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# EFFECT OF RHIZOGENIC BIOSTIMULATORS ON *ROSMARINUS OFFICINALIS* ROOTED CUTTINGS BIOCHEMICAL COMPOSITION

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***Abstract***

*Originally from the Mediterranean area, Rosmarinus officinalis (Fam. Lamiaceae) is a species widespread in most temperate regions of Europe and America with a significant economic impact due to the trivalent effect (ornamental, aromatic and medicinal). The purpose of these researches was to evaluate the impact of rhizogenic biostimulator Clonex-gel treatments on the rooting potential and some biochemical parameters of different types of cuttings in greenhouse conditions. The experiments carried out in the HORTINVEST greenhouses of USAMV Bucharest aimed to evaluate the rooting percentage, the development of the root system and the aerial part after rooting. Also, some biochemical parameters (assimilatory pigments and total soluble sugars content) were analyzed to assess the potential effect of the substances used to stimulate rooting on the biochemical composition of leaves formed on cuttings. The researches performed indicated that maximum percentage of rooting cuttings was determined in treated variants. The stimulation of the roots growth determined also an increased accumulation of assimilatory pigments and soluble glucids in the leaves.*

***Key words***: *carotenoids, chlorophyll, cuttings, rooting potential, rosemary.*

## INTRODUCTION

Rosemary (*Rosmarinus officinalis*) belongs to the family *Lamiaceae* (*Labiatae*) and is native to southern Europe and the Mediterranean area. Nowadays, rosemary is grown in almost all countries around the Mediterranean but also in England, USA and Mexico. In the countries of the Mediterranean, it is a popular spice especially in Italy and France, and less in Greece and Spain.

If for centuries it has been used as a herbal aroma, natural medicine and a basic ingredient in the perfume industry today rosemary can be defined as a trivalent species, in addition to its medicinal uses. So, it is cultivated as an aromatic plant in culinary art and also as a decorative plant for fragrances, protection from pests and gardening.

Rosemary has been longtime considered an important plant for its essential oil used not only in perfumes and as food spice (Moss et al., 2003), but also in medicine (Miguel et al., 2007).

The whole plant was reported to possess several medicinal properties like cardiac, antiseptic, antispasmodic, carminative, cholagogue, nervine spasmodic, astringent, stomachic, and tonic (Grieve, 1984; Polunin and Huxley, 1987; Chakravarti et al., 2005) and also it was used as a natural antimicrobial and antifungal against *E. coli, Pseudomonas, Aspergillus, Staphylococcus* or an insecticide (Duke, 2001). Besides this, it was reported that the leaves have high antioxidant activity attributed to its phenolic compounds (Singh and Guleria, 2013) therefore rosemary extracts have been widely used as a preservative in the food industry (Bozin et al., 2007).

One hundred grams of fresh rosemary leaves contain approx. 67.77 g of water, 3.31 g of protein, 20.70 g of carbohydrates, and 14.1 g of fiber. Among the minerals are Ca (317 mg), Mg (91 mg), P (66 mg), K (668 mg), Na (26 mg), Fe (6.65 mg), Zn (0.93 mg). Vitamins are mainly B6 (0.336 mg), Thiamin (0.036 mg), C (21.8 mg), Riboflavin (0.152 mg) and others (USDA, 2016).

Oils and vinegar are flavored with rosemary branches. It can be found in tea blends (Tisane) or spices (herbs of Provence). In combination with most Mediterranean herbs (oregano, basil etc.) it tastes good but can also be associated with garlic and thyme. The rosemary leaves from the culinary point of view can be used fresh, but also dry (the aroma is not lost, even if the plant is treated for a long time).

