Initial Culture of Shoot Tip in 'Sweet Calabash' Passionfruit (*Passiflora maliformis*L.)

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Keywords: rootstock, micropropagation, tissue culture, plant growth regulators

Summary

The purpose of this study was to develop a micropropagation protocol for 'Sweet Calabash' passionfruit, a potential rootstock for commercial cultivation. The primary new shoot tips with 0.3~0.5 mm in length from 3-year-old net house-grown were cultured *in vitro* combinations of different PGRs, MS levels, and types of medium to investigate their survival percentage and size of explants. Twenty days after the initial culture, the survival percentages reached over 70% in all of medium. When shoot tips cultured on paper bridge with different MS level, the length of explants was higher in MS and 1/2MS, but callus formation easily. When PGRs was added, the growth of explants was improved in mediums containing 0.2 mg/l BA, 0.02 mg/l IBA with 0.5 mg/l GA_3, or only 0.5 mg/l GA_3. These treatments also caused the formation of calluses. Thus, there was unhealthy of shoots which cultured on paper bridge medium. Furthermore, it was found that in experiment of medium types combined with different PGRs shoot tips cultured on paper bridge induced callus easily. Liquid and solid mediums contained 0.02 mg/l NAA also showed callus formation but there was less callus formation in medium with 0.2 mg/l BA supplementation. Moreover, the length and fresh weight with no callus formation of explants shoot tips cultured on the solid medium and supplemented with BA and GA_3 by deep culture into the medium. The initial culture in 'Sweet Calabash' passionfruit was achieved when shoot tips were cultured on solid medium with half-strength of MS.

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Introduction

Passionfruit is in the genus *Passiflora* belongs to the Passifloraceae family. It is popular subtropical and tropical countries in the world (Hickey and King, 1988; Isutsa *et al*., 2004) and is grown in Asia, Africa, Australia and Brazil (Drew, 1997; Dhawan *et al*., 2004). Passionfruit has great commercial value, especially for edible fruits for cooking and juice industry (McGuire, 1999; Prammanee *et al*., 2011).

Passionfruit general propagated by seeding cutting air layering and grafting but there are low and susceptible to a range of fungal and viral pathogens disease (Martin and Nakasone, 1970; Drew, 1997; Isutsa, 2004). 'Sweet Calabash' passionfruit which origin from Latin America (Vieira and Carneiro, 2005) which is well known its light-yellow and hard shell which less commercial significant the local market but of growing importance for breeding purpose. Grows and has a pleasing aromatic flavor. Additionally, it is very resistant to pest, diseases and tolerant to most passionfruit pathogens (Morton, 1987; Nyanzi *et al*., 2004; Yockteng *et al*., 2011). However, the cultivar was difficult to seeds and hard to propagation by seeding. Developing a valid tissue culture technique is important for improving the propagation for 'Sweet Calabash' passionfruit.

During the early years, the initial shoot tip culture was successfully been used in many fruit trees, such as the hybrid cultivar Tainong No. 1 passionfruit (Yu *et al*., 1993), *P. edulis* F. *Flavicarpa* Deg (Faria and Segura, 1997), *P. edulis* Sims., (Isutsa, 2004; Prammanee *et al*., 2011), *P. foetida*L. (Komathi *et al*., 2011; Ragavendran *et al*., 2012), and other species as loquat (*Eriobotya japonica* Lindl.) (Wang and Yang, 1999), carambola (*Averrhoe carambola* L.) (Lin and Yang, 2002). In this present study, 'Sweet Calabash' passionfruit was applied to the initial culture as the first step of micropropagation.

The current research investigated the initial culture of shoot tip in 'Sweet Calabash' passionfruit on the modified medium according to Yu *et al*. (1993) who successfully established the tissue culture of Tainong No.1 passionfruit by using the 1/2MS (Murashige and Skoog, 1962) medium supplemented with 0.2 mg/l BA, 0.02 mg/l IBA and 0.5 mg/l GA_3 on paper bridge medium. The purpose of present study was therefore to establish an efficient, high survival and callus formation decreasing on the initial shoot tip culture.

Materials and Methods

Plant materials

Three-years old tree of 'Sweet Calabash' passionfruit were grown in a net house at the Horticultural Research Station, National Chung Hsing University, Taichung, Taiwan, and were used as explants sources.

