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Full Length Research Paper

Improved growth and quality of *Dioscorea fordii* Prain et Burk and *Dioscorea alata* plantlets using a temporary immersion system

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The effects of temporary immersion system (TIS) culture on the growth and quality of *Dioscorea fordii* Prain et Burk and *Dioscorea alata* plantlets were investigated. Results indicate that TIS promoted the growth and quality of *D. fordii* and *D. alata* plantlets. Proliferation rate, shoot length, fresh weight (FW) and dry weight (DW) of shoots, and total biomass production were significantly (P≤0.05) higher in the TIS than in gelled and liquid medium, respectively. The TIS also promoted tuberization of *D. fordii*, and decreased vitrification of *D. alata* significantly. The healthy plantlets of *D. fordii* and *D. alata* obtained in the TIS would probably have positive effects on transplanting in large-scale commercial production.

Key words: *Dioscorea fordii* Prain et Burk, *Dioscorea alata*, culture system, TIS, micropropagation, tuberization.

INTRODUCTION

Dioscorea is a well known edible and traditional medicinal plant. The rhizomes of various species of *Dioscorea* had been used as an important ingredient for invigorating the spleen and stomach, promoting the body fluids, benefiting the lung and invigorating the kidney, in addition to its used as a food crop (Wang et al., 2006). Rhizome cuttings or aerial bulbils of *Dioscorea* are normally used to initiate a crop, but these are sensitive to viral disease infection, which finally degrade the cultivars and dramatically decrease the yield. To overcome such problems, *in vitro* propagation had been implemented for many *Dioscorea* species, such as *D. composita* Hemsl. and *D. cayenensis* Lam. (Viana and Mantell, 1989), *D. rotundata* (Balogun et al., 2006), *D. nipponica* Makino (Chen et al., 2007), *D. cayenensis-D. rotundata* complex (Ovono et al., 2010) and *D. prazeri* (Thankappan and Patell, 2011). In recent years, we have focused on the micropropagation of two elite cultivars, *viz.*, *Dioscorea fordii* Prain et Burk ("Guihuai No. 2") and *Dioscorea alata* ("Guihuai No. 6") (Yan et al., 2010, 2011), which had been spread widely in Southwestern China owing to their edible and medicinal dual functions.

The temporary immersion system (TIS), which was based on the principle of temporary contact between plants and liquid medium, had been extensively used for micropropagation (Alvard et al., 1993; Cabasson et al., 1997; Martre et al., 2001; Wawrosch et al., 2005; Alonso et al., 2009; Yan et al., 2010). Compared with gelled and liquid culture, the TIS had 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 *D. fordii* and *D. alata* using TIS had never been reported before. Hence, this research was conducted to improve the growth and quality of *D. fordii* and *D. alata* plantlets by using TIS.

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Abbreviations: **TIS**, Temporary immersion system; **SNCs**, single node leafy cuttings; **NTPs**, mean number of tubers per plantlet.

MATERIALS AND METHODS

Single-node leafy cuttings (SNCs) obtained from *in vitro* shoots of *D. fordii* and *D. alata* were used in all the experiments. *In vitro* shoot

