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Effects of Different Nutrient Solutions on the Acclimatisation of *in vitro* *Caladium* Plantlets using a Simplified Hydroponic System

(Kesan Larutan Nutrien yang Berbeza pada Pengikliman *in vitro* Anak Benih *Caladium* menggunakan Sistem Hidroponik Dipermudah)

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ABSTRACT

An efficient acclimatisation protocol is critical to improve the survival rate and promote growth of *in vitro* plantlets. Herein, tissue-cultured plantlets of 'Red Flash' *caladium* (*Caladium* × *hortulanum* Birdsey) were treated with deionised water and three nutrient solutions including Japanese garden formula (JG), South China Agriculture University formula B for leafy vegetables (SCAU-B) and Hoagland solution in a simplified hydroponic system under indoor environmental conditions. The results showed that all the plantlets survived after 30 days of hydroponic culture, and the three nutrient solutions showed significant positive effects on the plant growth and the content of chlorophyll a, chlorophyll b, total chlorophyll, carotenoid, soluble sugar and soluble protein compared to the deionised water treatment. No significant difference was recorded for the other growth parameters except the average leaf number per plantlet among the nutrient solution treatments. For the analysed physiological parameters, the plantlets treated by SCAU-B contained significantly higher content than those of the Hoagland treatment except the protein content. No significant difference was found between the JG and the SCAU-B treatment except the carotenoid content, and between the JG and the Hoagland treatment except the protein content. The plantlets acclimatised in the nutrient solutions also had a good performance when transferred to plastic pots. These results implied that water alone was not capable of maintaining healthy growth of *in vitro* *caladium* plantlets, and hydroponics approach using the three nutrient solutions could be a feasible method for acclimatisation of the plantlets.

Keywords: *Caladium* × *hortulanum* Birdsey; morphological characteristics; nutrient solution; physiological changes

ABSTRAK

Protokol pengikliman yang cekap adalah penting untuk meningkatkan kadar kemandirian dan menggalakkan pertumbuhan anak pokok secara *in vitro*. Dalam kajian ini, anak pokok kultur tisu bagi 'Red Flash' *kaladium* (*Caladium* × *hortulanum* Birdsey) telah dirawat dengan air ternyahion dan tiga larutan nutrien termasuk formula taman Jepun (JG), formula B South China Agriculture untuk sayuran berdaun (SCAU-B) dan Larutan Hoagland dalam sistem hidroponik mudah dalam keadaan persekitaran tertutup. Hasil kajian menunjukkan bahawa semua anak pokok bermandiri selepas 30 hari dalam kultur hidroponik, dan ketiga-tiga larutan nutrien menunjukkan kesan positif yang signifikan terhadap pertumbuhan tumbuhan dan kandungan klorofil a, klorofil b, jumlah klorofil, karotenoid, gula larut dan protein larut berbanding dengan rawatan air ternyahion. Tiada perbezaan yang signifikan direkodkan untuk parameter pertumbuhan lain kecuali bilangan purata daun bagi setiap anak pokok antara rawatan larutan nutrien. Untuk parameter fisiologi yang dianalisis, anak pokok yang dirawat oleh SCAU-B mengandungi kandungan yang lebih tinggi secara signifikan berbanding rawatan Hoagland kecuali kandungan protein. Tiada perbezaan yang signifikan antara rawatan JG dan SCAU-B kecuali kandungan karotenoid, serta antara rawatan JG dan Hoagland kecuali kandungan protein. Anak pokok yang diiklimkan dalam larutan nutrien juga mempunyai prestasi yang baik apabila dipindahkan ke dalam bekas plastik. Hasil kajian ini menunjukkan bahawa air sahaja tidak dapat mengekalkan pertumbuhan sihat anak pokok *caladium* secara *in vitro*, dan pendekatan hidroponik menggunakan ketiga-tiga larutan nutrien itu boleh menjadi kaedah yang sesuai untuk pengikliman tanaman.

