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## ENHANCED GROWTH AND DEVELOPMENT OF *CHRYSANTHEMUM MORIFOLIUM* IN MICROPONIC SYSTEM UNDER LIGHT-EMITTING DIODES

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### SUMMARY

This paper investigated the growth and development of *Chrysanthemum* shoots in microponic systems in comparison with shoots in micropropagation system. A microponic culture system, combining micropropagation and hydroponics, could reduce the drawbacks of micropropagation system such as reduction of infection, saving of labor, material, space, etc. In this study, *Chrysanthemum morifolium* shoots with 3 cm in length were cultured in MC (microponic system with circular container - 12 cm diameter at top, 9 cm diameter at bottom and 8.5 cm in height), MR (microponic system with rectangular box - 8.5 cm in height, 35 x 28 cm at top and 30 x 25 cm at bottom) and micropropagation system (MO - rectangular plastic box with 800 ml of half-strength MS medium containing 30 g/l sucrose and 8 mg/l agar). The results indicated that shoots pretreated with 500 ppm IBA, cultured in MC (15 shoots per container) and ventilated with millipore membrane (Milliseal<sup>TM</sup>, pore size 0.5  $\mu$ m of diameter 2 cm) under 70% red LED combined with 30% blue LED gave the best plant height, number of roots, fresh weight, and chlorophyll content (a, b and a+b) (5.18 cm, 12.50, 0.52 g, 28.19  $\mu$ g/g, 13.56  $\mu$ g/g and 41.75  $\mu$ g/g, respectively). The survival rates of plants derived from MC and MR in the greenhouse were higher than those in MO (100%, 100% and 85%, respectively). This study indicated that MR was an effective and simple system for large-scale production of *Chrysanthemum morifolium*.

**Keywords:** *Chrysanthemum*, circular container, LEDs, microponic, rectangular box

### INTRODUCTION

Micropropagation proves to be an effective method for multiplying plants in large numbers in a short time. The success of micropropagation method depends heavily on the success of acclimatization in nursery conditions. In *in vitro* stage, growth of plants is controlled while in the acclimatization stage; plants are exposed to numerous adverse external factors such as fungal diseases, temperature fluctuation, low humidity, and poor nutrition. These sudden changes reduce the survival rate of plants, significantly. In addition, when transferred to *ex vitro* conditions, physiological changes within the plant organs also lead to morphological and anatomical abnormalities. In a general micropropagation system, stomata of plants do not function properly, roots are weak, and the epidermal

layer is thin (Mathur *et al.*, 2008). During this adaptation stage, studies have demonstrated changes in sugar, starch, protein solubility, ribulose 1,5 - biphosphate carboxylase (Rubisco) activity and phosphoenolpyruvate carboxylase in oats (Valero-Aracama *et al.*, 2006), or increasing activity of L-gene-lactone oxidase gulono- $\gamma$  (GLOase) in potato (Hemavathi *et al.*, 2010). Therefore, understanding the physiological and biochemical changes in plants during adaptation is essential to devise measures for improving the survival rate, growth and development of plants in greenhouse.

Microponic system is a propagation approach which combines the advantages of micropropagation and hydroponics. This system was first described by Hahn *et al.* (1996, 1998, 2000), using the nutrient film technique (NFT) with a small pump for medium circulation through rockwool. Culture conditions

(temperature, CO<sub>2</sub>, air humidity, pH and electrical conductivity) were controlled so that the photosynthetic rate, fresh weight, dry weight, leaf size, leaf number, and stomatal density could be improved. However, previous studies on this system evaluated the morphological characteristics of plant growth only without further assessment of physiological changes as well as application for commercial production.

## MATERIALS AND METHODS

### Materials

#### *Plant material*

*Chrysanthemum morifolium* shoots with 3 cm in length and 2 pair's leaves were used as explant source. These shoots were obtained from a mass of shoots cultured *in vitro* on MS medium (Murashige, Skoog, 1962) with 8 g/l agar and 30 g/l sucrose after 40 - 45 days of cultured.

#### *Substrate*

Nylon films with size of 20 cm x 30 cm were wrapped around a test tube with an outer diameter of 1.5 cm. Then, they were welded by a heated metal rod.

Next, the test tube was removed, excess of nylon film was cut off and the long plastic tube was cut into short tubes with 2 cm in length. These tubes were used as the substrate in microponic system.