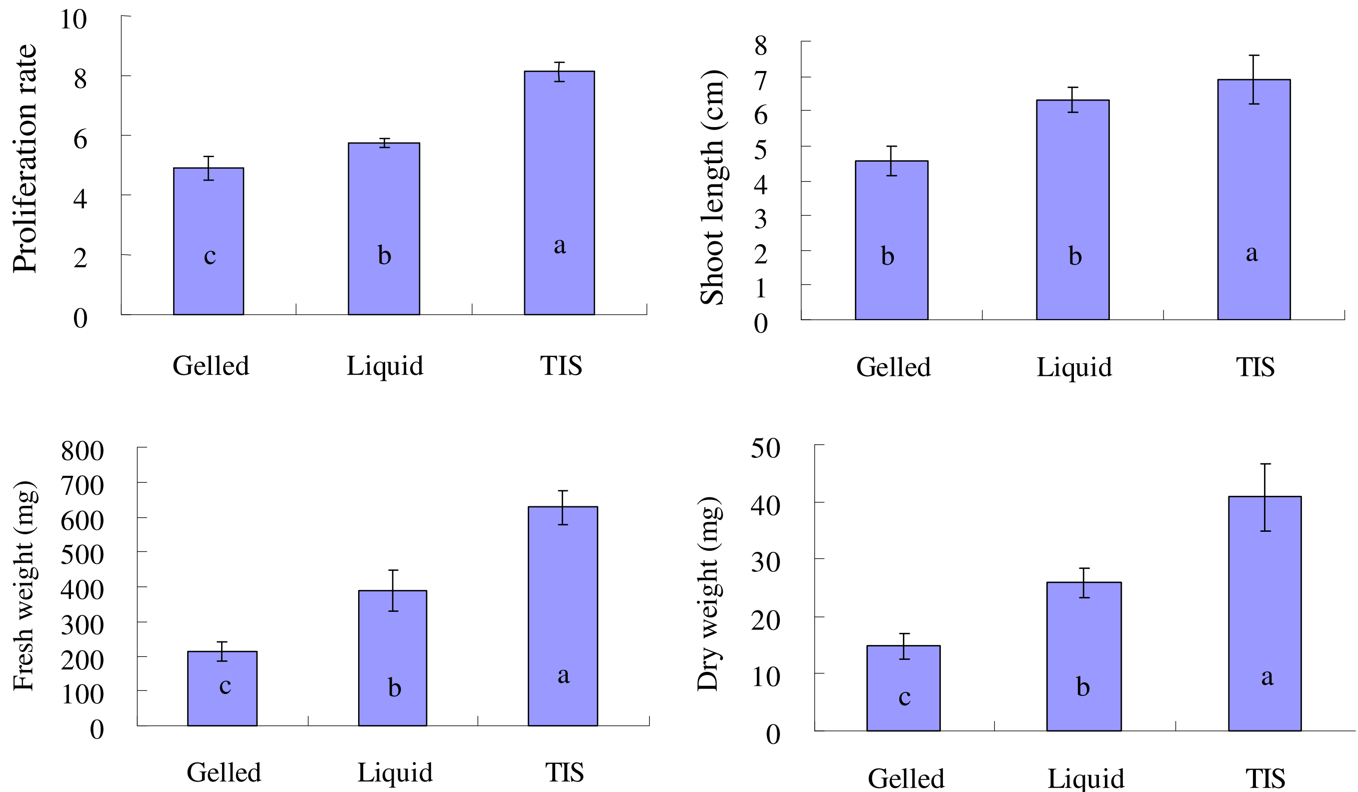

Figure 1. Comparative effects of gelled, liquid and temporary immersion system (TIS) on *D. alata* shoot proliferation with six weeks of culture. (Values with different alphabets within the bar are significantly different (P≤0.05), according to the *t* tests.)

regeneration methods had already been established (Yan et al., 2011). Using SNCs as explants, the effects of different culture systems (*viz*., gelled, liquid and TIS) on shoot proliferation of both *D. fordii* and *D. alata* were compared. As the gelled medium for proliferation, Murashige and Skoog (1962) (MS) medium was used and supplemented with 1.0 mg/L 6-benzylaminopurine (BA) (Sigma), 0.1 mg/L naphthalene acetic acid (NAA) (Sigma), 3% (w/v) sucrose (Nanning, China), 1.5 g/L activated charcoal (AC) (Nanning, China) and 4.0 g/L agar (Shanghai, China). The liquid and TIS medium for proliferation was the same as that 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 and 64 mm diameter).

The TIS used Plantima containers (A-Tech Bioscientific Co., Ltd., Taipei, Taiwan) with 250 ml medium in each container. The container comprised two compartments, an upper one with the plantlets and a lower one with the medium. The application of pressure in the lower compartment pushed the medium into the upper one. Plantlets were immersed as long as forced pressure was applied. During the immersion period, air was 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 3 min every 4 h by forced pressure, which propelled the liquid towards the plant material. The pH of all media used was adjusted to 5.8 ± 0.1 before autoclaving at 121°C for 20 min. All cultures were incubated at 25 ± 1°C under a 12/12 h (day/night) photoperiod with light supplied by white fluorescent tubes (25 µmol $m^{-2}s^{-1}$). After *in vitro* culture for six weeks, shoot length, proliferation rate, fresh weight (FW) and dry weight (DW) of shoot, frequency of tuberization and mean numbers of tubers per plantlet (NTPs) were scored. Shoot length was measured from the apical shoot tip to the base of stem. DW of shoots was determined after drying at 80°C for 72 h.

Statistical analysis

All the experiments were carried out in a completely randomized design with three replicates for each treatment, and using 18 SNCs in each replicate. The statistical analyses were performed using statistical analysis package (SAS version 8.01). Data were presented as mean ± standard error. When necessary, statistical significance was determined by using analysis of variance (ANOVA), the *t*- test and extreme deviation analysis.