Methods

Shoot tips in 5 cm length from vigorous growing primary new shoots were harvested to the tissue culture laboratory in October 2012. The leaves of explants were removed and then the shoot without leaves were surface-disinfected 1 cm in length with 1% Clorox and 1-2 drops of Tween 20 for 15 min. The surface-sterilized explants were rinsed 4 to 5 times in autoclaved double-distilled water under aseptic conditions in a laminar air flow cabinet; each rinse was 5 min in duration. Subsequently, 0.5 mm in length of shoot tips were cultured on medium containing various MS levels and PGRs (Table 1). Six replications of each treatment were cultured under aseptic conditions in a laminar air-flow cabinet. Fresh weight and shoots length were measured on the 20th day after culture. The amount of callus formation were account by the size with abound (+++), moderate (++), less (+) and no callus formation (-).These cultures were incubated in the culture room at 25±2°C and 70-80% relative humidity with 16/8-h light/dark photoperiod. Light was provided by cool white fluorescent lamp (Philips FL320, Taiwan) and agro lamps (Synvania Gro-Lux, 40W, USA) at 56 μmol $m^{-2}s^{-1}$ of photon flux density

Table 1. The medium establishments

Experiments[z]	Type of medium[y]			MS levels				PGRs[x]			
	PB	LQ	SL	1	1/2	1/4	1/8	BA	IBA	NAA	GA_3
1	o	x	x	o	o	o	o	o	o	x	o
2	o	x	x	x	o	x	x	o	o	x	o
3	o	o	o	x	o	x	x	o	x	o	x
4	x	x	o	x	o	x	x	o	x	x	o

[z] The experiments were examined on the 20th day after culture.

[y] PB: Paper bridge medium, LQ: Liquid medium, SL: Solid medium (agar 8 g/l). All of the medium in each experiment contained 30 g/l sucrose, pH was adjusted at 6.2.

[x] BA: 0.2 mg/l, IBA: 0.02 mg/l, NAA: 0.02 mg/l, GA_3: 0.5 mg/l.

Results

Experiment 1. Effects of MS levels in paper bridge medium

The modified medium by using different levels of MS medium supplemented with PGRs included 0.2 mg/l BA, 0.02 mg/l IBA and 0.5 mg/l GA_3 in paper bridge medium. On the 20th day after initial culture, all of treatments exhibited a high survival percentage (100%). Although 1/8MS showed the lowest callus formation but explants were too small. The results showed greatest in explants size and fresh weight in MS (8.5 mm, 28.4 mg) and 1/2MS (9.2 mm, 22.0 mg). However, the 1/2MS medium exhibited callus formation less than MS medium (Table 2). Thus, this result was shown that 1/2MS was better concentration for initial culture but explants which produced from this experiment were inappropriate to use for subculture because it was induced high callus formation developed from the base of explants which was difficult to culture to new medium.

Table 2. Effects of MS levels in paper bridge medium on initial culture of shoot tips in ' Sweet Calabash' passionfruit.

MS levels[z]	Survival (%)	Explants length (mm)	Fresh weight[y] (mg)	Callus amount
MS	100	8.5 a[x]	28.4 a	[w] +++
1/2MS	100	9.2 a	22.0 b	++
1/4MS	100	4.9 b	11.3 c	+++
1/8MS	100	2.7 c	5.1 c	+

[z] Medium contained BA 0.2 mg/l, IBA 0.02mg/l and GA_3 0.5 mg/l.

[y] Fresh weight included callus.

[x] Means in the column with the same letter are not significantly different by the LSD test at P=0.05.

[w] +++, ++, +, - : Abound, moderate, less and no callus formation.

Experiment 2. Effects of 1/2MS medium supplement with PGRs in paper bridge medium

Twenty days after initial culture, explants in each treatment had high survival percentage (100%). There was a significant difference on PGRs on explants growth (Table 3). Although the maximum length of explants were induced by contained PGRs (BA, IBA and GA_3) was 9.2 mm

and only GA_3 supplementation produced length of 11.2 mm. However, these regenerated explants had a high callus formation. Thus, the results suggested that IBA supplementation was unnecessary for regeneration in the initial culture. Although longer explants produced by the medium which supplement with GA_3 or PGRs (included GA_3 supplement) than another treatments, a higher amount of callus when compared to the control (non PGR) and IBA treatment.

Table 3. Effects of 1/2 MS medium in paper bridge medium on initial culture of shoot tips in 'Sweet Calabash' passionfruit.

PGRs	Survival (%)	Explant length (mm)	Fresh weight[y] (mg)	Callus amount
No PGR	100	5.1b[x]	6.2 d	[w]+
PGRs[z]	100	9.2 a	29.5 a	+++
BA	100	5.9 b	12.6 c	+++
IBA	100	5.3 b	13.7 c	+
GA_3	100	11.2 a	18.6 b	++

[z] PGR contained BA 0.2 mg/l, IBA 0.02 mg/l and GA_3 0.5 mg/l.

[y] Fresh weight included callus.

[x] Means in the column with the same letter are not significantly different by the LSD test at P=0.05.

[w] +++, ++, +, - : Abound, moderate, less and no callus formation.

