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Original Research Article

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

Abstract

There is a needs 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 succsfully established from mature seeds. Results showed that the germination rate of seeds was 90%. The plantlets requires MS basalt medium for growth. MS + 2 mg/L BAP was suitable for organogenesis and production of explant for other purposes.

Keywords: *Selinicereus megalanthus,* micropropagation, yellow pitaya.

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 of the genus *Hylocereus*, 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. polyrhizhus*) and yellow (*Selenicereus megalanthus*) varieties. These varieties are commercially grown in Taiwan, Nicaragua, Colombia, Vietnam, Israel, Australia and the USA. The red pitaya or popularly known as the dragon fruit is popular due to the red-purple colour as food products and antioxidant contents, the betacyanin (Wybraniec & Mizrahi, 2002). On the other hand, the consumer acceptance measured in the Brisbane market exhibited that yellow pitaya is better than red pitaya as fresh fruit (Jacobs, 1999). The musky smell and taste of the red variety may explain its apparently reduced appeal in this market. Comparatively, yellow pitaya have smaller size than the dragon fruit, but its flesh is pleasantly sweet. The yellow pitaya is a perennial climbing segmented cactus with triangular fleshy stems. It is an epiphyte, and its aerial roots attach themselves to various types of supports. The fruit is ovoid, spiny and yellow. 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 (Pendal et al., 2002).

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The shoot proliferation and somatic embryogenesis in yellow pitaya have been previously reported. Infante (1992), who used 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. Pendal and co-workers (2002) used the thidiazuron (TDZ). Objective of the current study is to develop the medium for organogenesis in yellow pitaya by means for application in genetic modification of the germplasm.

Material and Methods

Source of plant materials

Seeds of yellow pitaya were obtained from mature fruits bought at Harrods that sold as a produce of Colombia. The collected 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. Seeds were separately germinated on sterile petri-dishes containing solid basal MS (Murashige and Skoog, 1962) medium for 2 weeks. The number of germinated seeds were recorded. Subsequently, the two-weeks old seedling were randomly selected and cultured in 100mL Erlenmeyer flasks containing 30 mL of the following growth medium; i.) MSA (MS modified with addition of 100 mg/l of asparagine, arginine and glutamine, respectively; ii) WPM (woody plant medium (Lloyd and McCown, 1980); MSO (MS basalt salt); iii) MS2B (MS basal salt added with 2 mg/L 6-benzylaminopurine) or iv) MS2N (MS basal added with 2 mg/L α-Naphthalene acetic acid). 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 (day/night) 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, MS + 2mg/L BAP, MS + 5 mg/L BAP, MS + 2 mg/L NAA or MS + 5 mg/L NAA for 4 weeks and subsequently sub-cultured onto organogenesis medium (Table 1) for another 4 weeks culture. The number of branches or any changes on the plantlet was recorded. The data

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(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 Chlorox concentrations at 80 % (v/v) and above. The highest survival rate of explants was also obtained in 20 and 40 % (v/v) of Chlorox. The survival rate was declined in as the Chlorox 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 culture medium. The percentage of germinated seeds was increased to 90 % after the 16 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.

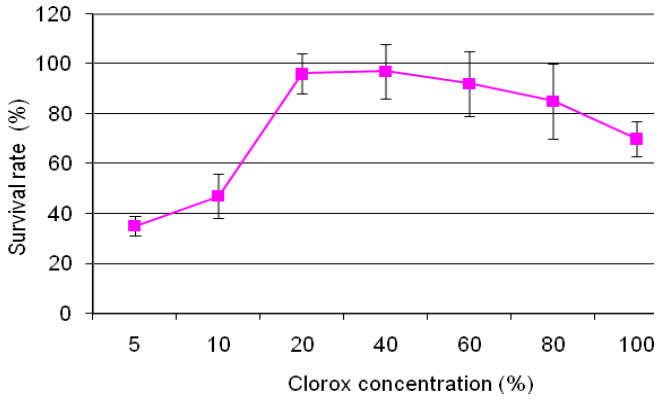

Figure 1: The survival rate of *S. megalanthus* seed cultured on MS medium treated with different concentrations Chlorox

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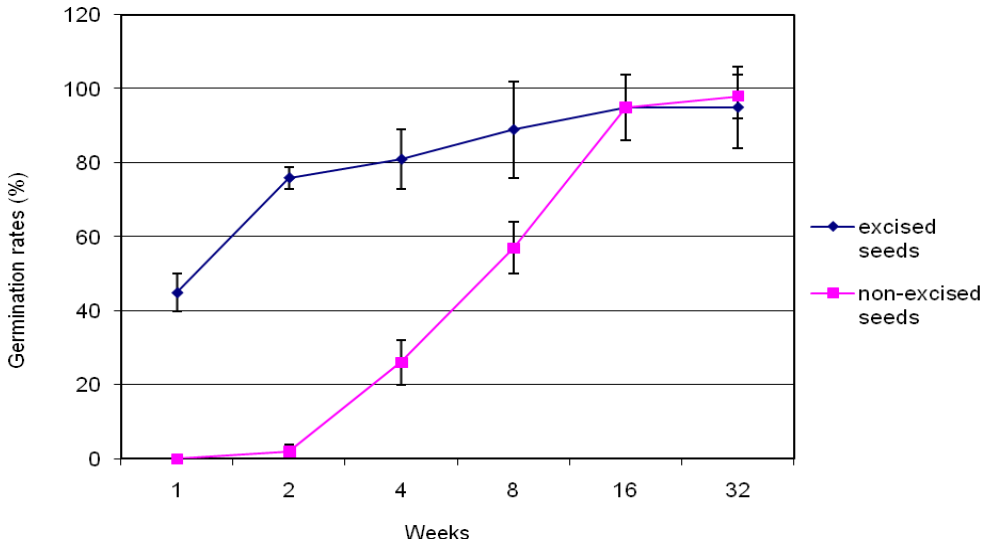

Figure 2: Germination rate of the excised and non-excised seeds of *S. megalanthus*

Growth and Organogenesis

The growth of plantlets was significantly influent 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 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).

