Original Research Article

Establishment of organogenesis protocol for genetic modification of 'yellow pitaya' *Selenicereus megalanthus* (Cactaceae)

Abstract

There is a need to improve the existing germplasm of yellow pitaya (*Selenicereus megalanthus*), particularly for fruit quality and traits. The genetic transformation requires the organogenesis and shoot regeneration protocols. Therefore, the *in vitro* culture of the plant was successfully established from mature seeds. Results showed that 90% of the seeds were germinated. The plantlets require MS basalt medium for growth. MS added with 2 mg/L BAP was suitable for organogenesis and production of explant for other purposes.

Keywords: *Selenicereus megalanthus,* micropropagation, yellow pitaya, Cactaceae

Abbreviation: MS0- phytohormone-free MS basal medium, MSA- MS medium added with 100 mg/l of asparagine, arginine or glutamine, MS2B -MS medium added with 2mg/L BAP, MS5B- MS medium added with 5 mg/L BAP, MS2N- MS medium added with 2 mg/L NAA, MS5N- MS medium added with 5 mg/L NAA. WPM- phtohormone-free woody plant medium,

Introduction

Pitaya is a common name applied to a broad variety of warm-climate cacti fruit from different species and genera. It represents an interesting group of under-exploited crops with potential for human consumption. Pitaya is a member of the Cactaceae includes several edible fruit species that known rich in micronutrients (Wu et al., 2006). It is native to the tropical forest regions of Mexico and Central and South America (Mizrahi et al., 1997). To date, the cultivated species are the white (*Hylocereus undatus*), red (*H. polyrhizus*) and yellow (*Selenicereus megalanthus*) varieties. These varieties are commercially grown in Taiwan, Nicaragua, Colombia, Vietnam, Israel, Australia and the USA. The red pitaya produces fruit with reddish-purple colour due to betacyanin contains high antioxidant properties (Wybraniec & Mizrahi, 2002). Comparatively, yellow pitaya have smaller size than the dragon fruit, but its fleshy pulp is pleasantly sweet. The yellow pitaya is a perennial climbing segmented cactus with triangular fleshy stems (Plume et al. 2013).. It is an epiphyte, and its aerial roots attach themselves to various types of supports such as wood and trees. The fruit is ovoid, spiny and yellow, with numerous small black seed embedded in a white pulp. Upon maturity the spines drop off

Pitaya is usually propagated through seeds or cuttings (Elobeidy, 2006). However, there is a need to improve the existing germplasm, particularly for fruit quality traits. Genetic

transformation may be used to introduce desirable genes but it is necessary first to develop protocols for the initiation of organogenesis and for regeneration of whole plants. The tissue culture technique also is promising for commercial cultivation as the demand for the planting materials increase in the near future (Pelah et al., 2002).

The shoot proliferation and somatic embryogenesis in yellow pitaya have been previously reported (Infante, 1992; Pelah et al., 2002). Infante (1992), used yellow pitaya epicotyls as explants in a culture medium containing various combinations of naphthaleneacetic acid (NAA) and 6-benzyldadenine (BA). With this method, certain difficulties were observed by us such as a lack of development or death of calli. Pelah and co-workers (2002) used the thidiazuron (TDZ) on in vitro culture of yellow pitaya. In the present study we develop the organogenesis in yellow pitaya by means for application in genetic modification of the germplasm.

Material and Methods

Source of plant materials

Seeds of *Selenicereus megalanthus* (K. Schum. ex Vaupel) Moran were obtained from mature fruits bought at Harrods Market that sold as a produce of Colombia. The seeds were thoroughly washed in 10 % (v/v) Decon™ solution for 10 min and followed sterile double-distilled water. In aseptic condition, the seeds were surface sterilized by immersion in 20 % (v/v) of commercial bleach for 30 min and followed by rinsed three times with sterile double-distilled water.

Establishment of culture

A total of 200 seeds were randomly divided into two groups. The seed coats were excised and removed from the first group, while second group remained intact. Each group was 100 seeds. Seeds were separately germinated on sterile petri-dishes containing solid basal MS (Murashige and Skoog, 1962) medium. After 2 weeks, the number seeds germinated were recorded. Subsequently, the two-week old seedling were randomly selected and transferred into 100mL Erlenmeyer flasks containing 30 mL growth medium. Five seedling per flask and three flask per treatment medium. The medium used were MSO, MSA, MS2B, MS5B and WPM. Media were supplemented with 3% (w/v) sucrose and solidified with 7g/L agar. All cultures were incubated in culture room at 24 ^{0}C under 16h/8h photoperiod provided by cool-white fluorescent lamps. After 4 weeks, the fresh weight of the plantlets was recorded for calculation of the percentage growth rate.

Organogenesis

The well-developed plantlets were randomly selected and transferred onto MS0, MS2B MS5B, MS2N or MS5N for 4 weeks and subsequently sub-cultured onto organogenesis

medium (Table 1). After 4 weeks the number of branches or any changes on the plantlet was recorded. The data (25 replicates per treatment) were subjected to one way analysis of variance (ANOVA) to assess treatment differences and interaction using the SPSS version 11.0. Significance of differences between means was tested by DMRT's Test ($p \leq 0.05$).