Pleasure-smelling foliage shrubs are appealing in the garden, especially for visually impaired people. Sometimes the scent feels only in the vicinity of the bushes, sometimes it has to touch or crush the leaves between the fingers to release the perfume (Noordhuis Klaas T., 2008).
The propagation by cuttings obtained from the stem is a frequently used way of vegetative multiplication of many ornamental plant species. Cutting is well-known as common and relatively cheap method because it overcomes the difficulties of multiplication by plant seeds (Elhaak et al., 2015).
It is well known that the plant-produced phytohormones like auxins play a key role in stimulating the adventitious root development of stem cuttings and the roots' branching. Treating cuttings with auxins aims to increase the percentage of rooting, root initiation, number and uniformity of rooting (Elhaak et al., 2015). Besides these, cytokinin and gibberellins also contribute to the intensification of metabolic processes, cell division or growth in length. Associating such compounds in rooting substrates allows the increase of the rooted percentage of the cuttings and in the rooting of cuttings (Georgescu et al., 2012).
Previous study related that rosemary has a good rooting ability and the effect of the root-forming solution (standard macrosalt formulation) induces better results, both in terms of the rooting percentage and the qualitative features of the new root system (Talia et al., 2004).
The purpose of these researches was to evaluate the impact of the treatment with rhizogenic stimulator on the rooting potential and on some biochemical parameters in different types of cuttings in greenhouse conditions.
The rooting percentage, the development of the root system and the aerial part after rooting together with the content of assimilatory pigments and sugars were analyzed to assess the effect of this treatment.

## MATERIALS AND METHODS

The experiment was conducted in greenhouses at the Hortinvest Center of the USAMV Bucharest (44º 26' N and 26º 06' E latitude and longitude, respectively).
Long stems from selected mother plants were harvested. Two types of stem cuttings were made: of the segment (4-5 cm long) and peak (length 5-6 cm).
After sampling the cuttings were cut out by removing the leaves from the lower node for rooting. A number of 15 cuttings of each category were used for each variant.
The substrate for rooting cuttings was river sand in alveolar plaques.
Experimental variants were established: 50% of the cuttings were subjected to a treatment by immersing the base in rooting stimulators (Clonex-gel, a powerful formula with a 0,3% concentration of 4-indol-3-yl-butyric acid, containing also vitamins and minerals). As control variants there were $V_2$ and $V_4$ (Table 1).

Table 1. Experimental variants

| Variants | Description | Number of plants |
|---|---|---|
| $V_1$ | Shoot peak cuttings treated with Clonex | 15 |
| $V_2$ | Shoot peak cuttings without Clonex | 15 |
| $V_3$ | Shoot segment cuttings treated with Clonex | 15 |
| $V_4$ | Shoot segment cuttings without Clonex | 15 |

Throughout the period of rooting there were applied specific works complex care for directing microclimate factors.
After rooting were made observations and measurements on rooted cuttings, on the percentage of rooting, root length, shoot length.
For the rooting percent comparison between variants, it was used the Fisher exact test and for comparison between variants for shoot length and root length it was used the Student test.
The biochemical parameters were analyzed using proper methods:
• *Determinations of the assimilatory pigments content* in the active leaves**:** *chlorophyll* and *carotenoid pigments* were extracted in 80% acetone and the absorbance of the extract was measured at three wavelengths (663 nm, 647 nm and 480 nm) with a UV/Visible ThermoSpectronic Helios spectrophotometer. The results were calculated using the extinction coefficients and equations described by Schopfer (1989) and were expressed in mg/g fresh weight.

• *Determination of total soluble sugars* was performed according to the Nelson-Somogyi method (Iordachescu, 1988; Somogyi, 1952). For determination of the total soluble glucid non-reducing glucids were first transformed in reducing glucids by hydrolysis with hydrochloric acid. The reducing glucids when heated with alkaline copper tartrate reduce the copper from the cupric to cuprous state and thus cuprous oxide is formed. When the cuprous oxide is treated with arsenomolybdic acid, the reduction of molybdic acid to molybdenum blue takes place. The measurements of absorbance were achieved at 540 nm with a UV/Visible ThermoSpectronic Helios spectrophotometer. The results were expressed in mg/g fresh weight.

## RESULTS AND DISCUSSIONS

The rhizogenesis process at *Rosmarinus officinalis* cuttings has been carried out quickly, so that after approximately 30 days since the placement of the cuttings on the rooting medium, they have formed roots.

The number of new plants (rooted cuttings) obtained differs depending on the variant. Also, as the rooting process takes place, there are new vegetative growths of the cuttings' terminal bud.

***Study of biometric parameters in the rosemary cuttings in all experimental variants***

The data on the number of cuttings obtained after rooting were summarized in Table 2.