Kata kunci: *Caladium* × *hortulanum* Birdsey; ciri morfologi; larutan nutrien; perubahan fisiologi

INTRODUCTION

Caladium (*Caladium* × *hortulanum* Birdsey) is an important ornamental plant valued for its long-lasting colorful foliage, and is commonly grown in containers and in the landscape (Deng 2018, 2012). A great deal of reports indicated that *caladium* could be propagated by *in vitro* culturing of leaf segments, meristematic tissues or

petiole segments (Ahmed et al. 2004; Cai & Deng 2016; Thongpukdee et al. 2010), and morphological variants were frequently observed among these regenerated plants (Cai et al. 2015; Cao et al. 2016). Recent studies found that chromosome gain or loss was a common type of cytological variation in *caladium* (Cai et al. 2015; Cao et al. 2016). Although the variations caused by *in vitro* culture may limit

the application of tissue culture technology in agriculture, valuable variations could be potential source materials for genetic improvement and breeding of caladium.

In vitro plantlets are commonly incubated in culturing vessels in an enclosed environment characterised by limited gas exchanges, constant temperature, high relative humidity and sterile condition, resulting in limited leaf surface wax layers, low stomata number, poor photosynthesis and weak root absorption of the plantlets (Pospóšilová et al. 1999). Compared to greenhouse plants, the leaves of *in vitro* plantlets had bigger stomata with larger pore area, higher stomata densities and higher epidermal densities (Asayesh et al. 2017). Due to these abnormalities, transition to *ex vitro* conditions frequently leads to a relatively high percentage of plant death due to serious environmental stresses (Kozai 1991; Pospóšilová et al. 1999). Therefore, it is an important step to improve the survival rate and promote healthy growth of the regenerated plantlets during the acclimatisation process for an efficient *in vitro* propagation protocol. In caladium, *in vitro* plantlets were commonly transplanted to containers or pots containing different substrate mixtures under controlled conditions such as an artificial climate box, where regular water and fertilisation for several weeks were needed during the acclimatisation process (Cai et al. 2015; Cao et al. 2016). The plantlets were gradually acclimated to ambient atmospheric conditions, which was a difficult stage and a labor intensive process.

Hydroponics is a soilless culture system for growing plants with the root systems emerging in the water solution containing appropriate mineral nutrients under controlled growth conditions, and it is considered as a highly efficient way and a water-saving method in numerous plants worldwide (Bradley & Marulanda 2001). Composition, concentration, pH, electric conductivity (EC), temperature and oxygenation of nutrient solution are important factors that may affect plant growth and development (Hosseinzadeh et al. 2017). Recently, advances have been made in investigating suitable hydroponic culture conditions for a variety of ornamental plants (Banijamali et al. 2018; Phantong et al. 2018; Sutthinon et al. 2015) including caladium (Li et al. 2010; Phonpho & Saetiew 2017; Xu & Cui 2014;). In addition, several reports suggested that hydroponics could be successfully applied in acclimatisation of *in vitro* produced plantlets, such as *Solanum tuberosum* L. (Nhut et al. 2006), *Stemona curtisii* Hook. f. (Palee et al. 2012) and *Grammatophyllum speciosum* Blume (Sutthinon et al. 2015). In these studies, hydroponics had been proved to be a feasible alternative to acclimatise *in vitro* plantlets in a clean, convenient and water-saving way. However, there has been little information about an efficient method for acclimatisation of *in vitro* caladium plantlets using a hydroponic system. Nutrient solution formula is an important factor affecting the growth process and final yield and quality of crops (Trejo-Téllez & Gómez-Merino 2012). Quite a few nutrient solution formulations have been published

to define the balanced concentrations of essential microelements and macroelements, such as Japanese garden formula (JG) included by Liu (2001), South China Agriculture University formula B for leafy vegetables (SCAU-B) described by Liu (2001) and Hoagland solution (Hoagland & Arnon 1950). The nutrient solution can be static or flow continuously to maintain a constant nutrient level and water status in the root zone. Static hydroponics is a type of hydroponic solution culture technique in which plants are grown in static reservoirs of nutrient solution, which is regarded as the oldest soilless and easiest culture technique (Öztekin et al. 2018). It is very popular for home gardening due to its simplicity and low cost. In this study, *in vitro* plantlets of caladium were grown in simplified hydroponic plant boxes filled with deionised water without any fertiliser, JG, SCAU-B and Hoagland solution (as control), respectively, and the morphological and physiological characteristics of these plantlets were studied to establish a feasible acclimatisation method in a static hydroponic system.

