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EVALUATION OF GERANIUM VEGETATIVE PROPAGATION UNDER THE INFLUENCE OF SOME BIOACTIVE SUBSTANCES

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Abstract

The study evaluated the vegetative propagation of geraniums, Pelargonium peltatum, under the influence of some bioactive substances. 10 products with biostimulatory effect were tested, along with a control variant: V1 (control, water treatment), V2 (Radifarm, 0.2%), V3 (Radistim, powder - cuttings treatment at planting), V4 (Ecopan-1, 1.5%), V5 (Legume extract 1, 1.5%), V6 (Aromatic plant extract, 1.5%), V7 (Legume extract 2, 1.5%), V8 (Ecopan-2, 1.5%), V9 (Bionat Plus, 1.5%), V10 (Dill Essential oil, 1/50), V11 (GMO Essential oil, 1/100). The uniform geranium cuttings, 10 cm long, and with a pair of leaves at the top, were placed on a peat-based rooting substrate. The rooting process was evaluated in terms of the roots number (RN) and the roots length (RL). The planting took place on February 20, and the determinations were made on March 6 (T1), March 13 (T2), March 20 (T3) and April 2 (T4). The variants V2 and V3 were highlighted, as roots number (RN_{V2}=18.5±1.21, respectively RN_{V3}= 11.5±1.21), and variants V10 and V6 as root length (RL_{V10}=17.20±0.20 cm, respectively RL_{V6}=16.10±0.20 cm). Strong, positive correlations were identified between RN-T3 and RN-T2 ($r=0.795^{**}$), between RL-T3 and RL-T2 ($r=0.801^{**}$), and between RN-T4 and RN-T3 ($r=0.756^{**}$), in statistical safety conditions (** $p<0.01$). Based on PCA, PC1 confirmed 42.128% of variance, and PC2 confirmed 33.487% of variance. The values of the parameters RN and RL at the T4 moment (considered the end of the rooting process) were evaluated in relation to intermediate values (T2, T3) by regression analysis and equations were obtained in statistical safety conditions (R^2 = 0.992 for RN, respectively R^2 = 0.981 for RL, p <0.001 in both cases).

Key words: *biostimulators, geranium, models, rooting process, vegetative propagation*

INTRODUCTION

The geranium is a perennial evergreen plant, from the *Geraniaceae* Family, *Geranium* L. Genus, which includes about 325 species [2, 19, 21, 35]. The great diversity of *Geranium* species has been studied in relation to the habitats and different adaptations to the growing conditions [3, 25, 27, 33].

Geraniums are of interest from an ornamental point of view, but also for bioactive compounds, essential oils with various uses in the pharmaceutical, medical or cosmetic field [5, 26].

Geraniums (*Pelargonium* spp.) represent a popular category of ornamental potted plants (indoor or outdoor, depending on the season and conditions), with a high market share (eg 25% in the French market) [11].

The composition of bioactive compounds, with biological and biotechnological effects, has made geraniums of interest for various studies in the pharmaceutical and medicinal field [20, 24, 29].

Due to the large number of species and varieties, the large distribution area, the geraniums show high ecological plasticity expressed by adaptation to various eco-climatic conditions [15, 18, 32, 33]. The relationship of geraniums has been studied in relation to different growing substrates, water regime, and nutrient conditions, or stressors [1, 7, 22, 30].

The biomass accumulation, the content of nutrients, certain reports in the process of plants growth and development, morphological, floral and biochemical parameters under the influence of the substrate and the conditions of geraniums growth were evaluated [1, 36].

Geranium propagation is suitable both by "*in vitro*" techniques (micropropagation), and by vegetative propagation (based on stem cuttings or leaves) [5, 11, 16, 26].