#### *Culture system*

##### *Microponic system:*

Microponic system MC was circular plastic containers with 12 cm diameter at top, 9 cm diameter at bottom, and 8.5 cm of height. 40 ml of half-strength sugar-free liquid MS medium was added to MC includes 15 tubes nylon film (Nhut *et al.*, 2005).

Microponic system MR was rectangular plastic boxes with a height of 8.5 cm, 35 x 28 cm at top and 30 x 25 cm at bottom. MR system contained 300 substrate tubes and filled with about 800 ml of half strength sugar-free liquid MS medium.

Shoots were pretreated with IBA solution at concentrations 500 ppm for 20 minutes. Types of ventilation condition including one Millipore membrane with Milliseal<sup>TM</sup>, pore size 0.5 μm, Nihon Millipore Ltd., Tokyo, Japan of diameter 2 cm. The leaves of shoots were not exposed to IBA solution before being placed in MC system.

##### *Micropropagation system:*

Micropropagation system (MO) was rectangular plastic box (boxes with a height of 8.5 cm, 35 x 28 cm at top and 30 x 25 cm at bottom) with 800 ml of half-strength MS medium containing 30 g/l sucrose and 8 mg/l agar added to the substrate. This medium was sterilized (autoclave for 20 min at 121°C and 1 atm) and poured into MO.

### Methods

#### *Combination of microponic system and light-emitting diodes*

Shoots were cultured in MC system after 2 weeks of culturing under different light-emitting diodes (LEDs) including Green - G (565 nm), Blue - B (450 nm), Red - R (660 nm), Yellow - Y (590 nm) (Steigerwald, 2002), and B combined with R of different ratios (10:90, 20:80, 30:70, 40:60, 50:50 and 60:40) at 40-45 μmol.m<sup>-2</sup>.s<sup>-1</sup>. Fluorescent lamp (FL) was used as the control.

#### *Large scale system*

Three hundred shoots were cultured in MR and MO. After 2 weeks of culture, plants were transferred into greenhouse for acclimatization.

#### *Plant acclimatization*

One hundred plants cultured in each system (MO, MC and MR) were transferred into greenhouse at 18-25°C with humidity of 65-70% and put in the 50% of natural light.

#### *Culture conditions and statistical analysis*

All shoots were cultured at 25±2°C with humidity of 55-60% and photoperiod of 12 hours/day under LEDs and FL.

Plant height, number of leaves, number of roots, root length, fresh weight of plant and content of chlorophyll a, chlorophyll b and chlorophyll a+b (= Chlorophyll a + Chlorophyll b) of leaf were recorded after 2 weeks of culture while survival rate was recorded after 2 weeks of acclimatization.

Content of chlorophyll a and chlorophyll b were determined based on maximum absorption spectrophotometer of chlorophyll a (662 nm) and chlorophyll b (645 nm) using UV-2900 spectrophotometer machine (Lichtentaler, Wellburn, 1985).

$$\text{Chlorophyll a} = (11.75 \cdot A_{662} - 2.35 \cdot A_{645}) \text{ (}\mu\text{g/g)}$$

$$\text{Chlorophyll b} = (18.61 \cdot A_{645} - 3.96 \cdot A_{662}) \text{ (}\mu\text{g/g)}$$

All treatments were in triplicates and each replicate with 10 culture vessels. Data were scored after 2 weeks of culturing and analysis of variance was performed. The means were compared using Duncan's multiple range Test using SPSS (Version 16.0) at  $\alpha$  value = 0.05 (Duncan, 1995).

## RESULTS AND DISCUSSION

### Combination of microponic system and light-emitting diodes

After 2 weeks of culture, the effect of different light sources on the growth and development of shoots was recorded (Table 1 and Fig. 1).

The results showed that the light quality effected to growth and development of plants. The fresh weights of plants under B + R at the ratio of 10:90, 20:80, 30:70, 40:60 and 50:50 were higher than those of plants under FL, G, R and Y. Especially for ratio of 30B + 70R, fresh weight (0.52 g), plant height (5.80 cm) and number of roots (12.50 root) were higher than other lighting conditions. Root length was highest in FL (1.27 cm).

**Table 1.** Effects of lighting condition on plant growth and development in MC after 2 weeks of culture. : \*Different letters within a column indicate significant differences at  $\alpha$  = 0.05 by Duncan's multiple range tests; \*\*: Necrotic plants.