MATERIALS AND METHODS

PLANTLET ESTABLISHMENT

Plantlets were produced *in vitro* from young leaf segments of 'Red Flash' caladium (*Caladium × hortulanum* Birdsey) according to the method described by Cai and Deng (2016). Briefly, after washed with running water for 30 min, the leaves were surface disinfected by soaking in 70% (v/v) alcohol for 15 s and then in 0.5% (w/v) sodium dichloroisocyanuric acid for 20 min, and rinsed with sterile water three times. The disinfected leaves were cut into small segments (about 0.5 × 0.5 cm) in a laminar flow cabinet, and then cultured on the callus induction medium, i.e., solidified Murashige and Skoog's medium (MS) supplemented with 1 mg/L thidiazuron (TDZ) and 1 mg/L 1-Naphthyl acetic acid (NAA). After 2 months of culture, the induced calli were transferred to MS basal medium with 1 mg/L 6-benzyladenine (6-BA) and 0.1 mg/L NAA (callus differentiation medium) for production of sufficient plantlets. Well-rooted plantlets were taken out from the culture medium and rinsed carefully under running tap water to remove the medium from the roots, and the plantlets of similar height were prepared for the present experiment.

HYDROPONIC APPARATUS

A simplified hydroponic plant box (length × width × height = 50 cm × 27 cm × 34 cm) equipped with two LED lamps (10 W each) and a solution tank of 4 L in volume (Haier Co., Ltd., Qingdao, China) was prepared for the plantlet growth, and there are 30 planting holes (3 cm height and 2.5 cm diameter) in the plastic cover plate as shown in Figure 2(A). A light intensity of about 3500 Lux was measured in the middle of the box, and it has no temperature control device and liquid flow system.

HYDROPONICS

To establish a feasible static hydroponic system for acclimatisation of *in vitro* plantlets of caladium, deionised water, without any fertiliser, and three universal nutrient solutions including full strength of Japanese Garden formula (JG), South China Agriculture University formula B for leafy vegetables (SCAU-B) and Hoagland solution (as control) were prepared. The pH value of all the treatments was adjusted to 5.8 each time during application of the culture solutions. Four hydroponic devices were used in this study, and each was filled with one type of these culture solutions, respectively. The plantlets were transplanted individually to the hydroponic plant box by pushing the roots through the planting holes into the hydroponic solutions, and a total of 30 plantlets were grown in each hydroponic apparatus.

The plantlets were maintained in the hydroponic boxes for 30 days. The experiment was conducted during the period from May to early July of 2018 in a Lab at the Yangtze University, Jingzhou, China (30°21'26.2" N, 112°08'57.2" E) with an ambient temperature between 18°C and 32°C, and relative humidity varied from 55% to 85%. The plantlets grew with a 14 h light/10 h dark photoperiod under natural temperature conditions without a cooling/heating system. The hydroponic solutions were replaced by fresh solutions once every 10 days. At the end of the experiment, five healthy individuals in each treatment were selected at random from the 30 plantlets for variable analysis and assays were repeated three times.

VARIABLE ANALYSIS

After 30 days of hydroponic culture, different growth parameters including the survival rate, plantlet height, number of leaves and roots, and fresh weight per plantlet were collected directly. Leaf tissue without main veins was used to determine the physiological changes including content of photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid), soluble sugar and soluble protein. Leaf pigments were extracted by soaking and grinding 0.2 g leaf tissue with a mortar in an 80% acetone buffer solution according to Li

(2000), and the absorbance of the extract was measured spectrophotometrically at 663 nm, 645 nm and 470 nm, respectively. The content of photosynthetic pigments was calculated using the equations provided by Lichtenthaler (1987). Total soluble sugar content was determined by using the anthrone colorimetry method at 630 nm according to Li (2000). Soluble protein content was measured by Coomassie Brilliant Blue G-250 method using bovine serum albumin (BSA) as standard (Bradford 1976).