RESULTS AND DISCUSSION

The TIS clearly promoted shoot formation (Figure 2A and B). Proliferation rate and shoot length of *D. fordii* plantlet in the TIS were significantly (P≤0.05) higher than those in gelled and liquid medium, respectively as shown by proliferation rate (2.1 and 1.1 times greater than those in gelled and liquid medium, respectively) and shoot length (2.9 and 1.3 times greater than those in gelled and liquid medium, respectively) (Table 1). Similar results are shown in Figure 1. FW (423.3 mg) and DW (39.4 mg) of *D. fordii* plantlet in the TIS were also significantly higher than those in gelled medium (72.2 and 6.3 mg, respectively) and

Figure 2. (A) *D. fordii* plantlets produced in gelled, liquid and TIS proliferation medium after six weeks of culture. (B) *D. alata* plantlets produced in gelled, liquid and TIS proliferation medium after six weeks of culture. (C) Various sizes of microtubers of *D. fordii* produced in TIS. (D) *D. fordii* plantlet with three microtubers produced in TIS. (E) The vitrificated plantlet of *D. alata* cultured in liquid medium. Bar = 2 cm.

those in liquid medium (306.8 and 28.2 mg, respectively) (Table 1). The positive effects of the TIS on shoot growth had been demonstrated by many authors in earlier studies (Alvard et al., 1993; Lorenzo et al., 1998; Etienne and Berthouly, 2002; Escalona et al., 2003; Yan et al., 2010). Under the same growth conditions, compared to gelled and liquid culture, plantlets in the TIS showed a significant ($P \leq 0.05$) increase in total biomass production expressed as DW and FW per plantlet (Table 1 and Figure 1). Alonso et al. (2009) confirmed that TIS was a promising method for biomass production of *Digitalis purpurea* by *in vitro* shoot multiplication. The total biomass

Table 1. Comparative effects of gelled, liquid and temporary immersion system (TIS) on *D. fordii* shoot proliferation with six weeks of culture.

Culture method	Proliferation rate	Shoot length (cm)	Fresh weight (FW) (mg)	Dry weight (DW) (mg)	The ratio of FW to DW	Frequency of tuberization (%)	Mean number of tubers per plantlet
Gelled	2.4 ± 0.4[b*]	2.0 ± 0.2[c]	72.2 ± 22.4[c]	6.3 ± 1.1[c]	11.3 ± 2.2[a]	8.3 ± 14.4[b]	0.1 ± 0.1[b]
Liquid	4.8 ± 0.8[a]	4.5 ± 0.6[b]	306.8 ± 57.6[b]	28.2 ± 6.5[b]	11.1 ± 1.8[a]	83.3 ± 8.3[a]	0.9 ± 0.1[a]
TIS	5.0 ± 0.1[a]	5.7 ± 0.3[a]	423.3 ± 54.6[a]	39.4 ± 4.8[a]	10.7 ± 0.3[a]	73.8 ± 2.1[a]	0.8 ± 0.1[a]

*Values with the different alphabets within the same column are significantly different ($P \leq 0.05$), according to the *t* tests.

of *Siraitia grosvenorii* plantlet cultured in TIS increased significantly, compared to that in gelled and liquid culture (Yan et al., 2010). The most important reason for the efficiency of the TIS was that it combined the advantages of both gelled culture (gas exchange) and liquid culture (increased nutrient uptake), which improved the growth of the plantlets (Etienne and Berthouly, 2002).

Compared to gelled culture, the liquid and TIS culture significantly ($P \leq 0.05$) promoted *in vitro* tuberization, indicated as frequency of tuberization and NTPs (Table 1). TIS could increase the tuber size and numbers of tubers per plantlet by extending the culture time without renewing the culture medium, compared to the liquid culture. After three months of culture, various sizes of microtubers of *D. fordii* (Figure 2C) were obtained, 70% of which were able to sprout in the seed bed (data not shown). There were three tubers produced on one plantlet occasionally (Figure 2D). Furthermore, vitrification often happened in the liquid culture of *D. alata* (Figure 2E), but not in TIS. Leaf malfunction in the liquid culture partly attributed to high relative humidity in the culture container (Ziv, 1991). By the combination of adequate culture ventilation and intermittent contact between shoots and the liquid medium, the microenvironment inside the temporary immersion container probably was improved, which contributed to improved shoot and root formation of *D. alata*. More also, *Calathea* plants from TIS presented more functional photosynthetic and respiratory apparatus, and could adapt more successfully to the environmental changes during *ex vitro* acclimatization (Yang and Yeh, 2008).

It is worth mentioning that the healthy plantlet obtained in TIS had well-developed roots, which probably shortened the time to transplant. The renewal of the head space in the TIS with every immersion led to the higher oxygen concentration (Roels et al., 2006), which probably contributed to the well-developed root formation of *D. fordii.* and *D. alata* plantlets. However, low dissolved oxygen concentration around the shoot base in the agar medium partially resulted in the poor rooting of sweet potato *in vitro* shoots (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.

Conclusion

This is the first report on the micropropagation of two Chinese yams, *D. fordii.* and *D. alata*, using TIS. Our results show that TIS could be used for shoot multiplication of these two yams, thereby providing a valuable way for propagation and seedling production. Meanwhile, TIS also provided an alternative valuable option for microtuber production of *D. fordii.* Therefore, with the availability of reliable microtuber production, the germplasm propagation, conservation and exchange of *in vitro* propagated, pathogen-tested elite clones would be facilitated (Balogun, 2009).

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