Light conditions	Plant height (cm)	No. of leaves	No. of roots	Root length (cm)	Fresh weight (g)
FL	4.73de <sup>*</sup>	7.00ab	9.70bc	1.27a	0.42bc
B	5.50ab	7.00ab	11.20ab	0.36 <sup>e</sup>	0.41bc
R	5.70a	7.00ab	9.10bc	0.86bc	0.38cd
10B + 90R	5.21bc	6.70ab	9.80bc	0.87bc	0.48ab
20B + 80R	5.08cd	6.80ab	9.50bc	1.01ab	0.49ab
30B + 70R	5.80a	7.20a	12.50a	1.12ab	0.52a
40B + 60R	5.30bc	6.80ab	8.30cd	0.79cd	0.48ab
50B + 50R	4.90cde	6.70ab	8.90cd	0.52de	0.44bc
60B + 40R	4.71 <sup>e</sup>	6.40b	6.80 <sup>e</sup>	0.42 <sup>e</sup>	0.33d
G	- <sup>**</sup>	-	-	-	-
Y	-	-	-	-	-

Shoots in MC under B and Y were necrotic. This may be due to the wavelength of the Y (590 nm) and G (565 nm) which is not consistent with the maximum absorption spectrophotometer of chlorophyll a (662 nm) and chlorophyll b (645 nm) (Lichtentaler, Wellburn, 1985). In this study, plants were cultured in MC under B (450 nm) gave the effected to growth and development (Table 1). Blue LEDs was the absorption peak of cytochrome and carotenoid. Moreover, this light source is capable of adjusting light intensity, together with the adjustment of CO<sub>2</sub> concentration, relative humidity, temperature, accumulation of chloroplasts, open stomata optimize growth and development of plants in greenhouses and in the tissue culture (Bula, 1991; Sakai *et al.*, 2001; Kinoshita *et al.*, 2001). B also had a role in the formation of chlorophyll in plants (Akoyunoglou, Anni, 1984). Plants in MC cultured under 30B:70R showed the

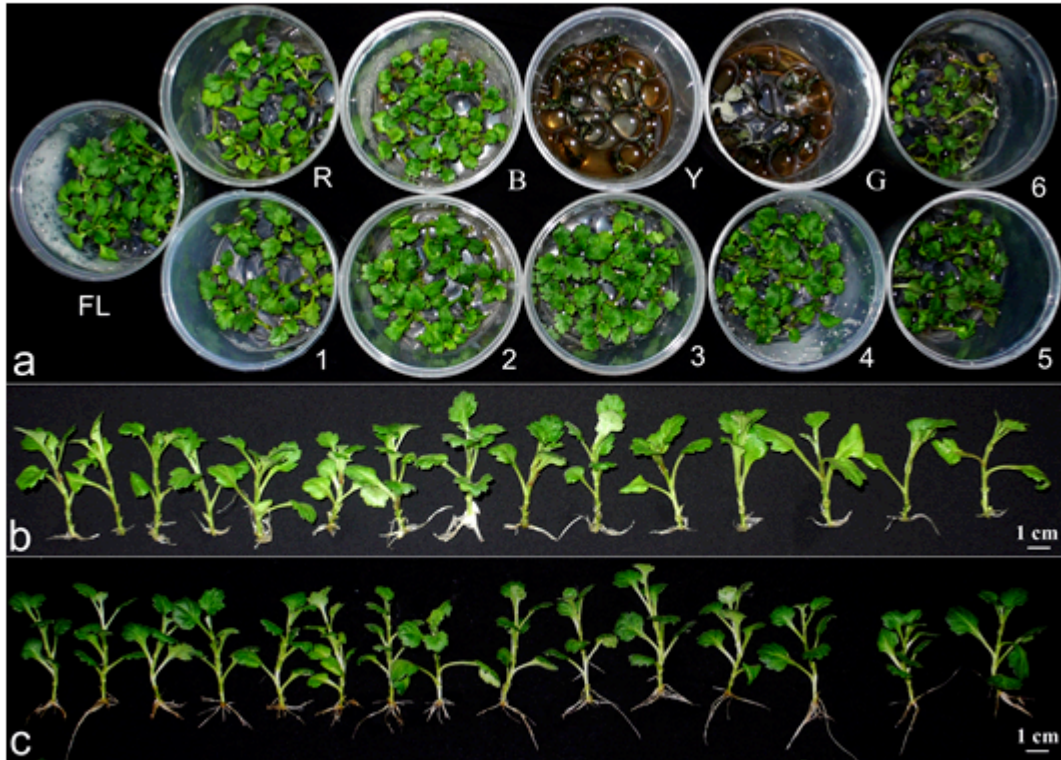
content of chlorophyll a, b and (a+b): 28.19, 19.56, 41.75  $\mu$ g/g, which were higher than other light condition, include other combined B and R LED (Fig. 2).