For a wide category of cultivators and lovers of ornamental plants, an accessible method of geraniums propagating is the vegetative one, based on stem or leaf cuttings, with the observance of some procedures for obtaining quality plants.
In order to stimulate the rooting process of the cuttings, the influence of different substances with biostimulatory role was evaluated [6, 23, 26].
Usually in the case of the vegetative propagation process based on cuttings, it is a combination of factors that contribute to obtaining good results. Thus, geranium propagation (*Pelargonium graveolens* L. Herit) was studied in relation to planting time, the position of cuttings (basal, middle, terminal - on source plant shoots), and treatments with rooting bioregulators, and it was found that the terminal cuttings treated with 750 ppm IBA, respectively the middle and basal cuttings treated with 1,000 ppm IBA, ensured good results in vegetative propagation [6].
The rooting substrate, biostimulating substances (rooting hormone), the planting depth of the cuttings (1.5 - 3 cm), the propagation season (summer, autumn), the type of cuttings (stem, leaf-bud cuttings) also influenced vegetative propagation process [26].
Eco-physiological factors in the period of root development (rooting process), it was also found to have an important role in obtaining quality plants by vegetative propagation in geraniums [8, 13, 14].
Recent concerns have focused on optimizing geraniums cultivation technologies by reducing fertilizers and expensive organic components in growing substrates, as well as environmental protection considerations [1].
Of importance is the hydric regime of the plants, in different systems and techniques of geraniums cultivation, associated both with the cultivation substrate and with the cultivation conditions (pots, open space) in order to ensure optimal water plants [4].
Studies on the resistance and also the response of geranium plants to diseases and pests are also of interest, in order to evaluate early aspects and formulate appropriate recommendations and treatments [9]. Imaging techniques in leaves studies are useful for the rapid, non-destructive evaluation of some aspects associated with the attack of diseases or pests, for the purpose of prophylactic and appropriate intervention [12].
The present study investigated the influence of some biostimulatory substances, in the vegetative propagation, by cuttings, of the geranium plants.

MATERIALS AND METHODS

The geraniums, *Pelargonium peltatum*, represented the biological material, used for the purpose of vegetative propagation based on cuttings of shoots (Photo 1). The cuttings were harvested and planted on February 20, 2019.
The geranium cuttings were uniform, 10 cm long, and with a pair of leaves on the upper part (Photo 2).
The experiment took place in protected conditions (greenhouse) within the Didactic and Research Base of the University of Life Sciences "King Mihai I" from Timisoara, Romania (Photo 2).
Ten products with biostimulatory effect were tested, along with a control variant: V1 (control, water treatment), V2 (Radifarm, 0.2%), V3 (Radistim, powder - cuttings treatment at planting), V4 (Ecopan-1, 1.5%), V5 (Legume extract 1, 1.5%), V6 (Aromatic plant extract, 1.5%), V7 (Legume extract 2, 1.5%), V8 (Ecopan-2, 1.5%), V9 (Bionat Plus, 1.5%), V10 (Dill essential oil, 1/50), V11 (GMO Essential oil, 1/100).
On each experimental variant, initial treatments were made for cuttings, with biostimulator substances, in the specified concentrations.
After treatment, the cuttings were planted on the rooting substrate. During the experimental period, at intervals of 7 days, foliar treatments were performed on each experimental variant (except variant V3), with the same concentrations of biostimulator products.
The rooting substrate used was Gramoflor, Cultivo 1 F.

Photo 1. Geranium plant, *Pelargonium peltatum*; plant from the cuttings source group
Source: original photo taken by authors.

(a) (b)

Photo 2. Aspects of the vegetative multiplication process; (a) - sample of geranium cuttings; (b) - geranium cuttings to rooting on experimental variants
Source: original photo taken by authors.

The substrate was based on peat (100% peat), fine-grained (0 - 10 mm), acid reaction (pH = 5.2 - 6.0), humidifying agent 1 L / m^3, content of organic matter 85 - 95%, content of nutrients in the form of macroelements (N = 140 mg, P_2O_5 = 160 mg, K_2O = 180 mg) and trace elements, according to the product presentation [31].

Specific to the vegetative propagation process, the roots number (RN) and the roots length (RL) in cuttings were evaluated on each experimental variant.

