



Full Length Article

High-efficiency Micropropagation of Dormant Buds in Spine Base of Red Pitaya (*Hylocereus polyrhizus*) for Industrial Breeding

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Abstract

By the systematical optimization on explants sterilizing, induction and multiplication of adventitious shoots, rooting and seedling transplanting, the high-efficient regeneration system and domestication transplanting technology for red pitaya (*Hylocereus polyrhizus*) were established using dormant buds in spine base as primary materials. Results showed that the optimal surface sterilization procedure of red pitaya explants (nodal segments with one spine base) was 0.2% mercuric chloride (HgCl₂) treatment for 7 min. The appropriate medium formulation for adventitious shoot induction and proliferation of sterilized red pitaya explants was Murashige and Skoog's medium (MS) supplemented with 5.5 mg/L 6-benzyladenine (6-BA) and 0.1 mg/L α -naphthaleneacetic acid (NAA), and maximum average number (6.4) of adventitious shoots per explant and maximum average plantlet height (2.8 cm) were achieved within 30 days. An optimal formulation of half-strength MS medium (1/2 MS) added with 0.5 mg/L NAA and 0.3 mg/L indole-3-butyric acid (IBA) produced an average of 7.21 roots per explant with highest (92%) rooting response when inoculating 4 adventitious shoots per bottle. The tissue-cultured seedlings of red pitaya display a survival rate of up to 100% by optimized procedure of domestication and transplantation. © 2017 Friends Science Publishers

Keywords: Red pitaya; Micropropagation; Dormant buds; Industrial breeding

Introduction

Red pitaya (*Hylocereus polyrhizus*) is a very popular kind of tropical and subtropical fruits belonging to Cactaceae family, and it is also named dragon fruit, red dragon fruit or red-fleshed pitaya (Yu and Wang, 2009; Chen *et al.*, 2003). As a dragon-like fruit, red pitaya is known worldwide and has been introduced into many countries, moreover, red pitaya has a great potential for diversifying by producing different types of fruit products with high nutritional value. The flesh of red pitaya contains high amounts of vegetable albumin, anthocyanins (Deng *et al.*, 2011), vitamin and water soluble meal fiber (Song, 2016), suggesting that red pitaya is an excellent pitaya variety with high nutritional value. Red pitaya is commonly known to contain the highest amounts of anthocyanins among all the pitaya varieties. Anthocyanins have a high ROS-scavenging ability and are used to effectively scavenge different free radicals, and to prevent degeneration of brain cells and treat dementia (Zhou *et al.*, 2011). Red and white pitayas (*H. polyrhizus* and *H. undatus*, respectively) are two major varieties in South China over the years (Li *et al.*, 2010). Although the flesh of red pitaya is more fragrant and delicious than that of white

pitaya, the former has lower fruit yield per year than the latter (Wang, 2008). As a characteristic fruit variety, the red pitaya cultivation industry with exploitation potential would be a new economic growth point for specific fruit forestry in China (Tang and Me, 2010). Therefore, a high-efficient micropropagation technology for industrial breeding should be developed for large-scaled seedlings cultivation of excellent red pitaya cultivars with good taste and higher yield (He *et al.*, 2004).

Rapid and high-efficient micropropagation system of red pitaya is a very significant step for industrial seedling production, which is critical to quality and survival rate of seedlings. This effect is associated with the economic benefits directly (Liu *et al.*, 2011). Many researches focused on white pitaya had been carried out on cultivation technology (Song *et al.*, 2013; Hao and Wang, 2014; Zhang *et al.*, 2016), tissue culture (Huang *et al.*, 2012; Liu *et al.*, 2012; Wang *et al.*, 2012; Shen *et al.*, 2014) and component extraction (Rui *et al.*, 2009; Cui *et al.*, 2011; Yang *et al.*, 2011) in recent years. However, researches on red pitaya were less reported, and were mainly involved with component extraction (Harivaindaran *et al.*, 2008; Rebecca *et al.*, 2008; Ariffin *et al.*, 2009), constituent

activity (Woo *et al.*, 2011; Hor *et al.*, 2012; María *et al.*, 2013; Suh *et al.*, 2014) and spray-drying technology (Lim *et al.*, 2012; Bakar *et al.*, 2013), but lacked reports on quite efficient micropropagation technology for red pitaya industrial breeding using dormant buds in spine base as primary materials.