Currently, LEDs are used in many areas of biological research on photosynthesis as chlorophyll (Tripathy, Brown, 1995), photosynthesis (Tennessen *et al.*, 1994) and morphogenesis (Hoenecke *et al.*, 1992). The response depends on light intensity and quality of light (light spectrum, photoperiod, lighting direction) and photoperiod (Taiz, Zeiger, 2007).

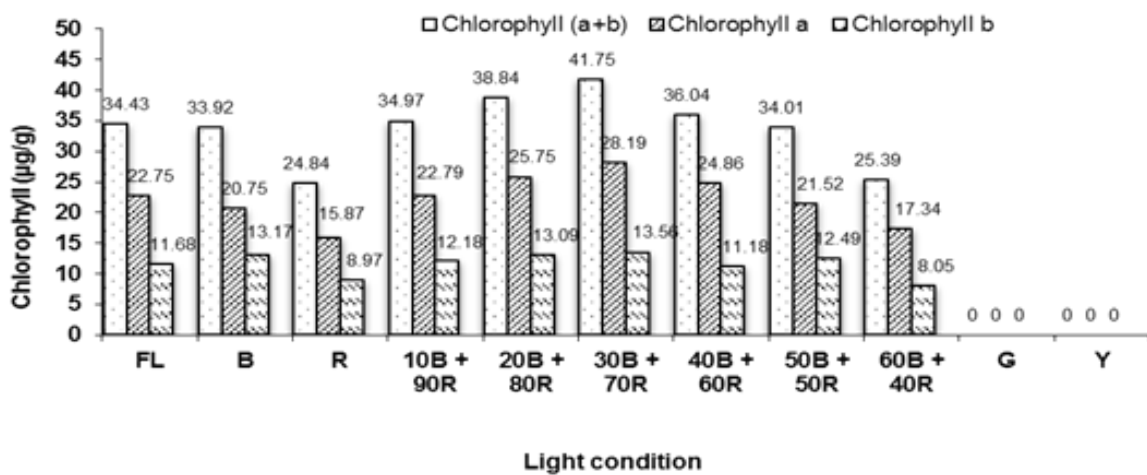
Nhut (2011) indicated that the shoots of Strawberry, *Eucalyptus*, *Cymbidium*, *Phalaenopsis* and Bananas under red LED were elongated and the concentration of chlorophyll decreased. In our studies, we used LED as artificial lighting source for MC and MR. *Chrysanthemum* shoots under R were

slender, yellowish and contained fresh weight (0.38 g), concentration of chlorophyll a (15.87 µg/g), chlorophyll b (8.97 µg/g) and chlorophyll a+b (24.84 µg/g) which were lower than the plants growing

under the combination of B and R (10:90, 20:80, 30:70; 40:60 and 50:50). Zeiger (1984) indicated that the low chlorophyll synthesis was controlled by B rather than R (10:90, 20:80, 30:70, 40:60 and 50:50).



**Figure 1.** Growth and development of plants in MC under different lighting conditions. **a.** MC under different lighting conditions [FL, R, B, Y, G and B + L (1: 10B+90R; 2: 20B+80R; 3: 30B+70R; 4: 40B+60R; 5: 50B+50R and 6: 60B+40R, respectively)]; **b.** Plants in MC under FL; **c.** Plants in MC under 30R+70B.



**Figure 2.** Effect of different lighting conditions on the ability of the plant synthesized chlorophyll chrysanthemum in the MC. [Chlorophyll (a + b) = (Chlorophyll a) + (Chlorophyll b)].

Therefore, the use of two types of LEDs emit light R and B in this model is essential for the normal growth of the seedlings. From the data obtained in this study, the use of B combined with R of the ratio of 30:70 increased the growth and development of plants as well as improved the plants quality.