The experiment was established on February 20, 2019. Periodic evaluations of the cuttings evolution (destructive sampling of cuttings) were made, regarding the emission of roots and their growth, under the influence of the applied biostimulators and growth substrate. The determinations have been made on March 6 (T1), on March 13 (T2), on March 20 (T3), and on April 2 (T4) respectively. In order to evaluate the cuttings evolution, in the vegetative propagation process, in relation to the applied treatments, the results were compared to the T1 determination.

The recorded experimental results were analyzed by appropriate mathematical and statistical methods (Anova test, regression analysis, PCA, cluster analysis), and for the safety of data and results appropriate statistical safety parameters were used (p, R^2, Coph. corr., and Coefficient of variation, CV). Excel, PAST software [17], and Wolfram Alpha (2020) [34] were used for data analysis and processing.

RESULTS AND DISCUSSIONS

Treatments with the biostimulator products on geranium cuttings have differentiated the rooting (formation and growth of roots) in order to plants vegetative propagation. The values recorded for the roots number (RN) and the roots length (RL), at the moments of determination (T1, T2, T3 and T4), are presented in Table 1.

The roots number (RN) and the roots length (RL), as quantifiable parameters of the vegetative propagation process in geraniums, evolved differently during the study period (moments of determination T1 to T4) in relation to the experimental variants, respectively the biostimulator treatments.

At the T1 evaluation (March 6), it was not found the start of the rooting process (RN-T1 = 0, RL-T1 = 0). Starting with T2 (March 13), the rooting process of the cuttings was found to have started, and variable values were recorded for the number of roots (RN) and the length of the roots (RL), in relation to the experimental variants.

The ANOVA test highlighted the presence of the variance in the experimental data set, and statistical safety (F >Fcrit, p <0.001, for Alpha = 0.001) (Table 2). The distribution of the values of the RN and RL parameters, in the form of Matrix plot, is presented in Figure 1.

Table 1. Data on the vegetative propagation of geranium cuttings under the influence of biostimulator treatments

Trial	Determination periods and values of RN and RL parameters							
	March 6, 2019		March 13, 2019		March 20, 2019		April 2, 2019	
	RN-T1	RL-T1 (cm)	RN-T2	RL-T2 (cm)	RN-T3	RL-T3 (cm)	RN-T4	RL-T4 (cm)
V1	RN-T1=0	RL-T1=0	1	0.40	6.5	0.46	8.5	11.10
V2			4	0.20	14.5	0.58	18.5	14.70
V3			7.5	0.40	19	0.78	11.5	14.80
V4			3.5	0.40	4.6	0.50	5	15.80
V5			4	0.40	7.2	0.70	10	12.20
V6			3	0.50	4.5	0.63	5.5	16.10
V7			3	0.18	4.2	0.49	5	12.51
V8			3.5	0.65	6.2	0.82	7.5	11.50
V9			1	0.25	3.7	0.48	8	13.80
V10			3	0.65	4.2	0.90	4.5	17.20
V11			1.6	0.10	4.5	0.41	8.5	14.20
SE			±0.54	±0.06	±1.49	±0.05	±1.21	±0.60

Source: original data from the experiment.

Table 2. ANOVA test, single factor

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	1526.937	5	305.3873	38.20116	2E-17	4.756521
Within Groups	479.6514	60	7.99419			
Total	2006.588	65				

Source: original data from the calculation.

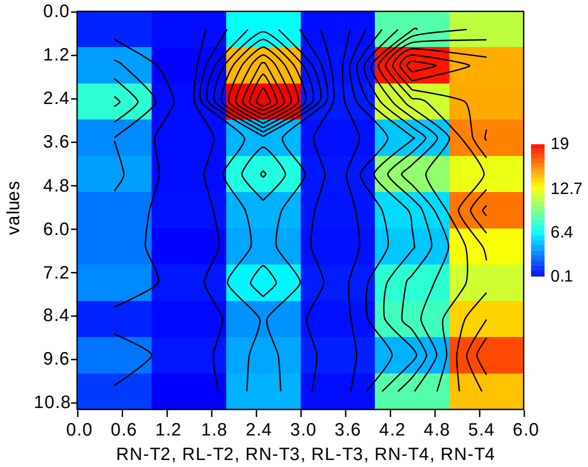

Fig. 1. Matrix plot for the RN and RL parameter distribution during the study period, *Pelargonium peltatum*
Source: iriginal graphic diagram, generated based on experimental data.

