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IMPROVING BIOLOGICAL AND PHYSIOLOGICAL PARAMETERS OF *NERIUM OLEANDER* L. CUTTINGS BY USING BIOSTIMULATING SUBSTANCES

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Abstract

Nerium oleander is a perennial shrub native to the eastern Mediterranean basin and Southeast Asia. As an ornamental plant grown in pots, it reaches heights of 1-2 m, with the appearance of a bushy shrub, with fragrant flowers, simple or double, colourful, blooming from spring until the end of autumn. With a very large adaptive potential, the species is one of the most popular in floral plants, as an indoor plant and where the climate allows, it can be grown on terraces and balconies, in hedges, alignments etc. The purpose of this study was to evaluate the impact of treatments with various rhizogenic biostimulators on the rooting potential of different types of Nerium oleander cuttings in greenhouse conditions. The experiment was performed in the HORTINVEST greenhouses of the USAMV Bucharest and there were determined the rooting percentage, the development of the root system and the aerial part, as well as some physiological indicators: photosynthesis, transpiration and stomatal conductance.

Key words: *cuttings, rhizogenic biostimulators, physiological indicators.*

INTRODUCTION

Nerium oleander L. is an evergreen shrub or small tree (with 2-5 m in height) from the *Apocynaceae* family, cultivated worldwide as an ornamental flowering plant, in different geographical and ecological places (Sinha and Biswas, 2016) thanks to an abundant and long-lasting flowering period from summer until late autumn (Argiropoulus and Rhizopoulou, 2013) and due to its moderate hardiness, tolerance to different soil characteristics and low nutrients consumption (Kiran and Prasad, 2014).

The plant value is due not only by its evergreen leaves, but also by the terminal flowers organized as clusters, with different beautiful colors, such as white, red, pink, salmon or light yellow.

Besides the above mentioned utilities, some of the secondary metabolites produced by this plant have also a pharmacological interests (Zibbu and Batra, 2010; Chaudhary et al., 2015) due to their antibacterial, antimicrobial, anti-inflammatory, antinociceptive, and antitumor activity (see review Sinha and Biswas, 2016). In addition, oleander has a promising potential for use in phytoremediation programs (Elloumi et al., 2017). Moreover, results obtained by Doganlar et al. (2012) and Vázquez et al. (2016) also highlights the importance of the species as a bio monitoring tool for airborne metal pollution in urban areas, thanks to its resistance to metals and its exclusion capacity.

Even if oleander is native to northern Africa and to the eastern Mediterranean region (Bailey, 1976), it is naturalized very easily (Zibbu and Batra, 2010) and grown as pot plant, on and around terraces and balconies, in hedges and screen plantings (Simion and Anton, 2009).

As Comeaux (1991) noticed, oleander may look like a small tree if the suckers are removed and a few stems are kept. Moreover, by growing it in container can be a good choice to plant grown even in cooler climates into greenhouses and conservatories, or as indoor plant, that can be grown outside during the summer, as it was practiced for many years.

Due to the great importance of this species and increased demands for seedlings, during the time, breeders have had many preoccupation to obtain new cultivars using different plant propagation techniques such as: generative propagation, vegetative propagation by cuttings (softwood or semi-hardwood cuttings in the spring or summer) or *in vitro* culture (Ochoa et al., 2003; Simion and Anton, 2009; Vila et al., 2010; Aryan and Rani, 2016), but are missing studies on the interaction between the multiplication methods and physiological performances of the obtained plants.

As regard as oleander leaves behavior, the researchers were concerned to study the influence of stress factors such as water stress (Björkman et al., 1981; Lenzi et al., 2009), freezing injury (Syros et al., 2005; Miralles-Crespo et al., 2011), ozone pollution (Lorenzini et al., 1999) or temperature acclimation (Badger et al., 1982) on some physiological and biochemical indicators.

To our knowledge, in our country has not until now done research regarding the oleander gas exchange indicators as related to the vegetative multiplication techniques. The objectives of the present study were to evaluate the cuttings type influence on rooting performance under different stimulation treatments and to determine some physiological indicators.