This study was conducted to develop a reliable and fast *in vitro* regeneration system of red pitaya via dormant buds, which will be suitable for commercial propagation and industrial seedling production. The result will facilitate the application of biotechnologies related to breeding exploitation for high-efficient multiplication of red pitaya.

Materials and Methods

Explant Preparation of Red Pitaya

The healthy nodal segments without disease spots and pest wormhole were collected from mature individuals of red pitaya cultivated in Hunan province, China, then were stored in the fridge at 4°C. Single-node cuttings with one spine base of red pitaya segments were used as primary explants, which were washed with soap solution for 15 min, and then surface dusts of primary explants were removed by running tap water for at least 10 min.

Medium Preparation and Illumination Condition

Agar (0.8%, w/v) and sucrose (3%, w/v) were used as gelling agent and carbon source. The pH of the medium was adjusted to 5.8 using 0.1 M HCl or NaOH before the medium was autoclaved at 121°C temperature and 104 kPa pressure for 20 min. All the cultures were maintained at 25±1°C with 12 h light/12 h dark photoperiod cycle between 1000–1500 lux in culture room.

Surface Sterilization of Explants

The cleaned primary explants of red pitaya were carried out in super-clean bench by the following treatment program: immersed in 75% ethanol for 45 sec, washed 3 times in sterile deionized water. Finally explants were sterilized with freshly prepared HgCl₂ of various concentrations (w/v) including 0.1% and 0.2% under various treatment time including 5 and 10 min. The explants were again washed 5–8 times in sterilized deionized water to completely remove the traces of sterilant. All the experiments were repeated for 40 replicates per treatment. Various parameters including percent response and survival rate were assessed after 7 days.

Induction and Proliferation of Adventitious Shoots

The surface-sterilized primary explants of red pitaya were inoculated in culture medium for adventitious shoot induction and proliferation. The medium formulation was optimized based on MS medium supplemented with plant

hormone combination of 6-BA (4 concentration levels: 4.0, 4.5, 5.0, 5.5 mg/L) and NAA (4 concentration levels: 0.06, 0.08, 0.10, 0.12 mg/L). All the experiments were repeated as 10 replicates per treatment. The multiplication rate and shoot numbers were assessed after 30 days.

Rooting Culture

After 1–2 months culture, the adventitious shoot growth of red pitaya was suitable for rooting induction. The strong shoots about 2.0 cm height were cut off and inoculated in half-strength MS (1/2 MS) medium added with plant hormone combinations of NAA (3 concentration levels: 0.3, 0.4, 0.5 mg/L) and IBA 3 concentration levels: 0.1, 0.2, 0.3 mg/L) for root induction. All the experiments were repeated for 6 replicates per treatment, and the inoculated shoot number per bottle was set in 3 levels: 2, 4 and 6. The root length, root number and relative indicators of plantlet were calculated after 30 days culturing period.

Domesticating and Transplanting of Seedlings

When the tissue-cultured seedlings of red pitaya grew up to the average height 2.5 cm and formed enough healthy roots, these strong plantlets were selected for domestication and transplantation. All plantlets in bottles were hardened for 4–5 days.

After hardening, plantlets were taken out from the culture bottles and washed in running tap water to remove the traces of agar. Subsequently, the plantlets were immersed in water and sterilized with 10% (v/v) fungicide (Bavistin) solution for 10 min, and then transplanted to small plastic pots containing sterilized humus soil after air dried. After transplanting, the seedlings were kept moist. The survival rate of transplanted seedlings was assessed after 15 days.