Results and Discussion

Results showed that hundred percent of successful in contaminant-free explants were obtained in Clorox concentrations at 80 % (v/v) and above. The highest survival rate of explants was also obtained in 20 and 40 % (v/v) of Clorox. The survival rate was declined in as the Clorox concentration used increases (Fig. 1). The right concentration of detergent is crucial to obtain high successful rate of surface sterilization and at the same time the number of survival explants also high. In most cases, high free-contamination is achieved in high concentration of detergent, but lower survival rate. Bleaching of tissue was the major phenomenon that contributed the lower survival rate. Comparatively, using seed as explants is more promising than the fragile explant such as young leaves, shoot tip or wounded explants.

The germination rate of seeds was varied between the two groups of explants. The excised seeds exhibited faster germination than the intact seeds (Fig. 2). Results showed, 70 % percent of the excised seeds were successfully germinated after 2 weeks on the growth medium. The percentage of germinated seeds was increased to 90 % after the 16th week for both types of explant used. These results indicated that removing of seed coat was facilitated the germination (Fig 3e-f). The embryos was directly exposed to the culture medium and allowed the water and nutrient uptake by embryos (Etienne et al., 2013).

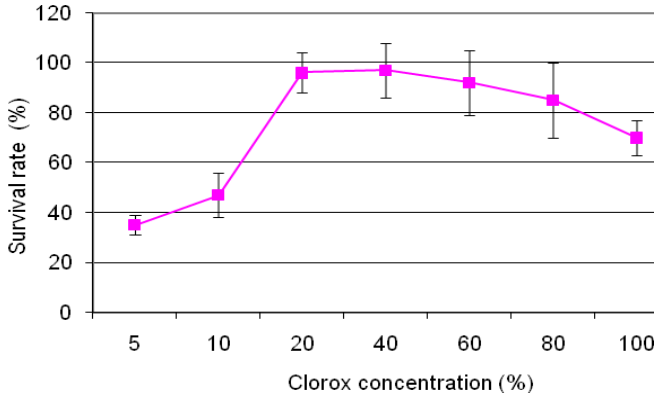

Figure 1: Survival rate of *S. megalanthus* seeds cultured on MS medium treated with different concentrations Clorox

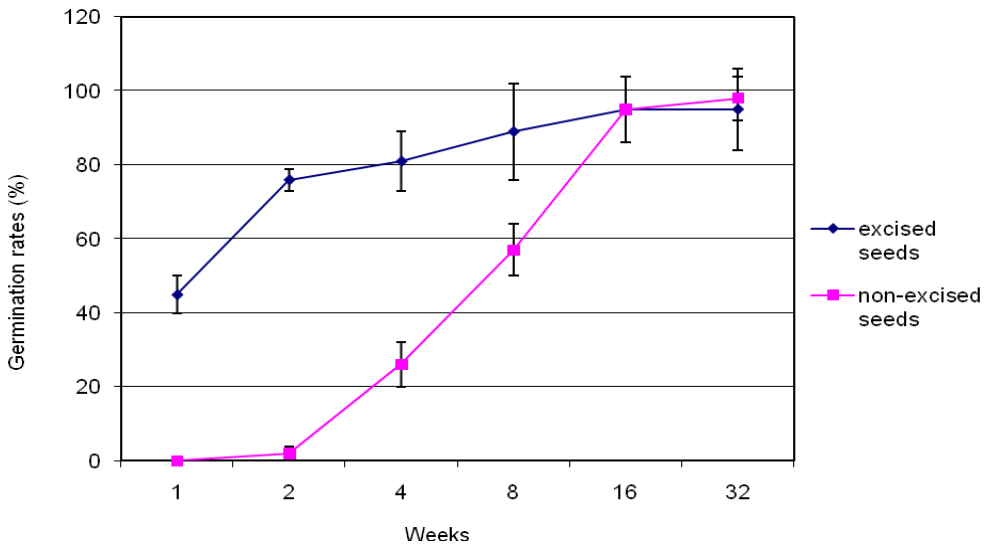

Figure 2: Germination rate of the excised and non-excised seeds of *S. megalanthus*

Growth and Organogenesis

The growth of plantlets was significantly influenced by the type of medium used (Fig. 4). The highest growth rate was in MSO basal medium followed with MSA and WPM. BAP added medium exhibited the lowest growth rate. The results indicated that basal MS medium was the most suitable medium for the growth seedling of yellow pitaya. On the other hand, the formation of organ on yellow pitaya was influence by the hormone, BAP added into the culture medium (Table 1). The highest number of branches was on explants cultures in 2 mg/L BAP for 4 weeks and in basal MS for another 4 weeks (Fig. 3i). Prolong the culture period on basal medium; for 8 weeks was enhanced the height of plantlet (Fig. 3h). Callogenesis was observed on explant culture in NAA containing medium for 8 weeks (Table 1). The capacity of callogenesis is genotype dependants and the plant growth regulator in the medium. Auxin, such NAA is normally applied for initiation of callus (Sani et al., 2012).

