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RESEARCH NOTE

Improved growth and quality of *Siraitia grosvenorii* plantlets using a temporary immersion system

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Abstract The effects of temporary immersion system (TIS) culture on the growth and quality of *Siraitia grosvenorii* plantlets were investigated. The TIS promoted the growth and quality of *S. grosvenorii* plantlets. Proliferation rate, shoot length, fresh weight (FW) and dry weight (DW) of shoots, and total biomass production were significantly ($P \leq 0.05$) higher in the TIS than in gelled and liquid medium, respectively. The TIS also decreased callus formation at the base of shoots. Callus diameter was significantly ($P \leq 0.05$) lower in the TIS (3.30 mm) than in gelled medium (6.31 mm) and liquid medium (6.77 mm), respectively. FW (50.83 mg) and DW (7.08 mg) of callus in the TIS were also significantly ($P \leq 0.05$) lower than those in gelled medium (80.00 and 10.56 mg, respectively) and liquid medium (218.75 and 23.75 mg, respectively). During rhizogenesis, minimal callus was evident at the base of shoots in the TIS, with a well-developed root system. However, the plantlets in gelled medium just produced thick, brown and easily broken roots with obvious callus and fewer secondary roots. The natural-like plantlets of *S. grosvenorii* obtained in the TIS would probably have positive effects on ex vitro rooting and transplanting in large-scale commercial production.

Keywords Micropropagation · Proliferation rate · Shoot length · Callus · Adventitious roots

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Abbreviations

BAP	6-Benzylaminopurine
DW	Dry weight
ETR	Electron transfer rate
Fv/Fm	Maximal photochemical efficiency of PSII
FW	Fresh weight
MS	Murashige and Skoog (1962) (medium)
NAA	Naphthalene acetic acid
PA	Photoautotrophic micropropagation
PM	Photomixotrophic micropropagation
TIS	Temporary immersion system

Siraitia grosvenorii (Swingle) C. Jeffrey is a herbaceous perennial vine that belongs to the genus *Siraitia* of the family Cucurbitaceae, and is mainly distributed in Yongfu County of Guangxi Province, China. Its fruit, called Monk fruit or East supernatural fruit, is a remedy for alleviating sunstroke, nourishing the lungs, removing phlegm, stopping coughing and aiding defaecation (Dai and Liu 1999). Moreover, key components of *S. grosvenorii* fruit act as an antioxidant (Shi et al. 1996), help to prevent cancer (Konoshima and Takasaki 2002) and are useful against the Epstein–Barr virus (Akihisa et al. 2007). The plant also contains a glycoprotein called momorgrosvin, which has been shown to inhibit ribosomal protein synthesis (Tsang and Ng 2001).

Conventional propagation of *S. grosvenorii* by using cuttings is time consuming and sensitive to viral disease infections, which degrade the cultivars and dramatically decrease yield. In recent years, plant tissue culture has been shown to be superior to traditional propagation methods for *S. grosvenorii* plantlet mass production, since the first

successful report on micropropagation of *S. grosvenorii* (Lin and Wang 1980). However, propagation of in vitro *S. grosvenorii* plantlets in fields showed some disadvantages such as low transplant survival rate and low yield after transplantation, raising doubt as to the quality of *S. grosvenorii* plantlets. It has been observed that massive callus formation happens at the base of shoots grown on both proliferation medium and rooting medium, but this was considered to be unavoidable in the micropropagation of *S. grosvenorii* (Lin et al. 2003). In order to solve the problem of callus formation and improve the quality of plantlets in *S. grosvenorii* micropropagation, photoautotrophic micropropagation (PM) of *S. grosvenorii* (Zhang et al. 2009) and ex vitro rooting methods (Yan et al. 2010) have been explored. Previous results indicated that plantlets developed via ex vitro rooting methods had an extensive root system with many secondary roots and without callus growth at the base of plantlets, just like the natural root system of *S. grosvenorii* (Yan et al. 2010).

The temporary immersion system (TIS), which is based on the principle that temporary contact between plants and liquid medium is preferable to permanent contact, has been extensively used for micropropagation (Alvard et al. 1993; Cabasson et al. 1997; Lorenzo et al. 1998; Martre et al. 2001; Wawrosch et al. 2005; Thomas et al. 2008; Alonso et al. 2009). Compared with gelled and liquid culture, the TIS has been proven to have quantitative advantages such as higher proliferation rate, higher somatic embryogenesis, improved morphological characteristics and reduced production cost (Etienne and Berthouly 2002). However, micropropagation of *S. grosvenorii* using a TIS has never been reported before. This research was conducted to improve the growth and quality of *S. grosvenorii* plantlets.