Results

Improved Surface Sterilization Treatment for Red Pitaya Explants

The efficiency of surface sterilization to red pitaya nodal segment explants with various sterilant concentrations and different treatment times are presented in Table 1. The result revealed that surface sterilization efficiency was directly associated with the concentrations of HgCl₂ treatment. After cultured for 7 days, among the test concentrations of HgCl₂ treatment, 0.2% (w/v) was found to be more efficient than lower concentration. Only 15% explants were contaminated by microbes and failed to survive. Similar situation was observed in sterilization time, survival rate did not increase significantly with sterilized for 7 min. However, in spite of higher survival rate under surface sterilization treatment with 0.2% HgCl₂ for 10 min, the activity of red pitaya explants became lower than other treatments.

In order to obtain more healthy and stronger seedlings, it was necessary to prevent browning effectively. In the present case, the optimal treatment of surface sterilization with 0.2% HgCl_2 (w/v) for 7 min can reduce the degree of browning and improve the survival rate of red pitaya obviously.

Condition optimization for Induction and Proliferation of Red Pitaya Adventitious Shoots

The morphogenetic responses of red pitaya nodal segment with one spine base to various concentrations of phytohormones are summarized in Table 2. During the culturing, regulatory effects of different phytohormone combinations on organ differentiation and development were significantly different. All the treatments could facilitate adventitious shoot differentiation from nodal segments. Swelling and growth of the dormant buds took place from the spine base within 15 days (Fig. 1), and then differentiated into multiple adventitious shoots within one month (Fig. 2). The analytical results showed that the average number of adventitious shoots presented a trend of anterior increase and posterior decrease when concentrations of 6-BA were unchanged, but the trend was the opposite when kept concentrations of NAA unchanged. Maximum average numbers (6.4) of adventitious shoots per explant, maximum average plantlet height (2.8 cm), and better vigor (3) were achieved within 30 days by the optimized medium formulation: MS medium added with 5.5 mg/L 6-BA and 0.1 mg/L NAA. Therefore, the optimal combination of 6-BA and NAA can be used as growth regulators for adventitious shoot induction and proliferation of red pitaya primary explants in the presenting investigation.

Highly-efficient Rooting of Red Pitaya Shoots

Influence of phytohormone combinations on shoot rooting: To optimize phytohormone combinations for MS-based medium formulation, different synthetic plant auxins (IBA and NAA) were tested at various concentrations. The rooting response of plantlets and relative indicators were recorded and analyzed (Table 3). When increasing IBA concentration and keeping NAA concentration unchanged, the rooting response, root number and other relative indicators of rooting also gradually increased, suggesting that NAA concentration insignificantly influenced the rooting of red pitaya shoots. By comparing the results of different phytohormone treatments on rooting of red pitaya, the combination of 0.3 mg/L IBA + 0.5mg/L NAA in 1/2 strength MS medium was found to be the most efficient for induction of healthy roots (Fig. 3). The highest average rooting response (92%), maximum average number of roots (7.21) and average root length (2.63 cm) was achieved in this medium formulation. These indicators had contributed to survive after transplanting of seedlings.

Table 1: Effect of different sterilization treatments on red pitaya explants

Concentration of HgCl_2 (%)	Sterilization time (min)	Survival rate (%)	Activity of explants
0.1	7	45±0.00a	strong
0.1	10	65±1.41b	strongest
0.2	7	80±4.24c	strong
0.2	10	85±2.83d	weak

Mean ± standard deviation. Values sharing same letters differ non-significantly ($P>0.05$)

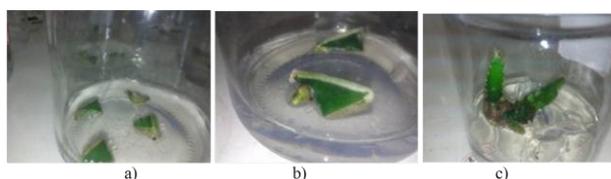


Fig. 1: Adventitious shoots induction from dormant buds in spine base of red pitaya

(a) Sterilized explants; (b) Swelling of dormant buds; (c) Growing of adventitious shoots



Fig. 2: High-efficient proliferation of red pitaya adventitious shoots



Fig. 3: Rooting of Adventitious shoots by optimal medium formulation

Influence of inoculation density on shoot rooting: The result showed that inoculation density of red pitaya shoots displayed a significant influence on its rooting rate, and revealed that rooting rate increased at first and then decreased gradually along with the increasing number of adventitious shoots (Table 4). The rooting rate of treatment 2 (4 inoculated shoots) was the highest (71%), and the roots were stronger and structurally uniform, this would provide better effect to improve posterior transplanting survival rate of tissue-cultured seedlings. Total number of roots was 91 in 1/2MS medium inoculated 6 red pitaya shoots, while the browning percentage of shoots significantly increased to 23%, which resulted in difficult posterior transplanting.

