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INFLUENCE OF CONTAINER VOLUME AND IRRIGATION SYSTEM ON PHOTOSYNTHESIS, WATER PRODUCTIVITY AND GROWTH OF POTTED *Euphorbia × lomi*

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ABSTRACT

The determination of an adequate container volume that maximize vegetative growth and the adoption of an efficient irrigation system in soilless culture, which improve water use efficiency without affecting crop performance, have become a priority in ornamental industry. A greenhouse experiment was conducted aiming to assess the effects of two container volumes (1 dm^3 or 3 dm^3) and two irrigation systems (closed drip-irrigation or subirrigation) on growth parameters, ornamental quality, SPAD index, leaf gas exchange, agronomical and physiological water use efficiency (WUE_A and WUE_P) of containerized *Euphorbia × lomi* Rauh. There were no significant differences in terms of plant growth parameters between the two irrigation systems. The subirrigation system was more efficient in terms of water use than the drip-irrigation system since it could save on average 27% of water. The WUE_A recorded with subirrigation in 3 dm^3 and 1 dm^3 containers were significantly higher by 43% and 81% compared with those recorded with drip-irrigation. The plant height, leaf number, leaf area, root length and shoot dry biomass were significantly lower by 45.7%, 39.5%, 45.5%, 35.1% and 43.1%, respectively when the *Euphorbia × lomi* plants were cultivated in the 1 dm^3 containers. The best crop performance recorded in the 3 dm^3 containers was related to a higher photosynthetic activity and higher leaf chlorophyll content (i.e. SPAD index) with respect to the plants grown in the 1 dm^3 containers.

Key words: leaf gas exchange, ornamentals, pot volume, chlorophyll content, recirculating nutrient solution, subirrigation, water use efficiency

INTRODUCTION

Soilless cultivation represents an important and alternative tool to soil culture, currently practiced all over the world allowing the achievement of high yield, precise control of root environment and also presenting many environmental benefits [Savvas 2003, Savvas et al. 2007]. Irrigation is one of the most important practices in soilless production due to the limited substrate volume [Rouphael and Colla 2005]. Different irrigation systems have been designed and developed for containerized horticultural crops, such as overhead, drip-irrigation and subirrigation [Frangi et al. 2011]. Drip-irrigation, is the most diffused method used in ornamental industry [Rouphael et al. 2008]. In this system the water and dissolved nutrients are applied in excess. Therefore the surplus of salts are accumulated in the recirculation

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solution, making necessary the discharge of nutrients (i.e. nitrate and phosphate), resulting in water and fertilizer losses [Juntunen et al. 2002]. In recent years, subirrigation has been proposed as an important alternative to the traditional drip-irrigation system [Ferrarezi et al. 2015]. Compared to drip-irrigation, subirrigation offers the possibility i) to reduce the cost of labor [Liu et al. 2012], ii) to increase the flexibility in pot spacing [Fascella and Rouphael 2015], iii) to decrease fertilizer and water use without affecting growth and quality of the product [Rouphael et al. 2008, Cardarelli et al. 2010] and iv) to simplify the nutrient solution management [Rouphael and Colla 2009]. On the other hand, the major handicap of subirrigation system is represented by the accumulation of unabsorbed salts in the upper part of the growing substrates, furtherly exacerbated by high fertilizer application rates and the use of low-quality water (i.e. saline) [Rouphael et al. 2006].

In ornamental floriculture industry, the substrate volume could significantly affect growth and ornamental quality of plants [Goreta et al. 2008]. Using smaller containers consents to produce more plants per unit area, and to reduce the substrate volumes and production costs, leading to an economic benefits for the growers [Girardi et al. 2005]. Nevertheless, the use of small container could also have biological constraints. For instance, large plants in small containers may have a large fraction of 'pot-bound' roots, with detrimental effect such as the reduction in plant biomass, pigment synthesis (i.e. chlorophyll), nutrients uptake, translocation and assimilation [Poorter et al. 2012]. Furthermore, root restriction often depresses the photosynthetic capacity even if the exact mechanism remain unclear [Goto et al. 2012]. The reduction in net CO_2 assimilation in root-restricted conditions has been often attributed to the imbalance in the supply and demand for carbohydrates as well as to stomatal closure caused by the internal water stress [Kharkina et al. 1999, Shi et al. 2008]. Small containers could also negatively affect the water status of plants as they have a limited water holding capacity leading to severe drought and a reduced water use efficiency [Poorter et al. 2012, Fascella and Rouphael 2015]. Besides resource availability, small containers are more sensible than large ones to thermic variations, in particular for greenhouse cultivation, with an increase of substrate temperature during spring-summer growing season.