### Large scale system

The results showed that plant height, number of leaves and fresh weight of plants, concentration of chlorophyll (a + b) and leaf width were not different from each treatments after 2 weeks of cultured (Table 2).

In MO (Fig. 3 c), the number of roots (17.17 roots) and root length (2.40 cm) were higher than those in MC (Fig. 3 b) and MR (Fig. 3 a). This may be due to the culture medium of MO containing sugar and agar, as favorable for the rooting.

MR (Fig. 3 a<sub>1</sub>) and MO - 300 plants were produced in MR and MO (35 × 28 = 980 cm<sup>2</sup>) (Fig. 3 a<sub>1</sub>, c<sub>1</sub>) saved space (½ of the area) in comparison with MC - 300 plants in 20 MC (20 × 6<sup>2</sup> × 3.14 = 2260.8 cm<sup>2</sup>) (Fig. 3 b<sub>1</sub>).

**Table 2.** Growth and development of plant in three different culture systems after 2 weeks of culture. Different letters within a column indicate significant differences at α=0.05 by Duncan's multiple range tests.

Culture systems	Plant height (cm)	No. of leaves	No. of roots	Root length (cm)	Fresh weight (g)	Leaf width (cm)	Chl a+b (µg/g)
MO	5.30ab	8.33a	17.17 a	2.40a	0.45ab	2.30a	42.45a
MC	5.80a	7.20ab	13.50b	1.40b	0.52a	2.26a	41.22a
MR	5.96a	7.35ab	13.33b	1.45b	0.55a	2.22a	42.37a

### Lowering production cost efficiency

In a commercial tissue culture room, the most lighting sources used for propagation are FL. FL is suitable for plant growth, but all FL is exothermic, hence it needs more energy in order to partially reduce the heat. Generally, *in vitro* plants were transferred after 4 - 8 weeks of culture. Standaert - de Metsenaere (1991) estimated labor for 65%, medium for 7%, maintenance of equipment and laboratories for 17% and other costs for approximately 11% of total production costs, so saving ½ of the space, saving material (substrate in microponic system is nylon film), production of large number of plants (300 plants), plant growth in unsterilized conditions (half strength sucrose and sugar-free liquid MS medium were added to the substrate) were reduce of production costs.

With the level of current science and technology, labor cost, tissue culture rooms cost, chemicals cost, equipment and other cost account for 35%, 17%, 13%, 9% and 7%, respectively, and normally are fixed for most process plants propagated *in vitro* (Tomar *et al.*, 2007). The cost reduction breeders can only be achieved while reducing energy costs (19%).

However, 15% of these are fixed costs for environmental appeal, cabinets; 25% on cooling costs and 60% of the remaining costs for lighting. Thus, LEDs are suitable for selection cost savings. Nam (2016) indicated that the energy saving LED's compare with FL's was 46.7%. For the *Chrysanthemum* plant, the cost of seedlings is reduced when using LED lighting as follows:

$$P = E \times L \times S = 19\% \times 60\% \times 46.7\% = 5.33\%$$

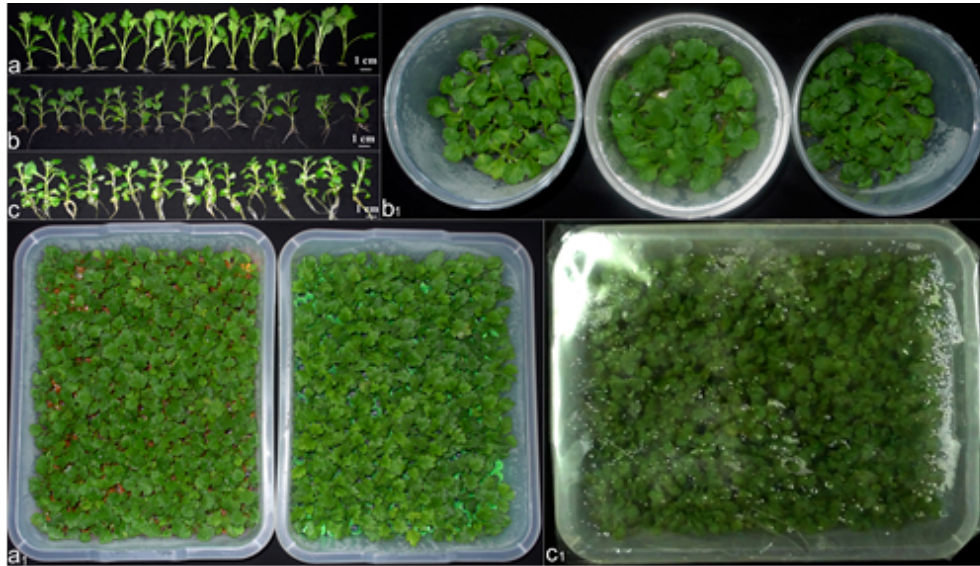
Where: P - Seedlings price reduction; E - Energy costs; L - Costs for lighting; S - Energy saving.