The correlation analysis between RN and RL parameters, in relation to the determination moment (T), evidenced strong, positive correlations, in statistical safety conditions ($p < .01$), between RN-T3 and RN-T2 ($r = 0.795$), between RL-T3 and RL-T2 ($r = 0.801$), and between RN-T4 and RN-T3 ($r = 0.756$) respectively (Table 3).

Table 3. Correlation table between RN and RL parameters at the evaluation moments

Categories		RN-T2	RL-T2	RN-T3	RL-T3	RN-T4	RL-T4
RN-T2	Pearson's r	—					
	p-value	—					
RL-T2	Pearson's r	0.214	—				
	p-value	0.528	—				
RN-T3	Pearson's r	0.795**	-0.082	—			
	p-value	0.003	0.811	—			
RL-T3	Pearson's r	0.576	0.801**	0.293	—		
	p-value	0.064	0.003	0.381	—		
RN-T4	Pearson's r	0.331	-0.374	0.756**	-0.032	—	
	p-value	0.32	0.257	0.007	0.926	—	
RL-T4	Pearson's r	0.227	0.150	0.038	0.245	-0.137	—
	p-value	0.502	0.659	0.911	0.468	0.689	—

* p < .05, ** p < .01, *** p < .001
Source: own data obtained from the calculation.

The variability of the studied parameters (RN, RL) was evaluated during the study period, based on the coefficient of variation (CV) and the graphical analysis. The root number (RN) parameter showed a higher variability compared to the root length (RL) parameter, at the three moments of determination, during the study period.

The values of the coefficient of variation were, in the case of the root number (RN) parameter, CVRN-T2 = 56.3311, CVRN-T3 = 68.9189, and CVRN-T4 = 47.8505, respectively. The coefficient of variation presented different values for root length (RL), according to determination moment (CVRL-T2 = 48.3304; CVRL-T3 = 26.8891; CVRL-T4 = 14.1539). The highest value of CV was recorded at T3 determination moment for RN parameter, and at T2 determination moment for RL parameter, respectively. The results obtained show that the two parameters, root formation (RN) and root growth (RL) in geranium cuttings are different. Graphic analysis provides similar results, in the form of Diversity profiles (Figure 2).

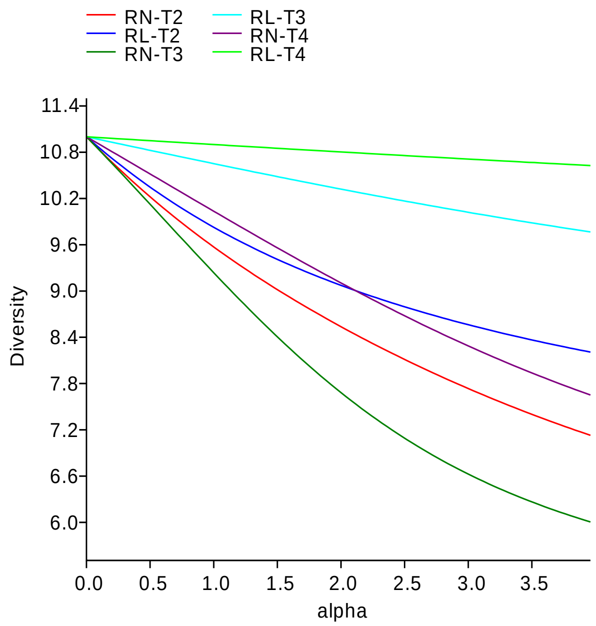

Fig. 2. Diversity profiles of RN and RL parameters in relation to the evaluation moment (T)
Source: original graphic diagram.