Euphorbia × *lomi* Rauh is an interspecific hybrid (*Euphorbia lophogona* Lamarck × *E. milii* Des Moulins) native to Madagascar and belonging to the Spurge family [Smoley 2000]. It is a succulent shrub, usually cultivated as potted flowering plant or as hedge in landscaping and xeroscaping [Fascella and Zizzo 2009, Fascella et al. 2011]. Nevertheless, few published data are available concerning the effects of container size (14 vs. 18 cm diameter) and irrigation system (subirrigation vs. drip-irrigation) in potted ornamentals [Fascella and Rouphael 2015]. The former authors demonstrated that the potted *Murraya paniculata* performance was similar between the two irrigation systems and the increasing container volume resulted in larger plants and higher water use efficiency [Fascella and Rouphael 2015]. However, since the response of potted ornamentals is species-specific, it is of great interest to understand the effect of irrigation system, container volume and their interaction on the crop performance of an important potted ornamentals such as *Euphorbia* × *lomi*.

The aim of the present study was to evaluate the effects of container volume (1 dm^3 or 3 dm^3) and irrigation system (closed drip-irrigation or subirrigation) on plant growth, leaf colour parameters, chlorophyll content, leaf gas exchanges, and water use efficiency of potted *Euphorbia* × *lomi*. These results can play an important role for the ornamental industry, which is very interested in assessing the effect of pot size on growth and to evaluate the subirrigation as an efficient technique to save water without affecting the economic value of the plant.

MATERIALS AND METHODS

Greenhouse conditions, treatments and experimental design. The experiment was conducted during the 2013 growing season, in an unheated east-west oriented greenhouse (34 × 16 m) with steel structure and polymethyl methacrylate cover, located at the Research Unit for Mediterranean Flower Species near Palermo – Italy (38°5'N, 13°30'E, 23 m a.s.l.). Inside the greenhouse, the air temperature was set at 28°C and 14°C for day and night, respectively. The ornamental plant tested for the present study was *Euphorbia* × *lomi* Rauh. On 1 August 2013, four months-old micropropagated plantlets of *Euphorbia* × *lomi* Rauh cv. 'Ilaria' were grown in 1 dm^3 and 3 dm^3 volume polyethylene containers (1 plant per container) filled with a mixture of peat and perlite (2 : 1, v/v). The 1 dm^3 and 3 dm^3 pots were placed on aluminium benches at a plant density of 10 and 8 plants m^{-2}, respectively.

Four treatments derived by the factorial combination of two irrigation systems (drip–irrigation or subirrigation) and two containers sizes (1 dm^3 or 3 dm^3) were compared. The treatments were arranged in a randomized complete block design with three replicates per treatment. Each experiment unit consisted of a separate bench containing 20 plants.

Nutrient solution composition and management. All plants were fed with the same nutrient solution having the following macro- and microelements composition (mg dm^{-3}): 180 N-NO_3, 50 P, 200 K, 120 Ca, 30 Mg, 1.2 Fe, 0.2 Cu, 0.2 Zn, 0.3 Mn, 0.2 B, 0.03 Mo. The electrical conductivity (EC) of the nutrient solution was 1.8 ±0.4 dS m^{-1}. When EC value exceeded the threshold of 2.5 dS m^{-1} imposed for potted ornamental plants [Rouphael et al. 2008], water was added to the fresh nutrient solution in order to restore the EC value to the original starting point. The pH of the nutrient solution was maintained between 5.8 and 6.3 by adding nitric acid (HNO_3). The nutrient solution in all tanks were prepared using a tap water having an EC value of 0.2 dS m^{-1}.

In both irrigation systems, the nutrient solution was pumped from independent tanks (one tank per experimental unit) having a volume capacity of 80 dm^3. In the drip irrigation system, the nutrient solution was supplied through one emitter per plant (flow rate of 2 dm^3 h^{-1}). The subirrigation system was equipped with a capillary mat and the nutrient solution was supplied through microperforated hoses integrated into the mat. The excess of nutrient solution in drip-irrigation and subirrigation systems was drained back to the individual tanks for recirculation. In both systems, irrigation scheduling was performed using electronic low-tension tensiometers (Tensioswitch, Tensio-Technik, Germany) that control irrigation based on substrate matric potential. In each treatment, three tensiometers (one per replicate) were installed at the midpoint of different pots in order to supply a representative reading of the moisture tension [Colla et al. 2012, 2013].