MATERIALS AND METHODS

Plant material

Oleander plants (*Nerium oleander* L.) were grown in a computer-controlled greenhouse of the HORTINVEST Centre at USAMV Bucharest, Romania (44º 26' N and 26º 06' E latitude and longitude, respectively).

The plants were obtained by vegetative propagation, using two different type of stem cuttings, the segment ones (4-6 cm long) and the peak ones (length of 8-10 cm).

After sampling, the cuttings were cut out by removing the leaves from the lower node for rooting. A number of 12 cuttings of each category were used for each variant.

As rhizogenic biostimulators were used three different commercial substances and three replicates were prepared:

- Clonex (a gel, a powerful formula of hormones, vitamins and minerals);
- Radistim (a powder containing non-hazardous bioactive preparations);
- BioRoot (contains vitamins, enzymes, organic acids, humic acids to stimulate root mass growth) (Table1).

The substrate for rooting cuttings was perlite, in alveolar plaques.

Table 1. Experimental distribution

Experimental variants	Description
Vm	Untreated
V_1	Clonex
V_2	Radistim
V_3	BioRoot

Rooting and Growth Parameters

The following parameters were determined: percentage of rooting, roots length, shoots length.

Leaf gas exchange physiological indicators such as photosynthesis rate (A_n), transpiration rate (E), and stomatal conductance (g_s), respectively have been quantified using the LCIpro + photosynthesis system equipped with a square analysis camera of 6.25 cm^{-2}, between 7:00 and 10:00 h a.m. All measurements were made in four replicates, three times, by analyzing the same leaves. The greenhouse temperature was below 32°C and the light intensity was maintained between 500-700 $\mu mol\ m^{-2}\ s^{-1}$ to avoid the photo inhibition process. The results were expressed as: A_n ($\mu mol\ CO_2\ m^{-2}\ s^{-1}$), E (mmols $H_2O\ m^{-2}\ s^{-1}$) and g_s (mol $H_2O\ m^{-2}\ s^{-1}$) and represent the mean values for the two cuttings types.

RESULTS AND DISCUSSIONS

Rooting and Growth

The experimental results show that oleander cutting rooting was improved by the stimulating substances.

The effect of the cuttings treatments was noticed for both cuttings types, and the medium and hormone applied to the cutting modified all the studied parameters.

The rooting percentage (Figure 1), for the peak shoot cuttings recorded the highest value on V_3 (100%), followed in decreasing order by V_2 (83.33 %), V_1 (75%) and Vm (66.67 %). The order for the other type of cuttings was the same, with the mention that on V_1 the value of the parameter was equal on both types of cuttings (75%). The rooting percentage for the peak shoot cuttings was higher, with the mentioned exception of V_1.

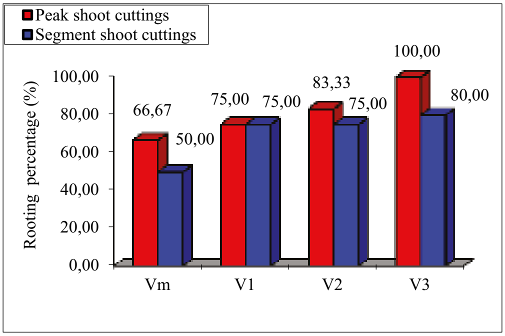

Figure 1. The rooting percentage of the cuttings

For the root length, as it is showed in the Figure 2, the value was highest on the same type of cuttings (the peak shoot ones), this time for all the experimental variants. The order was also the same as for the rooting percentage: V_3, followed by V_2, V_1 and Vm.

The difference between variants was from 21.49% (V_3 vs. Vm) to 12.40% (V_3 vs. V_2) on the new plants obtained from peak shoot cuttings and from 28.45% (V_3 vs. Vm) to 18.10% (V_3 vs. V_2) in the case of the segment shoot cuttings new plants.