Table 2: Effects of different phytohormone combinations on adventitious shoots induction and proliferation of red pitaya primary explants

Phytohormone combinations	Plant growth regulators		Average plantlet height (cm)	Average shoot number	Vigor (0–5)
	6-BA (mg/L)	NAA (mg/L)			
1	4.0	0.06	1.8±0.07e	3.0±0.71c	2±0.23b
2	4.5	0.06	1.7±0.16de	1.8±0.45ab	2±0.24b
3	5.0	0.06	1.5±0.10bc	1.2±0.45a	1±0.15a
4	5.5	0.06	1.9±0.14e	5.4±0.55fg	4±0.34d
5	4.0	0.08	1.2±0.12a	5.8±0.84fg	3±0.19c
6	4.5	0.08	1.6±0.07cd	4.2±0.84de	3±0.33c
7	5.0	0.08	2.1±0.17f	5.2±1.10ef	2±0.10b
8	5.5	0.08	2.7±0.17g	4.8±0.84ef	4±0.16d
9	4.0	0.10	1.8±0.07e	3.2±0.84cd	3±0.26c
10	4.5	0.10	1.5±0.19bc	2.8±0.84bc	2±0.05b±
11	5.0	0.10	1.4±0.10bc	4.2±0.84de	2±0.13b
12	5.5	0.10	2.8±0.10e	6.4±1.14g	3±0.08c
13	4.0	0.12	2.1±0.23f	1.8±0.84ab	3±0.28c
14	4.5	0.12	1.6±0.10cd	1.4±0.55a	2±0.10b
15	5.0	0.12	1.4±0.08b	2.6±0.89bc	2±0.22b
16	5.5	0.12	1.4±0.12bc	3.4±0.55cd	5±0.17e

Mean ± standard deviation. Values sharing same letters differ non-significantly (P>0.05)

Table 3: Influence of different phytohormone combinations on rooting of red pitaya shoots

Phytohormone combinations	Plant growth regulators		Response (%)	Average number of roots	Average root length (cm)	Vigor (0–5)
	NAA (mg/L)	IBA (mg/L)				
1	0.3	0.1	58±1.06e	4.00±0.07b	2.22±0.27bc	2±0.00b
2	0.3	0.2	50±0.49d	2.67±0.10a	2.42±0.13cd	1±0.14a
3	0.3	0.3	75±0.64g	3.86±0.41b	2.65±0.20d	2±0.08b
4	0.4	0.1	17±0.64a	5.71±0.16c	1.45±0.18a	3±0.04c
5	0.4	0.2	33±0.49c	5.75±0.04c	1.95±0.04b	3±0.12c
6	0.4	0.3	58±2.05e	7.00±0.71de	2.73±0.07d	3±0.19c
7	0.5	0.1	25±0.64b	4.00±0.28b	2.30±0.18bc	3±0.16c
8	0.5	0.2	67±0.21f	6.33±0.13cd	2.01±0.08b	4±0.04d
9	0.5	0.3	92±3.04h	7.21±0.42e	2.63±0.07d	5±0.18e

Mean ± standard deviation. Values sharing same letters differ non-significantly (P>0.05)

Table 4: Effects of different inoculation density on rooting of red pitaya shoots

No.	Inoculation density (Shoot number per bottle)	Total shoot number	Total root number	Rooting rate (%)	Browning rate (%)
1	2	12	7	58±0.14a	8±0.14a
2	4	24	17	71±0.07a	13±0.00a
3	6	36	23	63±0.05a	25±0.00a

Mean ± standard deviation. Values sharing same letters differ non-significantly (P>0.05)

The analytical result showed that inoculation density of red pitaya shoots affected the rooting induction, and 4 inoculated shoots per culture bottle was suitable (Fig. 4).