Growth and quality measurements. At the beginning of the experiment twenty plants were used for the initial determination of plantlets dry biomass. At the end of the experiment (30 November; 120 days after transplanting) ten plants per replicates were sampled and separated in shoots (leaves + stems) and roots. The dry mass of plant tissues was measured after oven-drying at 60ºC for 4 days.

The leaf area (LA) was measured using a digital area meter (WinDIAS 2; Delta-T Devices Ltd, Cambridge, U.K.). Plant height, number of leaves, longest root length and shoot-to-root ratio were also recorded. The Relative Growth Rate (RGR) was calculated according to the formula of Hoffmann and Poorter [2002]: $RGR = (\ln W_2 - \ln W_1)/(t_2 - t_1)$ where W_1 and W_2 are the total dry weight (g $plant^{-1}$) at the beginning (t_1) and at the end (t_2) of the experiment (days), respectively.

SPAD index, leaf colour and gas exchange measurements. On 30 September (60 DAT), the leaf chlorophyll content expressed as Soil Plant Analysis Development (SPAD) index was measured on fully expanded leaves by means of a portable chlorophyll meter SPAD-502 (Konica-Minolta corporation, Ltd., Osaka, Japan). Fifteen leaves were randomly measured and averaged to a single SPAD value for each treatment. Moreover, among the physical characteristics of ornamental plants that strongly influence the consumer preference and demand is the leaf colour. The leaf colour was performed on the Commission Internationale de L'Eclairage (CIE) color space parameters: L* a* b* using a Minolta CR-300 Chroma Meter (Minolta Camera Co. Ltd, Osaka, Japan). The measuring aperture diameter was 8 mm and the instrument was calibrated with Minolta standard white plate before sampling *Euphorbia* × *lomi* leaves. L* (lightness ranging from 0 = black to 100 = white), a* (ranging from green (–60) to red (+60)), b* (ranging from blue (–60) to yellow (+60)) readings were transformed to those of the L, a, b color space.

On the same date, measurements of leaf gas exchange were conducted within two hours (10.00 and 12.00) on the youngest fully expanded leaves, using five leaves per replicates. Leaf net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration (T_r) were determined using a portable photosynthesis system (LI-6200; LI-COR Inc., Lincoln, NE, USA). The LI-6200 was equipped with a stirred leaf chamber with constant-area inserts and fitted with a variable intensity red source (leaf temperature chamber was 28 ±2°C, leaf-air vapour pressure difference was 2.6 ±0.3°C, and CO_2 concentration was 365 ±10 µl dm^{-3}).

Physiological and agronomical water use efficiency. During the growing season (August to November), when the nutrient solution level in the independent tanks decreased owing to water lost by crop evapotranspiration, the tanks were replenished with fresh nutrient solution or water. The exact volume of the fresh nutrient solution or water was determined with a flowmeter (Spagnol, Treviso, Italy).

The physiological water use efficiency (WUE_P) was calculated as P_n/T_r, whereas the agronomical water use efficiency (WUE_A) was calculated as the ratio of plant total dry mass over the cumulative evapotranspired water [Rouphael et al. 2016].

Statistical analysis of data. Experimental data were subjected to analysis of variance (ANOVA) using SPSS statistical program and means were compared by Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$ significance level.

RESULTS

Plant growth and ornamental value. No significant effect among container volume (C) and irrigation system (I) treatments was observed for root dry weight (avg. 3.95 g $plant^{-1}$), root-to-shoot ratio (avg. 0.42) and the RGR (avg. 1.62 g g^{-1} day^{-1}) of *Euphorbia × lomi* plants (tab. 1). However, plant height, number of leaves per plant, leaf area, root length and shoot dry weight at the end of the growing cycle were only affected by container volume (tab. 1). *Euphorbia × lomi* plants grown in 3 dm^3 pots resulted in taller plants having more leaves. Particularly, plant height, leaf number, leaf area, longest root length and shoot dry biomass were significantly lower by 45.7%, 39.5%, 45.5%, 35.1% and 43.1%, respectively when the *Euphorbia × lomi* plants were cultivated in the 1 dm^3 pots (tab. 1).