### Plant acclimatization

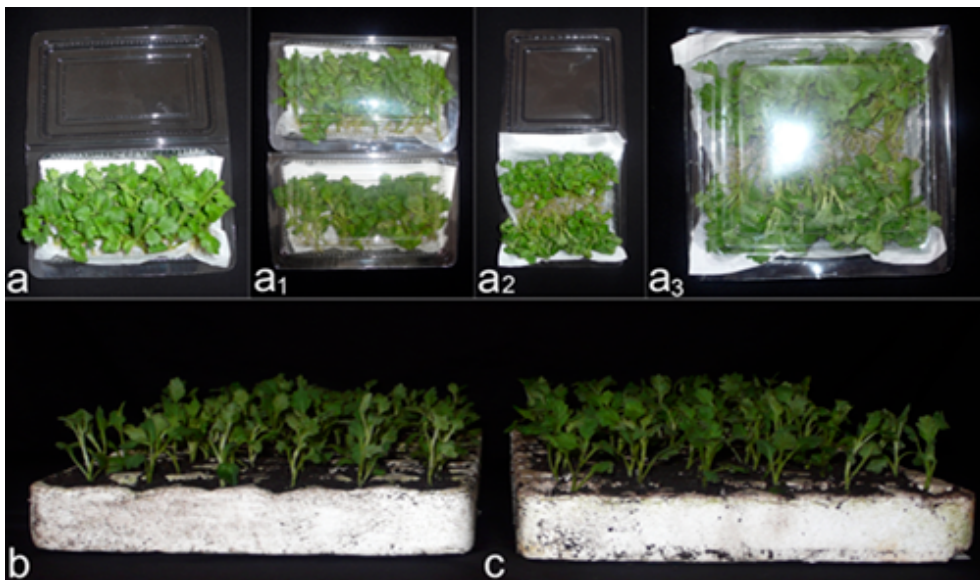
Plants delivered from MO, MC and MR (each system) were transferred to greenhouse to evaluate their growth and development as well as their survival rate. After 2 weeks in the greenhouse, the growth and development of plants in MC and MR were better than those in MO which included plant height, fresh weight and leaf width (Table 3). After transferred into greenhouse, the survival rate of plants cultured in microponic systems (MC and MR) were higher than that in micropropagation system (100% and 85%, respectively) (Fig. 4 b, c).

**Table 3.** Plants in microponic systems and micropropagation acclimated in greenhouse after 2 weeks of culture. Different letters within a column indicate significant differences at  $\alpha=0.05$  by Duncan's multiple range tests.

Culture systems	Plant height (cm)	No. of leaves	No. of roots	Root length (cm)	Fresh weight (g)	Leaf width (cm)	Chl a+b ( $\mu\text{g/g}$ )	Survival rate (%)
MO	9.17b	14.13a	50.87a	5.01a	2.34b	2.41b	45.39a	85
MC	12.70a	13.73ab	44.40b	4.82ab	3.10ab	3.20a	47.77a	100
MR	12.56a	13.87ab	47.47a	4.68ab	3.19a	3.14a	48.63a	100



**Figure 3.** Growth and development of plants in microponic systems and micropropagation system. **a, a<sub>1</sub>**. Plants in MR; **b, b<sub>1</sub>**. Plants in MC; **c, c<sub>1</sub>**. Plants in MO.



**Figure 4.** Plants in microponic system acclimated in greenhouse. **a, a<sub>1</sub>, a<sub>2</sub>, a<sub>3</sub>**. Plants in packed plastic box; **b**. Plants in MC acclimated in greenhouse; **c**. Plants in MR acclimated in greenhouse.

