitaya using thidiazuron (TDZ). In both cases, the numbers of regenerated plants were limited in number. The present study reports a novel method for the production of large number of plantlets from a single seedling.

Materials and methods

Ripe fruits of *S. megalanthus* were procured from the local market. The seeds were manually separated from the flesh by rubbing slices of the fruit on a fine sieve and centrifuging the resulting pulp at 2000 rpm for 10 min. The seed layer, settled at the bottom of the centrifuge tube, was collected after decanting the supernatant and the layer of the pulp. The seeds were surface sterilized by immersing them in 0.1% Bavistin (w/v) (A broad spectrum fungicide, Carbendizine) for 15 min and washed with distilled water. The seeds were then treated with 0.1% sodium hypochlorite solution (4% w/v available chlorine) for 3 min. They were washed in distilled water and kept in 0.1% mercuric

chloride for 3 min. The sterilized seeds were washed and rinsed thoroughly with sterile double-distilled water to remove any residue of the sterilents. The sterilized seeds were germinated on MS medium (Murashige and Skoog, 1962) supplemented with 100 mgL⁻¹ m-inositol, 3% w/v sucrose as the carbon source, pH was adjusted to 5.6, and solidified with 0.8% (w/v) agar. Seeds were inoculated into test tubes containing 10 mL of MS medium supplemented with different concentrations of BAP. Each test tube was inoculated with single seed of *S. megalanthus*. 30 seeds were taken for each treatment which was replicated three times. For rooting, the regenerated shoots were harvested and transferred to MS medium supplemented with various concentrations of 3-indole butyric acid (IBA). The cultures were maintained at a temperature of 25 ± 2°C and relative humidity of 75 ± 5%. A 16 hour photo period with photon flux density of 10-15 μEm⁻²s⁻¹ from cool daylight fluorescent tubes (40W) was provided.

Results

The seeds germinated on all media (Fig. 1A). When left on the same medium containing various levels of BAP, the shoot tip of the germinating seedling started callusing whereas the cotyledons and the hypocotyls remained unaffected (Fig.1B). After two weeks, the entire shoot apex gave rise to a friable callus, portions of which started becoming green and morphogenetic (Fig.1C). The morphogenetic callus regenerated shoots, their number depending on the amount of BAP present in the medium (Fig.1D). The highest number of shoots *i.e.* 18.6 were produced on medium having 1.5 mgL⁻¹ BAP after three weeks of culture (Fig. 1E). The shoots elongated on the same medium except on the medium containing 2.5 mgL⁻¹ BAP where they remained stunted. The best elongation was obtained with 1.5 mg/L BAP (Table 1). Shoots which had reached a length of about 3 to 4 cms were harvested.

Rooting: The harvested regenerated shoots were transferred to the rooting medium containing MS basal medium supplemented with 0.5, 1.0, 1.5 and 2.0 mg/L IBA. All cultures were incubated