Similarly to the plant growth parameters, the colour parameters: L* (brightness), a* (redness) were highly influenced by container volume but not by irrigation system; with no C × I interaction (tab. 2). Irrespective of irrigation system, cultivating the *Euphorbia × lomi* plants in small pots increases the leaf brightness and decreased the redness (tab. 2).

SPAD index and leaf gas exchange. The SPAD index and transpiration rate (T_r) were only affected by container volume, whereas the net photosynthetic rate (P_n) was highly influenced by both container volume and irrigation system with no C × I interaction (tab. 2). Moreover, the stomatal conductance (g_s) was significantly influenced by both treatments and their interaction (tab. 2). When averaged over irrigation treatment, the SPAD index, P_n and T_r values recorded in plants grown in 3 dm^3 pots were higher by 10.4%, 102.0% and 36.5%, respectively than those cultivated in 1 dm^3 pots (tab. 2). Finally, the highest values of g_s were observed in *Euphorbia × lomi* plants grown in 3 dm^3 pots with subirrigation, followed by drip-irrigation in 3 dm^3 pots, while the lowest values of g_s were recorded in 1 dm^3 pots with both drip-irrigation and subirrigation (tab. 2).

Electrical conductivity in the nutrient solution, water use and water use efficiency. The electrical conductivity (EC) of the nutrient solution for subirrigated plants exceeded one time the threshold value of 2.5 dS m^{-1}, whereas with drip-irrigation system the EC reached the imposed ceiling value three times during the growing cycle; the tank was replenished with fresh water in order to restore the EC to the original target values (data not shown). The water use was only influenced ($P < 0.05$) by the irrigation system, where the mean daily water use of potted *Euphorbia × lomi* with drip-irrigation (avg. 1.5 dm^3 $plant^{-1}$) was significantly higher by 36% in comparison with the subirrigation system (avg. 1.1 dm^3 $plant^{-1}$) (data not shown).

Table 1. Effects of container volume and irrigation system on plant height, number of leaves, leaf area, root length, shoot and root biomass dry weight, shoot-to-root ratio (S/R) and relative growth rate (RGR) of *Euphorbia* × *lomi*. Values are the means of three replicate samples

Container volume	Irrigation system	Plant height (cm)	Number leaves (no. plant^{-1})	Leaf area (cm^2 plant^{-1})	Root length (cm)	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	S/R	RGR (g g^{-1} day^{-1})
1 dm^3	Drip-irrigation	8.4	16.0	218.2	13.7	5.7	2.7	0.47	1.32
	Subirrigation	9.7	22.1	254.4	16.0	8.4	3.9	0.46	1.65
	Mean	9.0 b	19.0 b	236.3 b	14.8 b	7.0 b	3.3	0.47	1.48
3 dm^3	Drip-irrigation	15.8	28.5	401.1	21.2	10.8	4.4	0.42	1.74
	Subirrigation	17.3	34.3	465.6	24.5	13.8	4.8	0.35	1.79
	Mean	16.6 a	31.4 a	433.5 a	22.8 a	12.3 a	4.6	0.38	1.76
Significance[a]									
Container volume (C)		**	*	**	*	*	NS	NS	NS
Irrigation system (I)		NS	NS	NS	NS	NS	NS	NS	NS
C × I		NS	NS	NS	NS	NS	NS	NS	NS

[a] NS, *, ** – nonsignificant or significant at $P \leq 0.05$ and 0.01, respectively

Within columns, means followed by lower case letter are significant according Duncan's multiple range test. P = 0.05

Table 2. Effects of container volume and irrigation system on leaf color parameters L* (brightness), a* (+a* = red; –a* = green) and b* (+b* = yellow; –b = blue), Soil Plant Analysis Development (SPAD) index, net photosynthetic rate (P_n), stomatal conductance (g_s) and transpiration (T_r) of *Euphorbia* × *lomi*. Values are the means of three replicate samples