Plants were cultured in MO (non-ventilation), where diffusion of CO<sub>2</sub> concentration from outside was lower than MC and MR with 1M ventilation condition. Enhanced gas exchange in culture flasks leading to increase photosynthesis capacity of *in vitro* plants was reported by Kanechi *et al.* (1998). Nhut *et al.* (2011) indicated that ventilated conditions were responsible for the formation of secondary roots *in vitro* which could improve survival rate in greenhouse. In *in vitro* stage, growth of plants in MO is controlled while in the acclimatization stage; plants are exposed to numerous adverse external factors such as fungal diseases, low humidity, and poor nutrition. This is one of the main reasons for the low survival rate, growth, development and photosynthetic rate of plants in acclimatization (Grout, Donkin, 1987).

In addition, plants cultured in MC and MR conditions, put in packed plastic box were easy for storage and transportation (Fig. 4 a, a<sub>1</sub>, a<sub>2</sub>, a<sub>3</sub>).

Interestingly, the current study demonstrated that microponic system in combination with 70R + 30B LED light not only made the production cost lower, but also contributed to improve seedling quality.

## CONCLUSION

Microponic system is an efficient model in breeding of *Chrysanthemum*. Shoots were pretreated with IBA 500 ppm and cultured in MC (15 shoots per container) and ventilated with millipore membrane under 70% red LED combined with 30% blue LED gave the best for growth and development of plant.

MC and MR had many advantages such as (1) shortening the stages in the process of propagation by combining *in vitro* rooting and acclimatization into one stage; (2) reducing the products cost due to sugar- and agar-free culture mediums; (3) providing an open system for growth and development of plants were better, leading to increased survival rate (100%) of plants as well as rapid growth in greenhouse and (4) easily transportation and delivery.

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## GIA TĂNG SINH TRƯỞNG VÀ PHÁT TRIỂN CỦA CÂY CÚC (*CHRYSANTHEMUM MORIFOLIUM*) TRONG HỆ THỐNG VI THỦY CANH DƯỚI ĐIỀU KIỆN CHIẾU SÁNG LED

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### TÓM TẮT

Bài báo này đánh giá sự gia tăng sinh trưởng và phát triển của chồi cúc trong hệ thống vi thủy canh và vi nhân giống. Vi thủy canh là một phương pháp kết hợp giữa vi nhân giống và phương pháp thủy canh, phương pháp này có thể khắc phục một số hạn chế của hệ thống vi nhân giống như giảm nhiễm, tiết kiệm công lao động, vật liệu, không gian,... Trong nghiên cứu này, các chồi cúc (3 cm) được nuôi cấy trong hệ thống vi thủy canh hộp nhựa tròn (chiều cao 8,5 cm, đường kính miệng và đáy lần lượt là 12 cm và 9 cm) và hệ thống vi thủy canh hình chữ nhật (chiều cao 8,5 cm, 35 x 28 cm ở miệng hộp và 30 x 25 cm ở đáy hộp) và trong hệ thống vi nhân giống (hộp nhựa hình chữ nhật với 800 ml môi trường ½ MS có chứa 30 g/l sucrose và 8 mg/l agar). Kết quả cho thấy rằng, chồi được xử lý với 500 ppm IBA, nuôi cấy trong hệ thống vi thủy canh hộp nhựa tròn (15 chồi mỗi hộp) và thoáng khí với màng Millipore (Milliseal<sup>TM</sup>, kích thước lỗ 0,5 µm, đường kính 2 cm) dưới điều kiện chiếu sáng 70% LED đỏ kết hợp với 30% LED xanh cho chiều cao cây, số lượng rễ, khối lượng tươi, hàm lượng chlorophyll a, chlorophyll b và chlorophyll a + b cao nhất (5,18 cm; 12,50 rễ; 0,52 g; 28,19 µg/g; 13,56 µg/g và 41,75 µg/g; tương ứng). Tỷ lệ sống sót của cây có nguồn gốc từ hệ thống vi thủy canh hộp nhựa tròn và hình chữ nhật khi chuyển ra vườn ươm cao hơn so với có nguồn gốc từ hệ thống vi nhân giống (100%, 100% và 85%, tương ứng). Nghiên cứu cũng chỉ ra rằng hệ thống vi thủy canh hộp nhựa hình chữ nhật là một hệ thống hiệu quả và đơn giản cho sản xuất ở quy mô lớn của cây cúc.

**Từ khóa:** cây cúc, đèn LED, hộp nhựa hình chữ nhật, hộp nhựa tròn, vi thủy canh.