According to PCA (correlation matrix) was obtained the diagram shown in Figure 3, which shows the distribution of experimental variants (given by biostimulator treatments) in relation to biometric parameters (RN and RL, at different T moments) as biplot. PC1 explained 42.128% of variance, and PC2 explained 33.487% of variance. It was found the independent positioning of a group of four

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variants (V1, V7, V9 and V11) and the association of the other variants according to the considered parameters, and with various affinity levels.

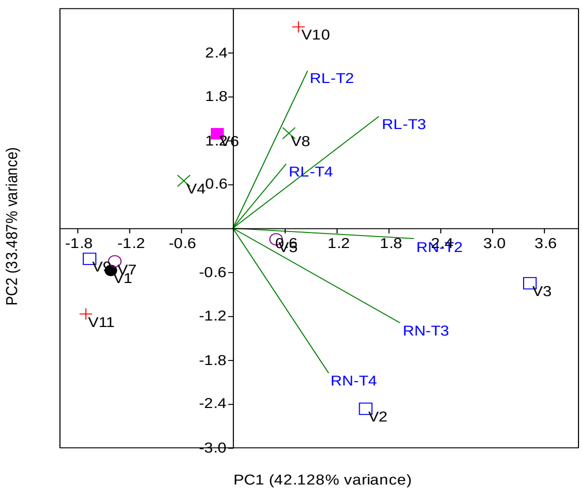

Fig. 3. PCA diagram
Source: original figure

Cluster analysis facilitated to obtaining the dendrogram in Figure 4, in statistical safety conditions (Coph. corr. = 0.972). In the dendrogram obtained, the experimental variants grouped according to the degree of similarity for generating the results in the two parameters considered (RN, RL) and the time moments (T). The formation of two distinct clusters was found. A C1 cluster comprises two variants (V2, V3) which in the PCA analysis showed high affinity for RN. Cluster C2 comprised two sub-clusters, C2-1 and C2-2, with several variants each. The highest level of similarity in the generation of results at evaluated parameters (RN, RL) and time T, was recorded between variants V4 and V6 (SDI = 0.7918), followed by variants V9 and V11 (SDI = 1.1989). The set of values for the calculated SDI is presented in Table 4.

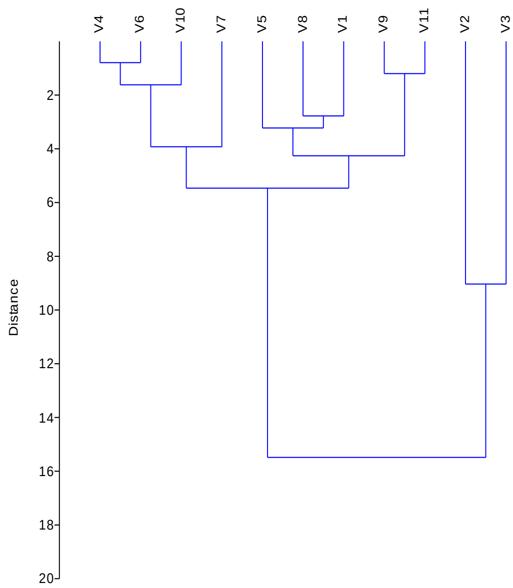

Fig. 4. Variant grouping dendrogram based on Euclidean distances
Source: original figure, obtained based on experimental data

At the T4 moment for determining the parameters RN and RL, it was estimated that geranium cuttings can be planted on more fertile substrates, for growth to obtain plants for ornamental and commercial purposes.

Table 4. SDI values in relation to the experimental variants and biometric parameters in geranium, in the study conditions