Container volume	Irrigation system	L*	a*	b*	SPAD index	P_n (µmol CO_2 m^{-2} s^{-1})	g_s (mmol H_2O m^{-2} s^{-1})	T_r (mmol H_2O m^{-2} s^{-1})
1 dm^3	Drip-irrigation	42.3	–14.3	22.2	36.8	3.01	11.4	0.44
	Subirrigation	41.9	–15.7	23.5	37.9	3.72	14.5	0.38
	Mean	42.1	–15.0	22.8	37.3	3.36	12.9	0.41
3 dm^3	Drip-irrigation	40.5	–15.8	24.3	39.3	6.67	23.2	0.60
	Subirrigation	39.2	–16.3	26.1	43.2	6.91	30.1	0.52
	Mean	39.8	–16.1	25.2	41.2	6.79	26.6	0.56
Significance[a]								
Container volume (C)		*	NS	*	*	**	**	*
Irrigation system (I)		NS	NS	NS	NS	*	*	NS
C × I		NS	NS	NS	NS	NS	*	NS

[a] NS, *, ** – nonsignificant or significant at $P < 0.05$ and 0.01, respectively

Within columns, means followed by lower case letter are significant according Duncan's multiple range test. P = 0.05

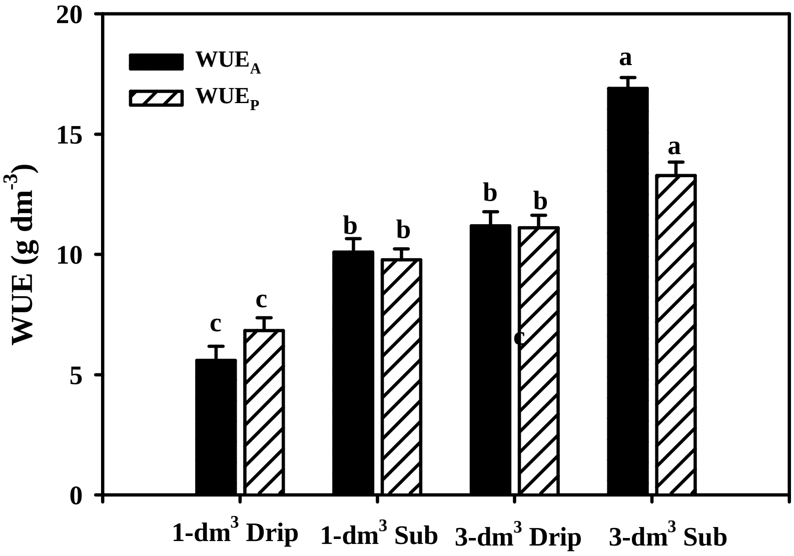

Fig. 1. Effects of container volume and irrigation system on agronomical water use efficiency (WUE_A = total crop dry weight/ total water use) and physiological water use efficiency ($WUE_P = P_n/T_r$) of *Euphorbia × lomi*. The values are means of three replicates. Vertical bars indicate ±SE of means. Different letters indicate significant differences according to Duncan's test ($P \leq 0.05$)

The WUE_A and WUE_P of *Euphorbia × lomi* were significantly affected by the C × I interaction ($P < 0.05$). The WUE_A and WUE_P ranged from 5.6 to 16.9 g dm^{-3} and from 6.8 to 13.3 g dm^{-3}, respectively (fig. 1). The highest WUE_A and WUE_P were recorded in 3 dm^3 pots with subirrigation, whereas the lowest values were observed in 1 dm^3 pots with drip-irrigation (fig. 1). Finally, the results of the current study indicated that WUE_A recorded with subirrigation in 3 dm^3 and 1 dm^3 pots were significantly higher by 43% and 81% compared with those recorded with drip-irrigation (fig. 1).

