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 (Argiropoulou 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.

Despite 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 
biactive 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>
<thead>
<tr>
<th>Experimental variants</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vm</td>
<td>Untreated</td>
</tr>
<tr>
<td>V1</td>
<td>Clonex</td>
</tr>
<tr>
<td>V2</td>
<td>Radistim</td>
</tr>
<tr>
<td>V3</td>
<td>BioRoot</td>
</tr>
</tbody>
</table>

**Rooting and Growth Parameters**

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

Leaves 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 
μmol m$^{-2}$ s$^{-1}$ to avoid the photo inhibition 
process. The results were expressed as: $A_n$ 
(μmol CO$_2$ m$^{-2}$ s$^{-1}$), $E$ (mmols H$_2$O m$^{-2}$ s$^{-1}$) and 
$g_s$ (mol H$_2$O 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 V3 
(100%), followed in decreasing order by V2 
(83.33 %), V1 (75%) and Vm (66.67 %). The 
order for the other type of cuttings was the 
same, with the mention that on V1 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 V1.
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: V3,
followed by V2, V1 and Vm.
The difference between variants was from
21.49% (V3 vs. Vm) to 12.40% (V3 vs. V2) on
the new plants obtained from peak shoot
cuttings and from 28.45% (V3 vs. Vm) to
18.10% (V3 vs. V2) in the case of the segment
shoot cuttings new plants.

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
V3 for the both type of cuttings (15.1/10.5 cm
peak shoot/segment shoot cuttings), followed
by V2 (13.75/10.25 cm), V1 (12.3/9.7 cm) and
finally Vm (11.1/7.6 cm).

Leaf gas exchange
Photosynthesis rate (Table 2) varied between
1.90 μmol CO₂ m⁻² s⁻¹ and 3.90 μmol CO₂ m⁻²
s⁻¹ and presented higher values in the case of V3,
as compared with the others treated variants
(V1 and V2), only in the case of the first
analysis data. Similarly, transpiration rate (E)
had higher values in the case of V3.

For stomatal conductance, the values were
generally low and near to those reported by
Delaney (2012) (0.03 mol H₂O m⁻² s⁻¹ - 0.06
mol H₂O 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
cuttngs on parapet and rooting system for
cuttngs 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)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Data</th>
<th>Control</th>
<th>V1</th>
<th>V2</th>
<th>V3</th>
</tr>
</thead>
<tbody>
<tr>
<td>$A_n$ (μmol CO$_2$ m$^{-2}$ s$^{-1}$)</td>
<td>25$^{th}$ May</td>
<td>3.30±0.30</td>
<td>3.00±0.36</td>
<td>3.00±0.15</td>
<td>3.90±0.11</td>
</tr>
<tr>
<td></td>
<td>11$^{th}$ July</td>
<td>3.62±0.29</td>
<td>3.05±0.25</td>
<td>3.21±0.24</td>
<td>3.89±0.41</td>
</tr>
<tr>
<td></td>
<td>12$^{th}$ Sept</td>
<td>2.65±0.66</td>
<td>1.90±0.38</td>
<td>2.51±1.33</td>
<td>2.18±0.10</td>
</tr>
<tr>
<td>$E$ (mmol H$_2$O m$^{-2}$ s$^{-1}$)</td>
<td>25$^{th}$ May</td>
<td>0.54±0.07</td>
<td>0.50±0.07</td>
<td>0.50±0.05</td>
<td>0.73±0.04</td>
</tr>
<tr>
<td></td>
<td>11$^{th}$ July</td>
<td>0.93±0.09</td>
<td>0.90±0.29</td>
<td>1.21±0.09</td>
<td>1.49±0.04</td>
</tr>
<tr>
<td></td>
<td>12$^{th}$ Sept</td>
<td>0.55±0.08</td>
<td>0.78±0.25</td>
<td>0.52±0.15</td>
<td>0.62±0.09</td>
</tr>
<tr>
<td>$g_s$ (mol H$_2$O m$^{-2}$ s$^{-1}$)</td>
<td>25$^{th}$ May</td>
<td>0.04±0.008</td>
<td>0.02±0.004</td>
<td>0.03±0.003</td>
<td>0.03±0.002</td>
</tr>
<tr>
<td></td>
<td>11$^{th}$ July</td>
<td>0.04±0.004</td>
<td>0.03±0.001</td>
<td>0.04±0.004</td>
<td>0.05±0.004</td>
</tr>
<tr>
<td></td>
<td>12$^{th}$ Sept</td>
<td>0.02±0.005</td>
<td>0.02±0.001</td>
<td>0.02±0.007</td>
<td>0.03±0.003</td>
</tr>
</tbody>
</table>

Legend: $A_n$ = 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 stomatal 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 fotoi nhibition 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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