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

Initial media	Second subculture	Number of plantlet	Plantlet description
MS Basal	MS Basal	2±0.06	Long branch
2 mg/l BAP	MS basal	7.5±1.4	Well growth
	2 mg/l BAP	0.5 ±0.01	Retarded
5 mg/l BAP	MS basal	4±0.23	Slow in growth
	5 mg/l BAP	1±0.05	Retarded and browning
2 mg/l NAA	MS basal	5±0.78	Well growth

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	2 mg/l NAA	4.5±1.04	Callus appeared
5 mg/l NAA	MS basal	4.5±0.56	small plantlets, Callus appeared,
	5 mg/l NAA	3.7±0.78	Slow growth, callus appeared

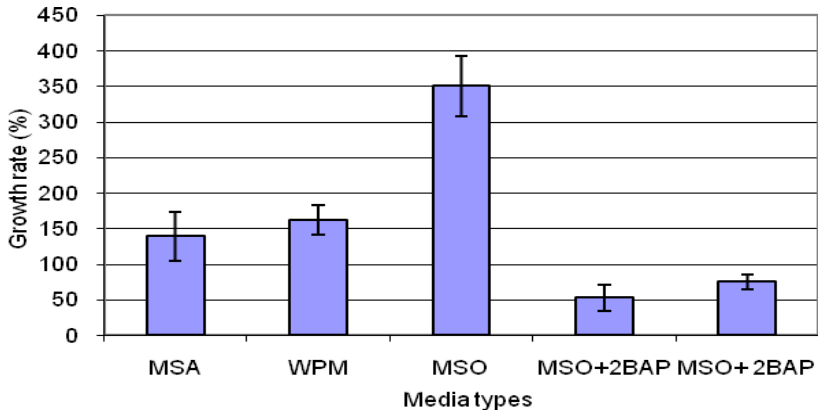

Figure 4: Effect of medium modifications on the growth of embryos into plantlets.

CONCLUSION

This present investigation demonstrated that the yellow pitaya, *S. megalanthus* successfully micropropagated through tissue culture technique using seeds as explant. The phytormone-free MS basal salt is suitable for establishment of the culture. MS medium with 2mg/L BAP is suitable for organogenesis that might be used as explant for genetic transformation.

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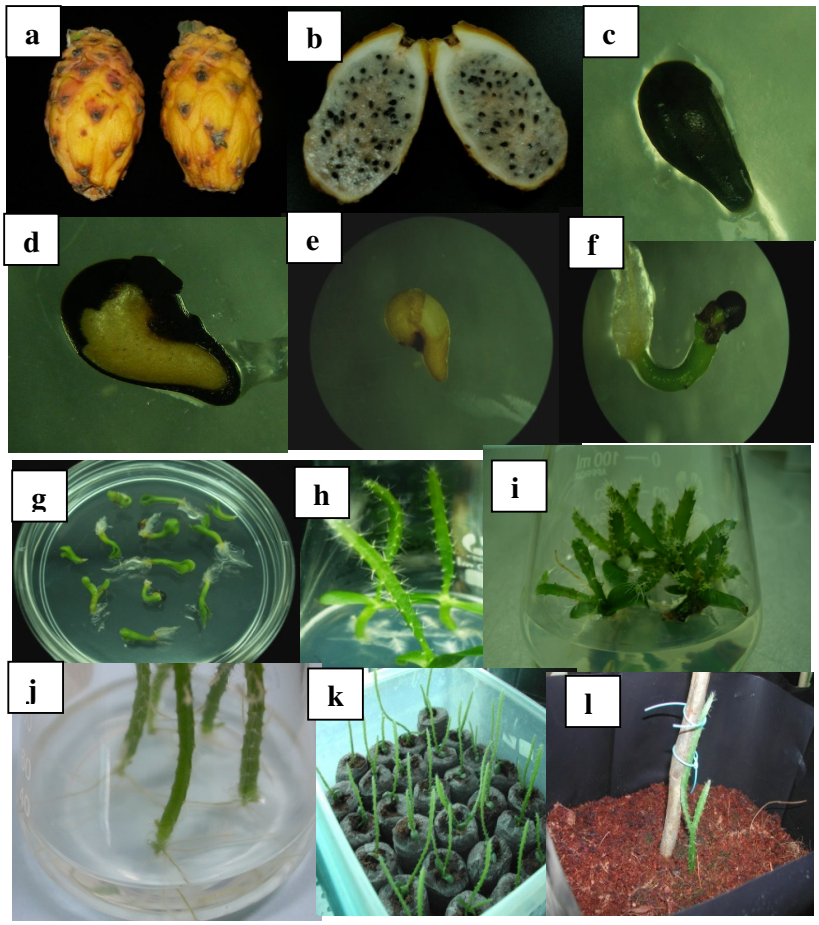

Figure 3: ripen-fruit of yellow pitaya (a), Cross- section of the ripe yellow pitaya fruit(b), seed of pitaya 10 x magnification (c, d), emergence of shoot tips on culture medium (e, f), germinated seeds (g), plantlets after four month (h), organ proliferation (i), rooting on the individual plantlets 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.

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