Table 2. The parameters of new plants obtained by cuttings

| Variants | Rooting percent (%) | Shoot length (cm) | Root length (cm) |
|---|---|---|---|
| $V_1$ | 93.33 | 10.84 | 13.22 |
| $V_2$ | 80.00 | 8.60 | 12.10 |
| $V_3$ | 86.66 | 9.43 | 13.14 |
| $V_4$ | 66.66 | 6.90 | 9.90 |

Thus, it can be seen that the values for the main parameters are superior in the variants treated with Clonex ($V_1$ and $V_3$). Regarding the values for each type of seedlings, it is noticeable that the peak ones ($V_1$) gave better results.

According to the Fisher exact test results, there is no significant difference for the rooting percent between variants. Comparing the shoot length mean with the Student test, there was nonsignificant differences between $V_1$ and $V_3$ (df = 28, $p = 0.10 > 0.05$).

Similar results have been obtained when comparing means between variants for root length.

Roots system is significantly involved in plant growth due to their role in water and nutrient uptake, therefore maintaining an active roots growth is essential for plant development and productivity.

It is expected that the bioregulators treatment to maintain viability and functionality of the roots system during the growth period.

***Study of biochemical parameters in the rosemary cuttings in all experimental variants***

*Assimilatory pigments*

*Chlorophylls* a *and* b represent the major photosynthetic pigments in plants, playing an important role in the photochemical reactions of photosynthesis (Taiz and Zeiger, 2009), while *carotenoids* are considered as accessory components providing photoprotection (Torres-Netto et al., 2005; Simkin et al. 2008).

Previous studies have been reported increased amounts of assimilatory pigments under applications of some growth biostimulators in tomato leaves (Khan et al., 2009), *Physocarpus opulifolius* stem cuttings (Pacholczak et al., 2016), dogwood cuttings (Pacholczak et al. 2012), wheat (Vician and Kováčik, 2013) or tomato (Mikiciuk and Dobromilska, 2014). On the contrary, Tsai and Arteca (1985) noticed that applications of gibberellic acid decreased the chlorophyll content in barley, squash, pepper, sorghum, pigweed and kochia, while in oat, wheat, mung bean, corn, millet and gomphrena it remained unchanged. Also, Vasca-Zamfir et al. (2012) found that treatment of the plants with root stimulators like Clonex, Coralite and Radistim did not significantly influence the content in assimilatory pigments in the leaves of *Pelargonium peltatum*, while Elhaak et al. (2015) made similar observation regarding the treatment with IBA (indole-3-butyric acid) of rosemary.

The researches made on rosemary cuttings showed that generally the biostimulator treatment influenced the contents of organic compounds. The contents of total chlorophyll in the cuttings treated with Clonex ($V_1$ and $V_3$) increased relative to the control plants ($V_2$ and $V_4$) in both experimental variants (Table 3).

However, the cuttings provided from peak registered higher values of the chlorophyll total content by comparison with shoot segment cuttings.

Table 3. The impact of rhizogenic biostimulator treatments on total chlorophyll and glucids content in rosemary cuttings

| Variants | Total chlorophyll (mg/g f.w.) | Total soluble glucids (mg/g f.w.) |
|---|---|---|
| $V_1$ | 2.03±0.04 | 1.32±0.03 |
| $V_2$ | 1.41±0.03 | 0.92±0.02 |
| $V_3$ | 1.48±0.05 | 0.82±0.05 |
| $V_4$ | 1.36±0.03 | 0.56±0.03 |

It can be noted higher amounts of both chlorophylls *a* and *b* in the cuttings which received stimulatory treatments, while the ratio chlorophyll *a*/*b* was not significantly influenced by the Clonex application (about 2.8 in the cuttings from the peak, respectively 2.9 in the cuttings from segment whether treated or not) (Table 4).

Table 4. Ratio of assimilatory pigments in the variants

| Variants | Chlorophyll a/b | Total chlorophyll / carotenoids |
|---|---|---|
| $V_1$ | 2.82 | 23.36 |
| $V_2$ | 2.88 | 20.67 |
| $V_3$ | 2.97 | 21.45 |
| $V_4$ | 2.95 | 20.90 |

Regarding the carotenoids content, the amounts determined showed a slight variation in the analyzed rosemary leaves in all experimental variants (Figure 1) indicating that Clonex treatment not affected this parameter.