Single-node microcuttings obtained from in vitro shoots of *S. grosvenorii* were used in all experiments. In vitro shoot regeneration methods have already been established (Yan et al. 2010). In the first experiment, the results for shoot proliferation obtained in gelled and liquid culture were compared with those achieved in the TIS. As the gelled medium for proliferation, Murashige and Skoog (1962) (MS) medium was used, supplemented with 0.1 mg/l 6-benzylaminopurine (BAP), 0.01 mg/l naphthalene acetic acid (NAA), 3% (w/v) sucrose and 4.0 g/l agar. The liquid and TIS medium for proliferation were the same as for gelled culture but without the 4.0 g/l agar. In the second experiment, the effects of different culture systems (viz., gelled, liquid and TIS) on rooting were compared. The gelled medium for rooting was the MS medium supplemented with 0.1 mg/l NAA, 3% (w/v) sucrose and 4.0 g/l agar. The liquid and TIS medium for rooting were the same as for gelled culture but without the 4.0 g/l agar. For the gelled and liquid culture, about 35 ml medium was dispensed into glass vessels (90 mm height, 64 mm diameter). The TIS used Plantima containers (A-Tech Bioscientific Co., Ltd., Taipei, Taiwan) with 250 ml medium in each container. The container comprises two compartments, an upper one with the plants and a lower one with the medium. Application of pressure in the lower compartment pushes the medium into the upper one. Plants are immersed as long as forced pressure is applied. During the immersion period, air is bubbled through the medium, gently agitating the tissues and renewing the air in the head space inside the culture container, with the forced pressure escaping through outlets in the upper part of the container. The explants were immersed for 4 min every 4 h by forced pressure, which propelled the liquid towards the plant material. The pH of all above-mentioned media was adjusted to 5.8 ± 0.1 before autoclaving at 121°C for 20 min. All cultures were incubated at 25 ± 1°C under 12/12 h (day/night) photoperiod with light supplied by white fluorescent tubes (25 μmol/m^2/s). After in vitro culture for 6 weeks, shoot length, proliferation rate, rooting percentage, callus diameter, fresh weight (FW), dry weight (DW), maximal photochemical efficiency of PSII (Fv/Fm) and electron transfer rate (ETR) were determined. Shoot length was measured from the apical shoot tip to the base of the shoot. FW and DW per plantlet were calculated by summing FW and DW of the shoot, callus and adventitious roots, respectively. The Fv/Fm and ETR of the third leaf from the apex were measured and calculated with a PAM-2000 portable chlorophyll fluorometer (Walz, Effeltrich, Germany).

There were three replicates for each treatment, and for each replicate, 18 single-node microcuttings were used. All statistical analyses were done using SAS software (version 8.01). Data are presented as mean ± standard error, and differences between the culture systems were compared by *t* test.

The TIS clearly promoted shoot formation (Fig. 1a). Proliferation rate and shoot length in the TIS were significantly ($P \leq 0.05$) higher than those in gelled and liquid medium, respectively, as shown by proliferation rate (2.4× and 6.7× greater than those in gelled and liquid medium, respectively) and shoot length (3.3× and 8.3× greater than those in gelled and liquid medium, respectively; Table 1). Similar results are shown in Table 2. FW (501.25 mg) and DW (60.83 mg) of shoots in the TIS were also significantly higher than those in gelled medium (168.89 and 21.94 mg, respectively) and those in liquid medium (76.48 and 9.26 mg, respectively; Table 1). The positive effects of the TIS on shoot growth have been demonstrated by many authors in earlier studies (Alvard et al. 1993; Lorenzo et al. 1998; Etienne and Berthouly 2002; Escalona et al. 2003). Under the same growth conditions, plantlets in the TIS showed a significant ($P \leq 0.05$) increase in total biomass production, expressed as DW and FW per plantlet