Experiment 3. Effects of medium types supplementation with PGRs

The survival percentage was 70-90% showed in the experiments and significant difference in length of explants (Table 4). Paper bridge medium produced a higher callus formation than that in liquid and solid mediums. Liquid and solid mediums decreased callus formation when supplemented with BA 0.2 mg/l. Supplementation of NAA can be promoted callus formation in each type of the medium. Explant growth induced by solid medium supplement with BA was better than liquid medium (Fig. 1). This result demonstrated that solid medium was better than other medium types for initial culture.

Table 4. Effects of medium types supplementation with PGRs on initial culture of shoot tips in 'Sweet Calabash' passionfruit.

Medium types[z]	PGRs[y]	Survival (%)	Explant length (mm)	Fresh weight[x] (mg)	Callus formation[v]
Paper bridge	No PGR	90	3.1 bc[w]	7.5 b	++
	BA	80	3.6ab	15.6ab	+++
	NAA	90	4.5 a	27.0 a	+++
Liquid	No PGR	90	2.6 bc	5.9 b	--
	BA	80	3.5 abc	6.3 b	++
	NAA	80	2.5 c	6.1 b	--
Solid	No PGR	90	3.6ab	5.4 b	--
	BA	70	3.7ab	9.2 b	++
	NAA	90	3.3 bc	31.0 a	--

[z] Medium included1/2MS. [y] BA; 0.2mg/l, NAA; 0.02 mg/l.

[x] Fresh weight included callus.

[w] Means in the column with the same letter are not significantly different by the LSD test at P=0.05.

[v] +++, ++, +, —: Abound, moderate, less and no callus formation, respectively.

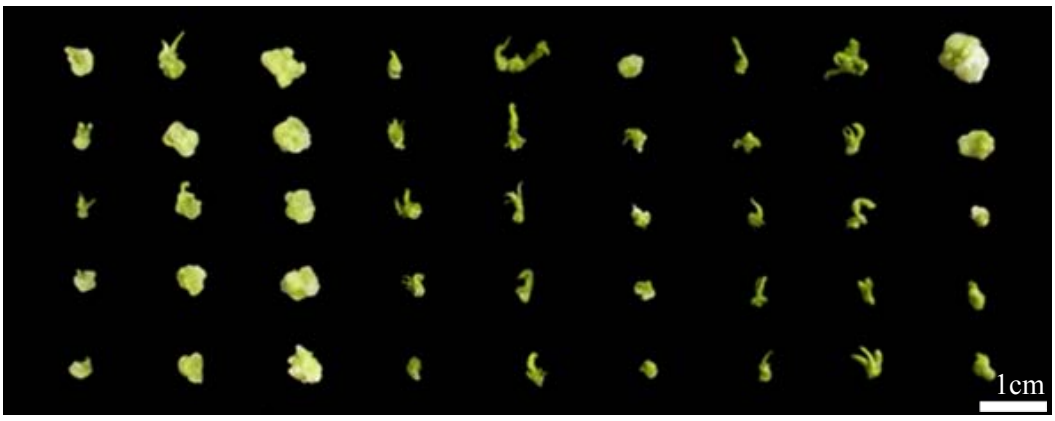

Fig. 1. Explants with callus formation from different types of medium supplemented with various PGRs.

Experiment 4. Effects of PGRs in 1/2MS solid medium

From the experiment 3 shown that solid medium with BA could induced normal shoots with a little callus formation, however we found that when placed the explants to the medium more deep can decreased callus formation. Thus, the survival percentage in each of medium reached 100%, the highest length and fresh weight of explants was induced by BA and GA_3 (Table 5). Moreover, there was no callus formation in the treatments (Fig. 2.) which indicated that explants can be easily to the subculture.

Table 5. Effects of half-strength MS solid medium supplement with various PGRs on initial culture of shoot tips in 'Sweet Calabash' passionfruit.

PGRs[z]	Survival (%)	Explant length (mm)	Fresh weight (mg)	Callus formation[x]
No PGR	100	3.1 c[y]	8.6 c	--
BA	100	5.3 b	20.2 b	--
GA_3	100	5.2 b	12.2 c	--
BA + GA_3	100	7.6 a	26.5 a	--

[z] PGRs included 1/2MS, BA 0.2 mg/l or/and GA_3 0.5 mg/l.

[y] Means in the column with the same letter are not significantly different by the LSD test at P=0.05.

[x] +++, ++, +, —: Abound, moderate, less and no callus formation, respectively.