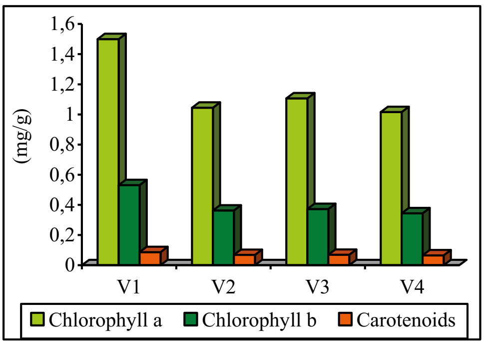


Figure 1. Accumulation of assimilatory pigments in the experimental variants

However, the chlorophyll (*a*+*b*)/carotenoids ratio increased in the variants treated with the biostimulator due to an increase of chlorophylls *a* and *b* rather than of carotenoids.
Increased chlorophyll (*a*+*b*)/carotenoids ratio found also Elhaak et al. (2015) in rosemary cuttings after soaking for one and three hours in IBA but the prolonged time of soaking (six hours) decreased the ratio probably because of an inhibition in the metabolism of chlorophylls *a* and *b*. Sosnowski et al. (2016) reported after treating *Medicago* plants with different growth regulators that the highest concentration of carotenoids was found in the *Medicago* leaves sprayed twice with cytokinin while auxin decreased their content and a mixture of auxin and cytokinin raised the ratio of total chlorophyll content to carotenoids.

*Total soluble glucids*

Sugars are considered important metabolites, is not only the first organic complex compounds formed in the leaves as a result of photosynthesis, but also a major respiratory substrate. Also sugars are involved in plant protection against wound and infections, as well as in cell detoxification (Kaur et al., 2000).
The results of the present researches indicated that the stimulation of the roots growth determined an increased accumulation of glucids in the leaves of rosemary cuttings (Table 3). Both experimental variants showed higher amounts of total soluble glucids in the stem cuttings treated with Clonex comparing to the control cuttings: about 1.43 times higher in the shoot peak cuttings, respectively 1.46 times higher in the segment cuttings.
Scientific literature noted that higher photosynthetic pigment concentrations in cuttings treated with growth regulators like auxin significantly contribute to increased photosynthetic activity and enhanced production of photoassimilates. Consequently, higher glucids levels during rooting are positively correlated with increased root formation and more nutrients uptake of cuttings (Krajnc et al., 2013).
Also, Pacholczak et al. (2016) found that the treatments with Goteo biostimulator increased total soluble and reducing sugar contents in *Physocarpus opulifolius* stem cuttings. Similar results obtained Rathore et al. (2009) in *Glycine max* treated with a preparation based

on alga *Kappaphycus alvarezii* and Sivasankari et al. (2006) in *Vigna sinensis* after application of seaweed extracts. Also, IBA (indole-3-butyric acid) applications led to increased chlorophyll accumulation in plant leaves (Ludwig-Muller, 2000).

## CONCLUSIONS

In conclusion, the analysis of the accumulated data allows the conclusion that treatment with Clonex led to obtaining of rosemary new plants of high quality and with strong roots, with real chances to solve the problem of setting up a commercial culture. It is available for the plants obtained on both types of cuttings.

The biochemical analysis made on rosemary cuttings showed that generally the biostimulator treatment significantly influenced the contents of organic compounds: total chlorophyll and total soluble glucids increased in the rosemary cuttings treated with Clonex both in the cuttings from the peak and from the segment. The carotenoids content in the leaves of rosemary cuttings was not influenced by the Clonex treatment but the chlorophyll (*a*+*b)*/carotenoids ratio increased in the variants treated with the biostimulator due to an increase of chlorophylls *a* and *b*.

In summary, it can be noted that all types of rosemary stem cuttings showed good growth and development regardless of which stem section they were provided, so the whole rosemary stem can be used for the obtaining of cuttings.

The results presented in this work show that biostimulators may enhance rooting and may be helpful for other researchers which aim to evaluate the effects of growth regulators on growth parameters and biochemical processes occurred during plants growth and development.

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