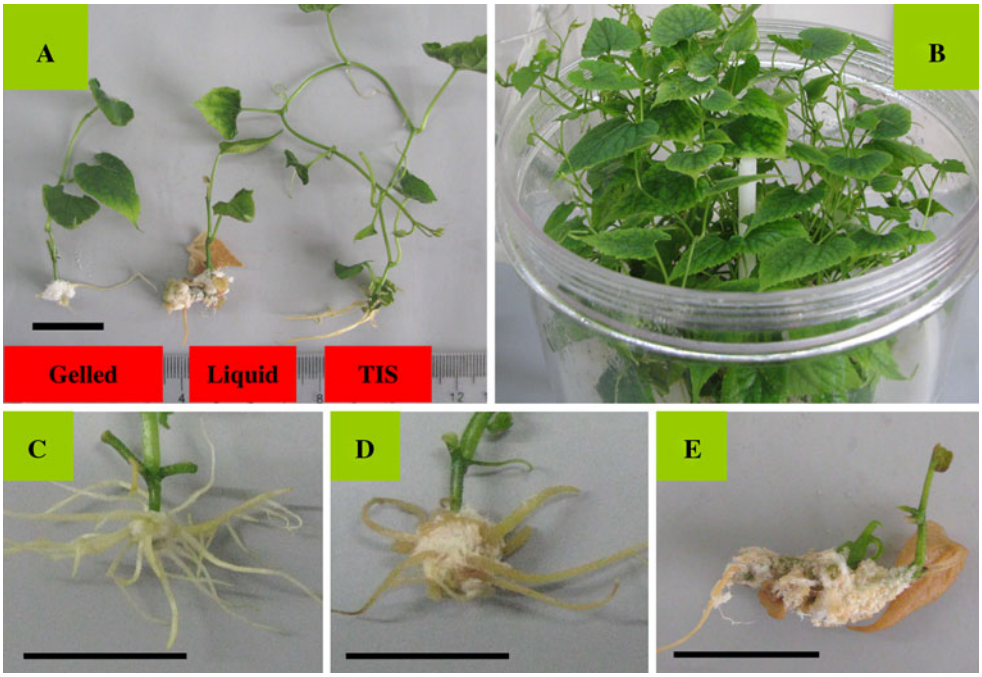

Fig. 1 **a** *S. grosvenorii* plantlets produced in gelled, liquid and TIS proliferation medium after 6 weeks of culture. **b** Natural-like plantlets produced in the TIS proliferation medium after 6 weeks of culture. **c** Adventitious roots produced in the TIS rooting medium. **d** Adventitious roots and callus produced in gelled rooting medium. **e** The base of an in vitro shoot surrounded by massive callus in liquid rooting medium (*bar* = 2 cm)

Table 1 Comparative effects of gelled, liquid and temporary immersion system (TIS) on *Siraitia grosvenorii* shoot proliferation with 6 weeks of culture

Culture method	Proliferation rate	Shoot length (cm)	Callus diameter (mm)	Callus		
				FW (mg)	DW (mg)	FW/DW
Gelled	3.72 ± 0.32 b*	5.57 ± 0.82 b	6.31 ± 0.53 a	80.00 ± 4.41 b	10.56 ± 1.73 b	7.67 ± 0.80 b
Liquid	1.30 ± 0.42 c	2.22 ± 0.97 c	6.77 ± 0.33 a	218.75 ± 9.25 a	23.75 ± 1.65 a	9.22 ± 0.26 a
TIS	8.75 ± 0.38 a	18.36 ± 1.07 a	3.30 ± 0.38 b	50.83 ± 12.77 c	7.08 ± 1.91 b	7.20 ± 0.32 b

Culture method	Shoot			Per plantlet		
	FW (mg)	DW (mg)	FW/DW	FW (mg)	DW (mg)	FW/DW
Gelled	168.89 ± 13.80 b	21.94 ± 1.27 b	7.69 ± 0.19 a	267.50 ± 13.10 b	34.44 ± 1.73 b	8.10 ± 0.64 b
Liquid	76.48 ± 34.45 c	9.26 ± 3.94 c	8.25 ± 0.57 a	295.23 ± 36.64 b	33.01 ± 4.31 b	7.77 ± 0.35 b
TIS	501.25 ± 6.96 a	60.83 ± 6.29 a	8.29 ± 0.73 a	598.75 ± 11.92 a	74.17 ± 4.39 a	8.95 ± 0.07 a

* Values with different letter within the same column are significantly different ($P \leq 0.05$)

(Tables 1 and 2). Alonso et al. (2009) confirmed that the TIS was a promising method for biomass production of *Digitalis purpurea* by in vitro shoot multiplication. The most important reason for the efficiency of the TIS is that it combines the advantages of both gelled culture (gas exchange) and liquid culture (increased nutrient uptake), which improves the growth of the plantlets (Etienne and Berthouly 2002).