STATISTICAL ANALYSIS

Data of different parameters were analyzed statistically by using one-way ANOVA in SPSS statistical software (version 23 for Windows), and significant differences between the means were evaluated using Duncan's multiple range test at $p < 0.05$. The data were expressed as the mean value \pm standard deviation.

RESULTS

CALLUS INDUCTION AND PLANTLET ESTABLISHMENT

Small amount of callus was observed around the cut surfaces of the explants about 1 month of culture on the callus induction medium, MS basal medium supplemented with 1 mg/L TDZ and 1 mg/L NAA, and the callus was compact with a light yellow color (Figure 1(A)). These calli proliferated slowly and several shoots and/or roots occurred after another month of incubation on the callus induction medium (Figure 1(B)). A large number of vigorously growing plantlets with healthy root system were regenerated from the callus after 3 months of culture on the callus differentiation medium, MS basal medium supplemented with 1 mg/L 6-BA and 0.1 mg/L NAA (Figure 1(C)). Thus, sufficient plantlets were established for the subsequent study.

PLANTLET GROWTH IN HYDROPONICS

Plantlets treated by the three nutrient solutions grew more vigorously than those cultured in the deionised water, and

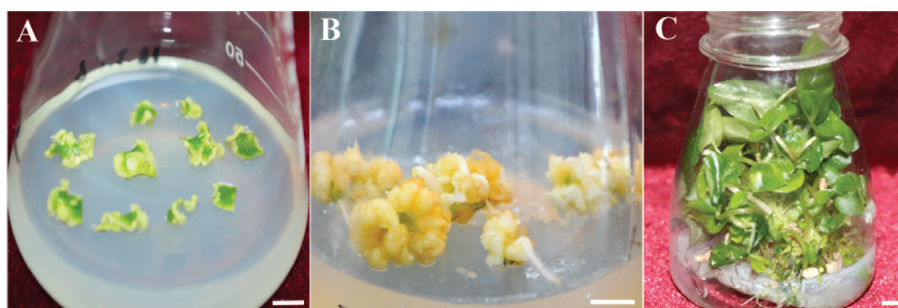


FIGURE 1. Plantlets regenerated from leaf segments of 'Red Flash' caladium (*Caladium × hortulanum* Birdsey). A. Callus initiation after about one month of culture on MS basal medium supplemented with 1 mg/L TDZ and 1 mg/L NAA (callus induction medium); B. Regenerated calli with shoots and/or roots after two months of culture on the callus induction medium; C. Plantlets regenerated from the calli after three months of culture on MS basal medium supplemented with 1 mg/L 6-BA and 0.1 mg/L NAA (callus differentiation medium). Scale bars=1 cm

each developed several heart-shaped leaves with red main veins, white blotches, green margins and a red-purple petiole (Figure 2(A)). Compared to the deionised water treatment, all the plantlets cultured in the nutrient solutions showed a higher height and developed more roots and leaves (Figure 2(B)). Moreover, although the plantlets grown in the deionised water showed a poor growth response, it was found that the caladium plantlets treated by not only the nutrient solutions but also the deionised water, all survived in this study.

SURVIVAL RATE AND MORPHOLOGICAL CHARACTERISTICS OF THE *IN VITRO* CALADIUM PLANTLETS IN HYDROPONICS

The survival rate of the hydroponic plantlets in the deionised water and the three nutrient solutions all reached 100% (Table 1), suggesting that hydroponic method was feasible and efficient for acclimatisation of the *in vitro* plantlets of caladium. Data pertaining to the morphological traits after 30 days of hydroponic culture influenced by the different treatment solutions were presented in Table 1. Effects of the hydroponic solutions on the plantlet height were in the following order: SCAU-B>Hoagland>JG>deionised water, and the plantlets treated by SCAU-B reached a mean height of 22.06 cm, which was nearly two times higher than that of the deionised water treatment. Statistical analysis suggested that all the three nutrient solution treatments significantly increased the plantlet height compared to the

deionised water treatment, while it was not significantly different among the three nutrient solution treatments for this growth parameter (Table 1). The mean leaf number per plantlet reached 5.08 when treated with JG, which was significantly higher than that of the other treatments, and no significant difference was found between the SCAU-B and the Hoagland solution treatments (Table 1). For the root number per plantlet, the results showed that there was no significant difference among the three nutrient solution treatments, while all of them significantly increased the root number compared to the deionised water treatment (Table 1). Plant fresh weight is an important parameter related to the growth activity and the nutrient absorption ability of the root system. The highest plantlet fresh weight was observed in the SCAU-B treatment (5.77 g per plant), which was 2.35 times higher than that of the deionised water treatment, while there was no significant difference among the three nutrient solution treatments (Table 1).