Domesticating and Transplanting of Red Pitaya Seedlings

The transition period for domesticating and Transplanting of red pitaya seedlings from bottle environment to natural environment was regarded as the most crucial step for tissue-cultured seedlings. These red pitaya seedlings with healthy roots and strong stems were selected for domesticating by hardening in bottles for 4–5 days. After fungicide treatment for 10 min, the domesticated seedlings were air dried for 12–24 h, then transplanted to small plastic pots containing sterilized humus soil. Bagging treatment of

seedlings was performed to keep suitable moisture for 15 days. According to the improved procedure of domesticating and transplanting (Peng *et al.*, 2015), 100% survival rate of the tissue-cultured red pitaya seedlings was achieved (Fig. 5).

Discussion

Micropropagation techniques of plant tissue culture can provide possibility to introduce new approaches for direct regeneration depending on strong competence of the genotype and artificial culture condition (Ochatt *et al.*, 2013). Efficient surface sterilization, right choice of explants and optimal combinations of plant growth regulators (phytohormones) must be considered (Raaman and Punitha, 2016).

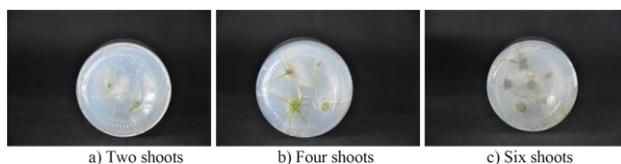


Fig. 4: Effects of different inoculation density on rooting of adventitious shoots



Fig. 5: Domesticating and transplanting of tissue-cultured red pitaya seedlings

In the present case, surface sterilization with 0.2% HgCl_2 (w/v) for 7 min can effectively reduce browning of red pitaya explants and increase their survival rate obviously. The optimal formulation of MS medium supplemented with 5.5 mg/L 6-BA and 0.10 mg/L NAA provided profuse and high percentage shoot regeneration in this study. The success of 6-BA in promoting efficient root induction has been reported for *Ipomoea sepiaria* Roxb, *Cryptolepis sanguinolenta*. (Monney *et al.*, 2016) and *Caralluma bhupenderiana* (Ugraiyah *et al.*, 2011; Cheruvathur *et al.*, 2015; Fatima *et al.*, 2015). In this study, addition of 6-BA along with auxin considerably increased morphogenetic response of red pitaya shoots as well as shoot number. Interestingly, the phytohormone combinations for the micropropagation of white pitaya (*Hylocereus undatus*) (Mohamed-Yasseen, 2002; Hua *et al.*, 2015) were distinctly different from our findings.

Theoretically, the osmotic pressure of culture medium should directly influence the formation and maturation of roots (Mafatlal *et al.*, 2016). Therefore, the rooting induction was performed on 1/2 strength MS medium. IBA was helpful for induction of roots in *Prunus dulcis* (Choudhary *et al.*, 2015) and *Cerasus yunnanensis* (Ye *et al.*, 2016). In our study, significant root induction was observed when proper concentration of IBA was combined with 0.5 mg/L NAA, which contributed to the development of strong tissue-cultured plantlets of red pitaya. This is the first report about influence of inoculation density on rooting of pitaya adventitious shoots. This optimal inoculation density of shoots, four shoots per culture bottle, may be recommended to reduce browning rate for the large-scaled industrialized production of red pitaya seedlings. The tissue-cultured seedlings of red pitaya display a survival rate of up to 100% by optimized procedure of domesticating and transplanting.

Conclusion

The optimized technology system for red pitaya micropropagation was established firstly using dormant buds in spine base as primary materials as following. 1) explant surface sterilization procedure: 0.2% HgCl_2 treatment for 7 min, 2) medium formulation for adventitious shoot induction and proliferation: MS with 5.5 mg/L 6-BA and 0.1 mg/L NAA, 3) rooting culture procedure: 1/2 MS with 0.5 mg/L NAA and 0.3 mg/L IBA by inoculating 4 shoots per bottle. These results would contribute to the industrial breeding of red pitaya by large-scaled industrialized production.

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