In contrast with the plantlets of *S. grosvenorii* produced in gelled and liquid culture, natural-like plantlets were produced in the TIS, as indicated by grown natural-like leaves and stems (Fig. 1b). It was also found that Fv/Fm and ETR in the TIS were slightly higher than those in gelled and liquid medium, respectively (data not shown), which suggested that plantlets obtained in the TIS have more functional photosynthetic leaves. *Calathea* plants

Table 2 Comparative effects of gelled, liquid and temporary immersion system (TIS) on *Siraitia grosvenorii* plantlets rooting with 6 weeks of culture

Culture method	Rooting percentage (%)	Proliferation rate	Shoot length (cm)	Callus diameter (mm)	Per plantlet		
					FW (mg)	DW (mg)	FW/DW
Gelled	100.0 ± 0 a*	3.61 ± 0.19 b	6.64 ± 0.57 b	6.61 ± 0.33 a	425.69 ± 9.58 b	48.82 ± 2.56 b	8.73 ± 0.39 ab
Liquid	96.30 ± 3.21 a	2.31 ± 0.24 c	4.33 ± 0.86 b	7.14 ± 0.77 a	454.12 ± 34.77 b	49.80 ± 5.92 b	9.16 ± 0.66 a
TIS	100.0 ± 0 a	7.68 ± 0.19 a	15.11 ± 1.91 a	2.63 ± 0.31 b	670.97 ± 8.94 a	81.53 ± 2.44 a	8.23 ± 0.22 b

* Values with different letter within the same column are significantly different ($P \leq 0.05$)

produced by TIS presented more functional photosynthetic and respiratory apparatus, and could adapt more successfully to environmental changes during ex vitro acclimatization (Yang and Yeh 2008).

The TIS also decreased callus formation at the base of shoots and promoted a well-developed root system. Callus diameter was significantly ($P \leq 0.05$) lower in the TIS (3.30 mm) than in gelled medium (6.31 mm) and liquid medium (6.77 mm). FW (50.83 mg) and DW (7.08 mg) of callus in the TIS were also significantly ($P \leq 0.05$) lower than those in gelled medium (80.00 and 10.56 mg, respectively) and those in liquid medium (218.75 and 23.75 mg, respectively; Table 1). In the rooting medium, callus diameter was also significantly ($P \leq 0.05$) lower in the TIS than those in gelled and liquid medium (Table 2). Minimal callus was evident at the base of shoots in the TIS, with a well-developed root system (Fig. 1c). McAlister et al. (2005) also reported that there is minimal callus evident at the base of plantlet stems in the TIS, compared with that in the gelled system. However, the plantlets in gelled medium just produced thick, brown and easily broken roots with obvious callus and fewer secondary roots (Fig. 1d), which caused problems (such as difficulty of transplanting owing to massive callus, easily broken roots when washing off the agar and longer time for the transplant survival) at the acclimatization stage. In the liquid medium, the base of many plantlets was surrounded by massive callus, which resulted in stunted shoot growth (Fig. 1e). The ventilation in the culture vessels probably affected the quality of the root system (Jackson 2003). Zhang et al. (2009) reported that, compared with the photomixotrophic micropropagation (PM) plantlets of *S. grosvenorii* grown on sucrose-containing medium without forced ventilation, PA plantlets with forced ventilated CO_2 as carbon source had a more developed rooting system, better shoots and no callus at the base of shoots. The poor rooting of sweet potato in vitro shoots partially resulted from low dissolved oxygen concentration around the shoot base in the agar medium (Zobayed et al. 1999). Similarly, in the liquid medium, the base of shoots was totally immersed in the liquid medium during the whole culture period, and so the concentration of oxygen around the root system was also limited. However, renewal of the head space in the TIS with every immersion led to higher oxygen concentration (Roels et al. 2006), which probably contributed to the well-developed root formation of *S. grosvenorii* plantlets.

In conclusion, by the combination of adequate culture ventilation and intermittent contact between shoots and the liquid medium, the microenvironment inside the temporary immersion container was improved, which contributed to improved shoot and root formation of *S. grosvenorii*. The natural-like plantlets of *S. grosvenorii* obtained in the TIS would probably have positive effects on ex vitro rooting and transplanting in large-scale commercial production.

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