VARIANCE OF PHYSIOLOGICAL PARAMETERS OF THE *IN VITRO* CALADIUM PLANTLETS IN HYDROPONICS

Variance of physiological parameters related to plantlet growth and development treated by different hydroponic solutions was shown in Table 2. Results showed that the plantlets grown in SCAU-B yielded the highest content of chlorophyll *a*, chlorophyll *b*, total chlorophyll, carotenoid, soluble sugar and soluble protein after 30



JG: Japan Garden formula; SCAU-B: South China Agriculture University formula B for leafy vegetables

FIGURE 2. Growth of *in vitro* 'Red Flash' caladium plantlets after 30 days of acclimatization in Hoagland nutrient solution by using a static hydroponics system (A) and plant morphological characteristics of the plantlets after 30 days of hydroponic culture in JG, SCAU-B, Hoagland solution and deionized water, respectively (from left to right) (B)

TABLE 1. Survival rate and morphological characteristics of the caladium plantlets after 30 days of acclimatization in the different hydroponic solutions by using a static hydroponic system

Treatments	survival rate (%)	plantlet height (cm)	Leaf number/plantlet (no)	Root number/plantlet (no)	Fresh weight/plantlet (g)
Deionized water	100 a	12.00±3.41 b	3.29±0.19 c	12.29±1.54 b	2.46±0.29 b
JG	100 a	17.62±2.67 a	5.08±0.26 a	16.00±1.77 a	4.31±0.92 a
SCAU-B	100 a	22.06±1.91 a	4.42±0.36 b	15.88±1.57 a	5.77±1.28 a
Hoagland	100 a	21.61±2.44 a	4.33±0.44 b	15.04±2.70 a	5.74±1.44 a

Means ± standard deviation within a column followed by the same letters are not significantly different according to Duncan's range test at $p < 0.05$. JG: Japan Garden formula; SCAU-B: South China Agriculture University formula B for leafy vegetables

days of hydroponic culture, which increased by 51.4%, 47.9%, 50.5%, 50.8 %, 41.1% and 32.7%, respectively, compared to the deionised water treatment. The plantlets grown in deionised water yielded the lowest content of these physiological parameters, which were all significantly lower than those of other nutrient solution treatments, respectively. The content of photosynthetic pigments including chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid when treated with SCAU-B was all significantly higher than that of the Hoagland solution treatment, and no significant difference was observed between the JG and the SCAU-B treatments for these variances except the carotenoid content. For the soluble sugar content treated by the three nutrient solutions, there was no significant difference between the JG and the Hoagland solution treatments. For the soluble protein content, no significant difference was observed between the SCAU-B and the Hoagland solution treatments, but the two treatments significantly increased the soluble protein content compared to the JG treatment (Table 2).

DISCUSSION

In caladium, callus, shoots and roots were readily induced from a range of explant types such as leaf segments on a suitable medium (Ahmed et al. 2004; Cai & Deng 2016; Thongpukdee et al. 2010). In this study, callus formation was also observed when the leaf segments were cultured on the MS basal medium supplemented with 1 mg/L TDZ and 1 mg/L NAA. The induced callus was easily differentiated into shoots and/or roots, and could regenerate into plantlets after culturing for several months. Similar results have also been found by Cai and Deng (2016) when developing a procedure for producing friable callus with sustained regeneration potential in caladium.