DISCUSSION

Root restriction has been reported to disturb morphological, physiological and biochemical processes leading to growth inhibition and product quality losses [Poorter et al. 2012]. Particularly, in *Euphorbia × lomi* plants, the reduction of container volume from 3 to 1 dm^3 causes inhibition in plant growth parameters such as plant height, leaf number, total leaf area, root length and shoot dry biomass (tab. 1). The findings that the reduced rooting volume (e.g. 1 dm^3) impairs plant growth are in agreement with the results of several greenhouse experiments on vegetables and ornamentals such as poinsettia [Goreta et al. 2008], tomato [Shi et al. 2008], oakleaf hydrangea [Hagen et al. 2014] and *Murraya paniculata* [Fascella and Rouphael 2015]. Moreover, in a recent meta-analysis based on the effects of rooting volume on plant growth, Poorter et al. [2012] showed that on average doubling the substrate volume results in 43% more biomass production in herbaceous and woody plants. The lower agronomical performance of *Euphorbia × lomi* under root restriction could be attributed to the reduced photosynthetic activity and chlorophyll synthesis [Poorter et al. 2012]. Our results are consistent with the findings of several authors [Kharkina et al. 1999, Shi et al. 2008, Fascella and Rouphael 2015] who demonstrated that root restriction-induced limitation to photosynthesis in cucumber, tomato and *Murraya paniculata*. The decrease in CO_2 assimilation and photosynthetic productivity have been attributed to several mechanisms including (i) stomatal closure caused by internal water stress [Pezeshki and Santos 1998], (ii) imbalance in the supply and demand for carbohydrates [Kharkina et al. 1999] and (iii) a decrease in the carboxylation efficiency of the Calvin cycle [Thomas and Strain 1991]. In this study, the reduction in net photosynthetic rate was largely dependent on stomatal factors, because the lowest photosynthesis recorded in *Euphorbia × lomi* plants grown in 1 dm^3 containers was accompanied by a reduction in both stomatal conductance and transpiration (tab. 2). Another possible reason for the photosynthesis and growth reduction in the 1 dm^3 pots could be the nutrient availability and translocation. A smaller substrate volume may decrease the macronutrient availability (i.e. N, P and K) in the container, leading to a reduction in net photosynthetic rate and biomass production. In the current study, SPAD index, a non-destructive measurement of chlorophyll content (i.e. N status) was significantly reduced by 10% in 1 dm^3 containers in comparison to the 3 dm^3 ones which may have influence the photosynthetically active radiation (PAR) absorption and photosynthetic rate.

Our findings also show that potted *Euphorbia × lomi* can be successfully grown using closed subirrigation system, due to the similar agronomical performance to drip-irrigation. The lack of changes between the two irrigation systems on agronomical traits, are in agreement with previous studies on ornamental plants such as tropical hardwood *Metrosideros polymorpha* [Dumroese et al. 2006], *Pelargonium × hortorum* cv. 'Real Mintaka' [Rouphael et al. 2008] and *Petunia × hybrida* cv. 'Giove' [Cardarelli et al. 2010].

One of the main drawbacks regarding the use of subirrigation is the accumulation of salts in the upper portion of the media due to the capillarity force and bulk flow, which may result in EC increasing, leading to growth inhibition [Reed 1996]. This was not observed in the current experiment since no significant differences between the two irrigation systems were observed for the substrate EC (data not shown).

Concerning the water balance, the subirrigation system was more efficient in terms of water use than the drip-irrigation system since it could save on average 27% of water. These results are in agreement with the findings of Rouphael et al. [2008] who showed an 11% water saving for subirrigated *Pelargonium × hortorum* in comparison to drip-irrigated plants. Similarly, Frangi et al. [2011] and Davis et al. [2011] reported that subirrigation allowed 77% and 45% of water saving for three cultivars of *Rosa* hybrids 'Bad Birnbach', 'Innocentia' and 'Mainaufeuer' and also for *Acacia koa* seedlings, respectively, compared to the traditional overhead irrigation system. Finally, we found that relationship between the water use and total dry biomass (i.e. WUE_A) as well as the relationship between the net photosynthetic rate and transpiration (i.e. WUE_P) were affected by the irrigation system. Particularly, the WUE_A values recorded with subirrigation in 3 dm^3 and 1 dm^3 containers were significantly higher by 43% and 81% compared with those recorded with drip-irrigation. Under our conditions, subirrigation should be adopted among floricultural industries, especially that in an era of water scarcity growers have to improve the management practices (i.e. irrigation system) aiming at saving water and maximizing productivity [Rouphael et al. 2016].

CONCLUSIONS

It can be concluded that no significant difference was observed between the two irrigation systems in terms of agronomical and quality traits. However, by adopting closed subirrigation system, it is possible to simplify nutrient solution management, reduce water use and increase WUE_A and WUE_P of containerized *Euphorbia × lomi* plants. Our results also indicated that larger container volume induced more vigorous vegetative growth and improved aesthetic value (i.e. colour) of this ornamental hybrid. The superior crop performance recorded in the 3 dm^3 containers were related to a better photosynthetic activity and higher leaf chlorophyll content (i.e. higher SPAD index) with respect to the plants grown in the 1 dm^3 containers.

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