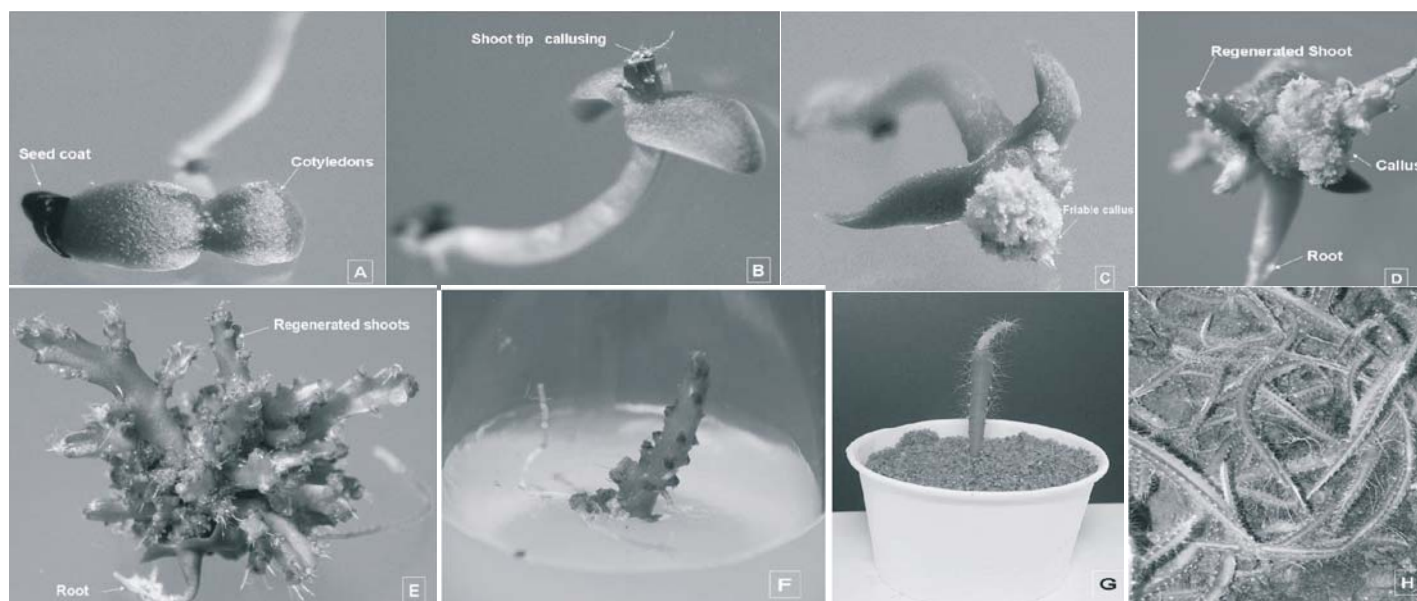


Fig. 1. Plant regeneration of *S. megalanthus*. A. Germination of seeds, B. Initiation of shoot tip callusing, C. Entire shoot tip forming friable callus, D. Shoot initiation from callus, E. Formation of multiple shoots, F. Rooting of the harvested shoot, G. Rooted plant transferred to soil, H. Plants after two months.

Table 1. Effect of different concentrations of BAP on *in vitro* shoot multiplication of germinating seedlings of *S. megalanthus*

BAP(mg/L)	Number of shoots/ seed	Shoot length (cm)
0 mg/L	4.4±2.4	2.3
0.25 mg/L	3.8±1.9	3.1
0.5 mg/L	3.0±1.5	2.8
1.0 mg/L	2.8±0.8	4.3
1.5 mg/L	18.6±6.0	6.0
2.0 mg/L	8.4±4.1	5.4
2.5 mg/L	1.4±0.5	5.0
5.0 mg/L	1.0±0.3	2.0

The data are represented as the mean ±SD from 30 samples in three independent experiments.

at 25 ± 2°C with 16-h photoperiod (10-15 μEm⁻²s⁻¹). After one week of culture rooting was observed in IBA 1.0 mg/L (Fig.1F). Two weeks after transfer, 100% of the shoots had rooted.

Hardening and acclimatization of rooted plants: After 4 weeks the rooted plants were removed from the culture medium and thoroughly washed with autoclaved double distilled water and transferred to sterile plastic cups containing a mixture of sterilized soil: sand (1:1) (Fig. 1G). The cups were covered with polythene bags for 10-12 days and later transferred to the green house for acclimatization. Two months old established plants were transferred to pots. Survival of the transferred plants was 95% after 2 months (Fig.1H).

Discussion

S. megalanthus is not only grown commercially for its fruit, but is also an impressive ornamental vine producing large yellow flowers. Few studies have been done on *in vitro* multiplication of this useful horticultural plant (Infante,1992; Pelah *et al.*, 2002). Excised epicotyls were used as explants in both these studies. Infante (1992) used NAA and BAP whereas Pelah *et al.* (2002) used TDZ to induce multiple shoots. The number of shoots obtained in both cases were few. TDZ has been shown previously

Table 2. Effect of different concentrations of IBA on *in vitro* rooting of *S. megalanthus*

IBA(mg/L)	Rooting (%)	No of roots/shoot*	Root length (cm)
0 mg/L	0	0	0
0.5 mg/L	26	2.2	1.9
1.0 mg/L	80	3.5	2.6
1.5 mg/L	62	1.8	2.74
2.0 mg/L	54	1.4	1.7

*Total of 50 micro shoots involved in each treatment

to induce bud formation in the cactus *Cercis candensis*, but the buds showed only slight or no elongation (Yusnita *et al.*, 1990). In our study, we have used the entire germinating seedling as explant. We could induce callus and obtain regeneration of a large number of shoots as well as their elongation using only BAP. The use of a single medium for all the 3 steps is an advantage in commercial micropropagation. About 19 shoots could be obtained from a single seed with 1.5 mgL⁻¹ BAP.

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