After well-rooted plantlets established, the most crucial stage for an efficient tissue culture protocol is to improve the survival rate and enhance growth of the regenerated plantlets during the acclimatisation process (Kozai 1991; Pospóšilová et al. 1999; Sutthinon et al. 2015). Different systems including hydroponics have been developed for acclimatisation of *in vitro* grown plantlets (Nhut et al. 2006; Palee et al. 2012; Sutthinon et al. 2015). In this study, a 100% survival rate and a superior performance

were observed for the *in vitro* caladium plantlets treated by the three nutrient solutions (Table 1). Physiological analysis showed that the nutrient solution treatments remarkably increased the content of photosynthetic pigments including chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoid (Table 2). It is widely known that chlorophyll content is an important indicator of photosynthesis activity and plant status (Bailey et al. 2004). The significant increase in several morphological characteristics of the plantlets grown in the tested nutrient solutions may be related to higher chlorophyll content in the plantlet leaves. Sucrose is one of the primary products of photosynthesis in the leaves of most plants (Kötting et al. 2010), and a relatively high content of chlorophyll is one of preconditions of intensive photosynthesis, which could be the reason why the soluble sugar content increased with increasing chlorophyll content in the plantlet leaves in this study (Table 2). Most soluble proteins are enzymes that participate in various metabolisms of plants, and are considered as one of the most important physiological parameters reflecting the overall metabolic level in plants (Doganlar et al. 2010). In this study, analysis of the protein content showed that the plantlets treated with the three nutrient solutions have significantly higher metabolic levels than that of the deionised water treatment (Table 2). The results of the physiological parameters showed that the caladium plantlets could grow well with normal physiological metabolism in the three nutrient solutions, which is a strong proof of the feasibility of the static hydroponic method for acclimatisation of *in vitro* caladium plantlets. Similar results have also been reported by Nhut et al. (2006) on *S. tuberosum* L. and Palee et al. (2012) on *S. curtisii* Hook. f.

Caladium is very sensitive to low temperature and chilling stress (Deng 2012). Due to the limitation of temperature conditions, its growth and ornamental period are usually short when cultivated in open fields in most of China. Therefore, hydroponic culture of *in vitro* or *in vivo* caladium plantlets indoors might extend its ornamental period. Several aspects of this technique still need to be investigated to optimise this hydroponic system. For example, ornamental value is an important evaluation factor for foliage plants, and therefore effects of nutrient solutions on foliar characteristics such as color of leaf main

TABLE 2. Variance of physiological parameters (mg/g FW) of the caladium plantlets after 30 days of acclimatization in the different nutrient solutions by using a static hydroponics system

Treatments	Chlorophyll <i>a</i> content	Chlorophyll <i>b</i> content	Total chlorophyll content	Carotenoid content	Soluble sugar content	Soluble protein content
Deionized water	1.46±0.11 c	0.48±0.06 c	1.94±0.17 c	0.31±0.02 c	3.67±0.19 c	27.26±0.60 c
JG	1.97±0.31 ab	0.64±0.12 ab	2.61±0.43 ab	0.38±0.08 b	4.38±0.34 b	32.93±1.71 b
SCAU-B	2.21±0.10 a	0.71±0.03 a	2.92±0.12 a	0.46±0.02 a	5.18±0.44 a	36.18±1.19 a
Hoagland	1.82±0.13 b	0.57±0.03 b	2.40±0.17 b	0.39±0.05 b	4.11±0.49 b	36.15±1.28 a

Means ± standard deviation within a column followed by the same letters are not significantly different according to Duncan's range test at $p < 0.05$

JG: Japan Garden formula;

SCAU-B: South China Agriculture University formula B for leafy vegetables

vein, blotches and margins need further study. Besides, minituber production by means of hydroponics has been realised in potato (Nhut et al. 2006; Ritter et al. 2001). Commercial production of caladium plants in the world is mostly forced from tubers. In this study, the plantlets did not form minitubers during the acclimatisation process, which may be due to the growth period which was too short to form minitubers. Establishment of an ideal hydroponic system for caladium tuber production using *in vitro* plantlets is needed in future study.

CONCLUSION

In summary, hydroponic culture has been proved to be an efficient way for acclimatisation of the *in vitro* grown caladium plantlets in this study. Water alone was not capable of maintaining healthy growth of the plantlets, and hydroponics using the three nutrient solutions could be a feasible method for acclimatisation of *in vitro* caladium plantlets in the static hydroponic culture system. Effects of different nutrient solutions on the ornamental value and minituber production of *in vitro* caladium plantlets during the acclimatisation process need further study.

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