	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11
V1		13.6390	14.8760	6.6484	3.6066	6.4837	4.8557	2.7735	3.9247	7.9219	3.7500
V2	13.6390		9.0327	16.7860	11.4820	16.4940	17.1510	14.1650	15.3850	17.5970	14.3540
V3	14.8760	9.0327		16.3310	12.6690	16.3770	16.9390	14.3800	17.0210	17.1500	15.9580
V4	6.6484	16.7860	16.3310		6.7089	0.7918	3.3590	5.2407	4.4814	1.6860	4.3044
V5	3.6066	11.4820	12.6690	6.7089		6.6155	5.9320	2.8402	5.2802	8.0841	4.4129
V6	6.4837	16.4940	16.3770	0.7918	6.6155		3.6538	5.3253	4.0330	1.5477	3.8443
V7	4.8557	17.1510	16.9390	3.3590	5.9320	3.6538		3.4424	3.8625	4.7576	4.1435
V8	2.7735	14.1650	14.3800	5.2407	2.8402	5.3253	3.4424		4.2797	6.7636	3.9065
V9	3.9247	15.3850	17.0210	4.4814	5.2802	4.0330	3.8625	4.2797		5.3288	1.1989
V10	7.9219	17.5970	17.1500	1.6860	8.0841	1.5477	4.7576	6.7636	5.3288		5.2529
V11	3.7500	14.3540	15.9580	4.3044	4.4129	3.8443	4.1435	3.9065	1.1989	5.2529	

Source: original data, obtained from the calculation

Regression analysis was used to assess the growth ratio and the level of RN and RL at T4 moment, depending to the values of the two parameters from previous moments (T2 and T3), during the studied period.

The better the values of the roots of the cuttings (RN and RL) from the first stages of propagation, the more developed the roots were at the time of planting (T4).

This leads to the appreciation that the biostimulators with favorable influence at the start of the cuttings by vegetative propagation will confer an advantage and will ensure the obtaining of vigorous plants, under practical aspect (propagation process), ornamental and commercial.

From the regression analysis, equation (1) was obtained which described the variation of RN-T4 in relation to RN-T2 and RN-T3, in statistical safety conditions (R^2 = 0.992, p <0.001).

Graphically distributed RN values were represented as 3D model (Figure 5), and on isoquants format (Figure 6).

$$\mathrm{RN-T4} = ax^2 + by^2 + cx + dy + exy + f \quad (1)$$

where: RN-T4 - roots number at the T4 moment
x – RN-T2, roots number at the T2 moment;
y – RN-T3, roots number at the T3 moment;
a, b, c, d, e, f – coefficients of the equation (1);
a= -1.59861945; b= -0.16582312;
c= 1.82859570; d= 1.64152727;
e= 0.81829463; f= 0.

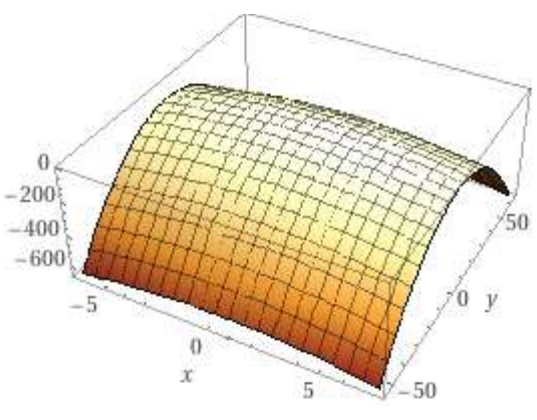

Fig. 5. 3D model of variation of RN-T4, depending to RN-T2 (x-axis) RN-T3 (y-axis)
Source: original graph generated based on experimental data.

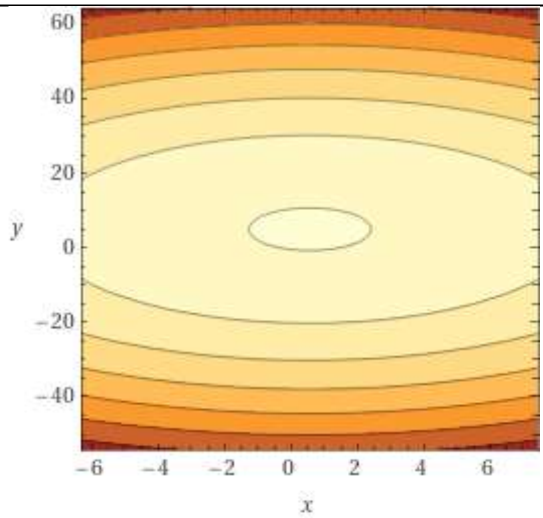

Fig. 6. Model in the form of isoquants regarding the variation of RN-T4 depending on RN-T2 (x-axis) RN-T3 (y-axis)
Source: original graph generated based on experimental data.