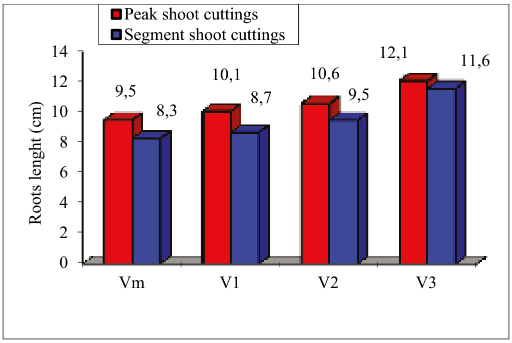

Figure 2. The roots length on the new plant

Regarding the result for the third parameter, the shoot length on new plants obtained from cuttings (Figure 3) it can be seen that the tendency is preserved: the highest value are on V_3 for the both type of cuttings (15.1/10.5 cm peak shoot/segment shoot cuttings), followed by V_2 (13.75/10.25 cm), V_1 (12.3/9.7 cm) and finally Vm (11.1/7.6 cm).

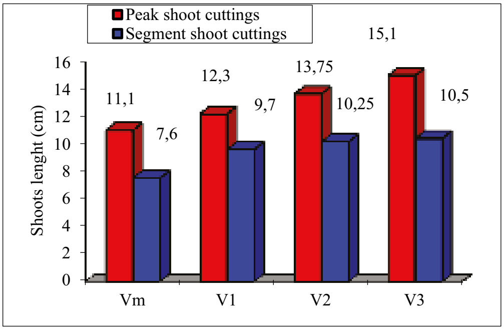

Figure 3. The shoots length on the new plant obtained by cuttings

Leaf gas exchange

Photosynthesis rate (Table 2) varied between 1.90 µmol CO_2 m^{-2} s^{-1} and 3.90 µmol CO_2 m^{-2} $s^{-1}$and presented higher values in the case of V_3, as compared with the others treated variants (V_1 and V_2), only in the case of the first analysis data. Similarly, transpiration rate (E) had higher values in the case of V_3.

For stomatal conductance, the values were generally low and near to those reported by Delaney (2012) (0.03 mol H_2O m^{-2} s^{-1} - 0.06 mol H_2O m^{-2} s^{-1}).

As regard as oleander multiplication, previous studies found that the seeds germination percentage varied between 82% and 100% (corresponding to two determination periods) and the germination time was five days in the both study cases.

A better rooting was obtained in the case of tip cuttings on parapet and rooting system for cuttings with temperature control, in peat and perlite (2:1) in 22°C warming culture medium (Simion and Anton, 2009).

As authors emphasized, the best period for micro propagation was July, on the Murashige & Skoog medium.

According to Ochoa et al. (2003) the larger root growth was obtained by using basal cuttings, while in the case of the apical cuttings, a longer roots number and a higher homogeneity in their distribution was noticed.

Micro propagation by using axillary shoot breaking of wild plants and commercial cultivars allowed higher multiplication rates than the propagation by cuttings, and rooting and acclimatization did not limited the efficient production of plants (Vila et al., 2003).

Table 2. Leaf gas exchange parameters (mean values ± standard errors)

Parameter	Data	Control	V_1	V_2	V_3
A_n (μmol CO_2 m^{-2} s^{-1})	25th May	3.30±0.30	3.00±0.36	3.00±0.15	3.90±0.11
	11th July	3.62±0.29	3.05±0.25	3.21±0.24	3.89±0.41
	12th Sept	2.65±0.66	1.90±0.38	2.51±1.33	2.18±0.10
E (mmol H_2O m^{-2} s^{-1})	25th May	0.54±0.07	0.50±0.07	0.50±0.05	0.73±0.04
	11th July	0.93±0.09	0.90±0.29	1.21±0.09	1.49±0.04
	12th Sept	0.55±0.08	0.78±0.25	0.52±0.15	0.62±0.09
g_s (mol H_2O m^{-2} s^{-1})	25th May	0.04±0.008	0.02±0.004	0.03±0.003	0.03±0.002
	11th July	0.043±0.004	0.039±0.01	0.046±0.004	0.05±0.004
	12th Sept	0.028±0.005	0.027±0.01	0.02±0.007	0.03±0.003

Legend: An= Net photosynthesis rate; E= Transpiration rate; g_s = Stomatal conductance.