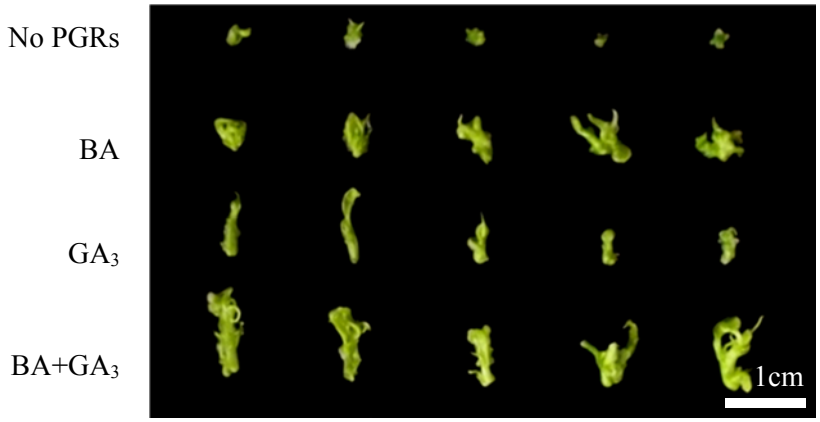

Fig. 2. Explants regeneration without callus formation in the initial shoot tip culture on 1/2MS solid medium supplemented with various PGRs.

Discussions

The aim of this study was to establish an *in vitro* culture method for 'Sweet Calabash' passionfruit. Effects of medium composition included levels of MS medium, types of medium and supplement of PGRs on explant growth were investigated.

In the present study, there were 70-100% of survival in the treatments. Similar to the findings of shoot tip culture in Tainong No. 1 passionfruit (Yu *et al*., 1993) and also resulted in high survival percentage such as loquat (Wang and Yang, 1999), Wutai persimmon (Hwang and Yang, 1998), and carambola (Lin and Yang, 2002). While some plants species have low survival percentage because of shoot tip browning on guava (Lee and Yang, 1994), Indian jujube (Yeh and Yang, 1999) and custard apple (Panchal *et al*. 2012). Results were showed that the rootstock cultivar 'Sweet Calabash' was easy to develop without the browning problem on micropropagation by shoot tip culture with high survival percentage.

In addition of culture medium, half-strength MS solid medium induced explant development greater than paper bridge medium for shoot tip culture. Moreover, auxins supplement also showed to be unappropriated for explants development. These results were similar to those reported in yellow passionfruit (Faria and Segura, 1997; Isutsa, 2004), purple passionfruit (Isutsa, 2004; Prammanee *et al*., 2011) and *P. foetida* L. (Ragavendran *et al*., 2012). The current study suggested that the culture medium for initial culture of 'Sweet Calabash' passionfruit were different from Tainong No.1 passionfruit, thereby the suitable of medium type and supplementations may be dependent on species of plants.

Callus formation exhibited on the medium was inappropriate on explants developed and difficult to subculture. Callus is defined as an unorganized tissue mass forms as a result of injury (Fuller and Gibor, 1987), which grows and forms on plants in response to explants type, genotype of plants, wounding, infestations and plant hormones (Sutan *et al*. 2012; Ikeuchi *et al*., 2013). However, it was unclear in the mechanism and effects of the paper bridge culture medium on callus formation.

A high survival rate with high efficiency of explants growth was found in this study when cultured on half-strength MS solid medium supplemented with BA and GA_3 for the initial shoot tip culture of 'Sweet Calabash' passionfruit, which can be used for subculture of micropropagation.

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'Sweet Calabash' 百香果(*P. maliformis* L.)之莖頂初代培養

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關鍵字: 砧木、微體繁殖、組織培養、植物生長調節劑

摘要：為建立百香果砧木 'Sweet Calabash' 之微體繁殖方法，本研究以 3 年生植株主梢 0.3-0.5mm 之莖頂為材料，探討其培植於不同的植物生長調節劑、MS 濃度及培養基形態後的成活率與培植體大小。由試驗結果發現，各種不同組合培養後 20 日的培植體成活率均可高達 70%以上。以濾紙橋配合不同 MS 濃度培養後，以 MS 或 1/2MS 培養者之培植體呈現較的生長，但不論 MS 濃度的高低，培植體均易形成癒傷組織。當莖頂於 1/2MS 的濾紙橋添加不同植物生長調節劑培養後發現以添加 0.02 mg/l IBA、0.2 mg/l BA 及 0.5 mg/l GA_3 者或單添加 0.5 mg/l GA_3 者之培植體長度增加較多、因此以濾紙橋培養者皆會形成不健康的枝條。另外，以不同形態培養基添加不同植物生長調節劑培養莖頂的結果可知，以濾紙橋培養者皆會形成癒傷組織，液態或固態培養基若添加 0.02 mg/l NAA 亦會產生癒傷組織，而單添加 0.2 mg/l BA 者的培植體、但是還出現一些癒傷組織且生長。其中固態培養基添加 BA 者，若再添加 0.5 mg/l GA_3，將培植體放到培養基進行深層培養，則不僅未有癒傷組織且生長較佳、培植體之長度及鮮重皆可達最高。

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