Table 1: Effect of medium types on the branching of *S. megalantus*

Initial media	Second subculture	Number of plantlet	Plantlet description
MSA	MSO	2±0.06	Long branch
MS2B	MSO	7.5±1.4	Well growth
	MS2B	0.5 ±0.01	Retarded
MS5B	MSO	4±0.23	Slow in growth
	MS5B	1±0.05	Retarded and browning

MS2N	MSO	5±0.78	Well growth
	MS2N	4.5±1.04	Callus appeared
MS5N	MSO	4.5±0.56	small plantlets, Callus appeared,
	MS5N	3.7±0.78	Slow growth, callus appeared

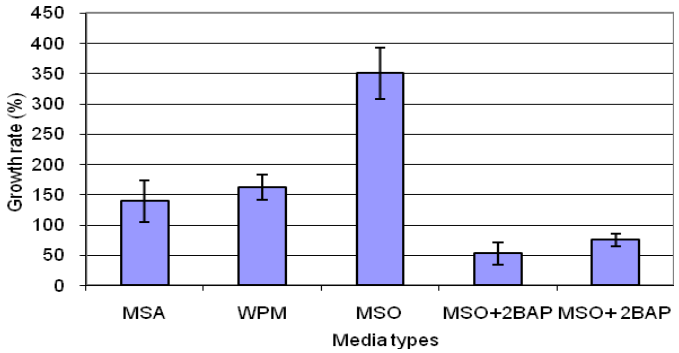

Figure 3: Effect of medium modifications on the growth of embryos into plantlets.

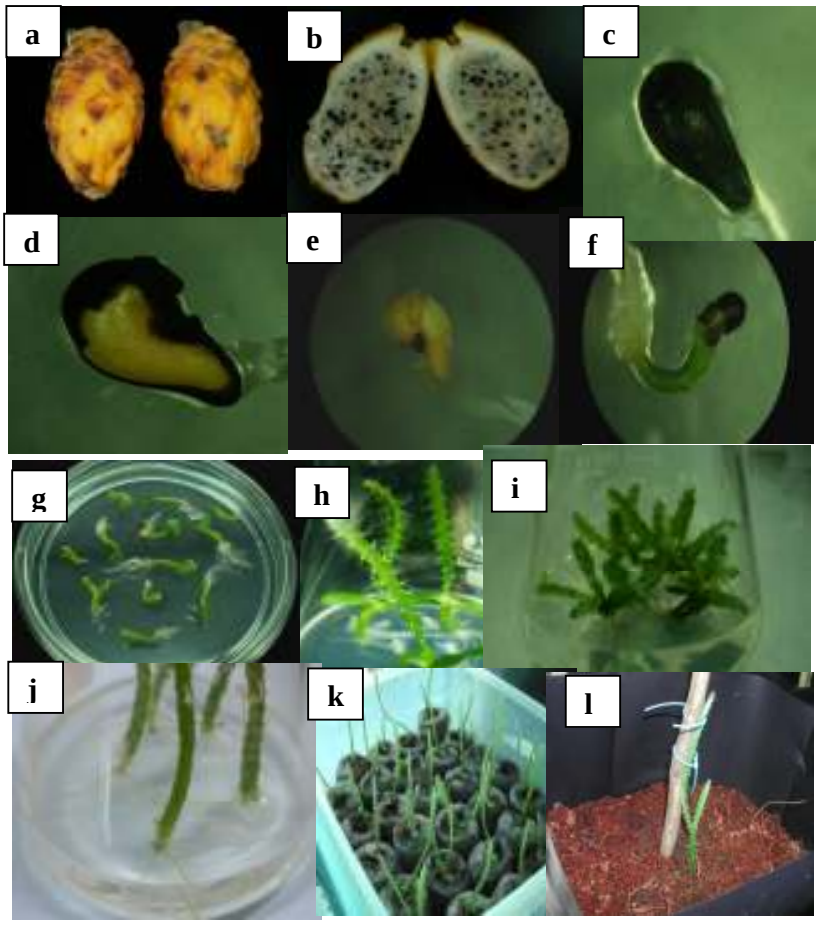

Figure 4: ripen- fruit of yellow

pitaya (a), Cross- section of the ripe yellow pitaya fruit(b), seed of pitaya 10 x magnification (c, d), emergence of shoot tips on culture medium (e, f), germinated seeds (g), plantlets after four month (h), organ proliferation (i), rooting on the individual plantlets in phytohormone-free MS medium (j), plantlets that directly transplanted into Jiffy for acclimatization of the seedling (k) and plantlets on pellets in a simple high humidity mist chamber (l) with 100% survival rate. The Jiffy pellets increased the growth rate of plantlets, compared to the traditional sand mix.

CONCLUSION

This present investigation found that the yellow pitaya successfully micropropagated through tissue culture technique using seeds as explant. The phytohormone-free MS BAP is suitable for establishment of the culture. MS medium with 2mg/L is suitable for organogenesis that might be used as explant for genetic transformation. BAP at this concentration also useful for multiplication of the explants.

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