The four studied variants showed some minor differences in leaf gas exchange parameter. Temperature increase determined a higher oleander leaves photosynthesis rate thanks to improved stability of enzymes involved in photosynthesis (Badger et al., 1982).

Transpiration is performed mainly by stomata complexes and their position, density and opening degree have generally a major impact on this process rate, interacting with the stomata conductance. Given that the oleander leaf stomata are located in crypts filled with trichomes, it was expected that water loss to be reduced, but studied carried out by Losch et al. (1982) emphasize that as compared with other species, stomatal crypts were not necessarily linked with greater leaf resistance.

According to the results of Roth-Nebelsick et al. (2009), in the case of water stress situations at the soil level, when the stomata will close to preserve the absorbed water, crypts influence is one minor. However, if the growing conditions are some that allow a higher stomata conductance, crypts presence and their effect on reducing water loss are important factors. In such a context, in a previous paper, Gollan et al. (1985) noticed that there was not a critical relationship between leaf water potential and leaf conductance, thus, gas exchange decreased was a consequence of soil water content, while making reference to abscisic acid hypothesis, about the influence on stomatal movement and indirectly on photosynthesis. According to Miralles-Crespo et al. (2011), a rapid method to characterise the impact of freezing injury in oleander is measuring chlorophyll fluorescence. Also, Syros et al. (2004) tested the ability of six-month-old oleander grown in pot, and obtained from propagation by cuttings, to cold hardening. The values obtained for the photosynthesis rate in the case of the control plants were positive, around 2.21 (photosynthesis higher than respiration) and negative values in the case of temperature levels under 0°C. Also, in the case of transpiration, the values were positive (0.32), while at negative temperatures the values were negative, up to -0.72 at -8°C. Higher photosynthesis rate was reported by Lenzi et al. (2009) (above 8 μmol CO_2 m^{-2} s^{-1}) in the case of fully irrigated plants and in the case of Angiolo Pucci cv. it was registered the highest carbon dioxide assimilation (An- 10.97 μmol CO_2 m^{-2} s^{-1}), as well as the highest transpiration rate of 3.24 mmol m^{-2} s^{-1} was registered. In the same time, for this cv. stomatal conductance was higher too, 114.72 mmol m^{-2} s^{-1}.

It was reported a significant decrease of photosynthesis in *Nerium oleander* in the case of ozone treatments and this behavior was associated to a partial stomatal closure (Lorenzini et al., 1999). In terms of light intensity impact, the fotoinhibition effect on oleander chloroplast activity caused by a decrease in leaf water potential was lower in the case of shade conditions, compared to the plants growth of in full sun (Bjorkman and Powles, 1981). On the other hand, experiments performed by Badger et al. (1982) led to the conclusion that in the case of oleander, temperature acclimation it was not required the activation state of Rubisco enzyme. The influence of different temperatures was explained as follows: at low temperatures it has accumulated a higher amount of proteins involved in photosynthesis, to ensure higher rates of photosynthesis and on the other hand, leaves grown at higher temperatures have managed to achieve higher photosynthetic rate, due to increased heat tolerance of some enzymes, which are active in the carbon dioxide reduction cycle (Badger et al., 1982).

CONCLUSIONS

The experimental data show that oleander cutting rooting was improved by the stimulating substances and the best results were particularly obtained by BioRoot.

The effect of the cuttings treatments was noticed for both cuttings types, and the culture medium and hormone applied to the cutting modified all the biological studied parameters.

The physiological indicators varied especially in relation to the leaf age, but generally higher values were registered in the case of BioRoot stimulation, in a close interrelation with the particularities of plant rooting and growth.

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