To describe the variation of RL-T4 in relation to RL-T2 and RL-T3, the regression analysis has facilitated to obtaining the equation (2), in statistical safety conditions (R^2 = 0.981, p <0.001). Graphically distributed RN values were represented as 3D model (Figure 7), and on isoquants format (Figure 8).

$$\mathrm{RL-T4} = ax^2 + by^2 + cx + dy + exy + f \quad (2)$$

where: RL-T4 - root length at the T4 moment;
x – RL-T2, root length at the T2 moment;
y – RL-T3, root length at the T3 moment;
a, b, c, d, e, f – coefficients of the equation (2);
a= -29.44934422; b= -61.31811298;
c= -19.50434508; d= 55.47547725;
e= 66.96517294; f= 0.

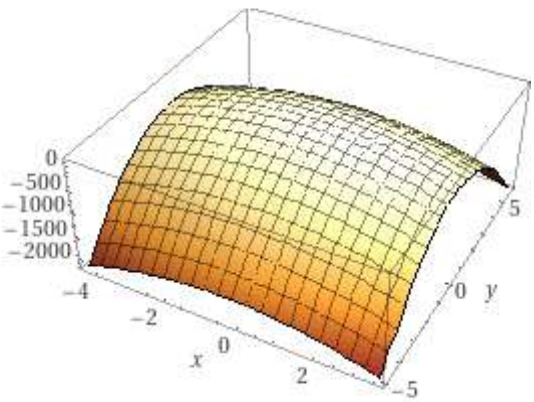

Fig. 7. 3D model of RL-T4 variation, depending to RL-T2 (x-axis) RL-T3 (y-axis)
Source: original graph generated based on experimental data.

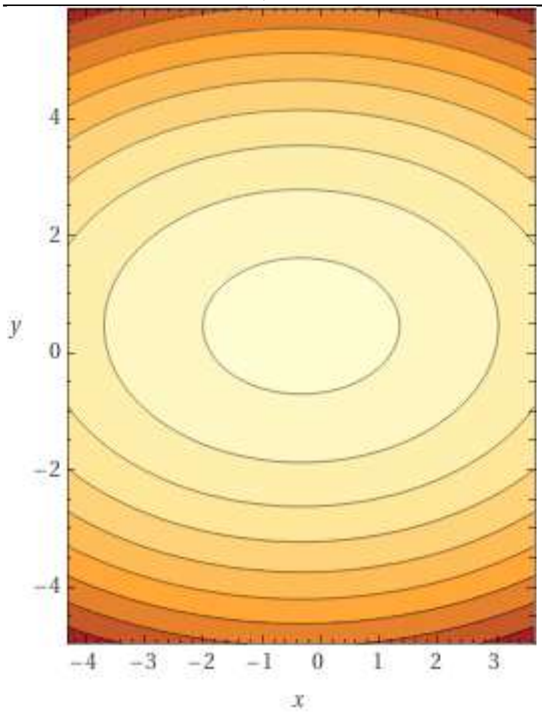

Fig. 8. Model in the form of isoquants regarding the variation RL-T4 depending on RL-T2 (x-axis) RL-T3 (y-axis)
Source: original graph generated based on experimental data.

The differences regarding RN and RL were calculated on the experimental variants, at the T4 moment, in relation to the average values of the experiment. The recorded values (positive and negative) are presented in Figure 9.

For the cultivation of plants on artificial nutrient media, different components of organic, organomineral or mineral type are used, in singly or in a mixture, or in the form of nutrient solutions can be used [28].

For rooting cuttings, light-textured substrates are recommended to facilitate root growth and development. Sand is an accessible substrate, but mixtures with other organic components (eg peat, coconut) ensure better results in the rooting process.

Pholo et al. (2013) [26] reported very good results by using a mixed substrate with sand and coconut, planting cuttings at 3 cm depth, and appropriate treatments with rooting biostimulators.

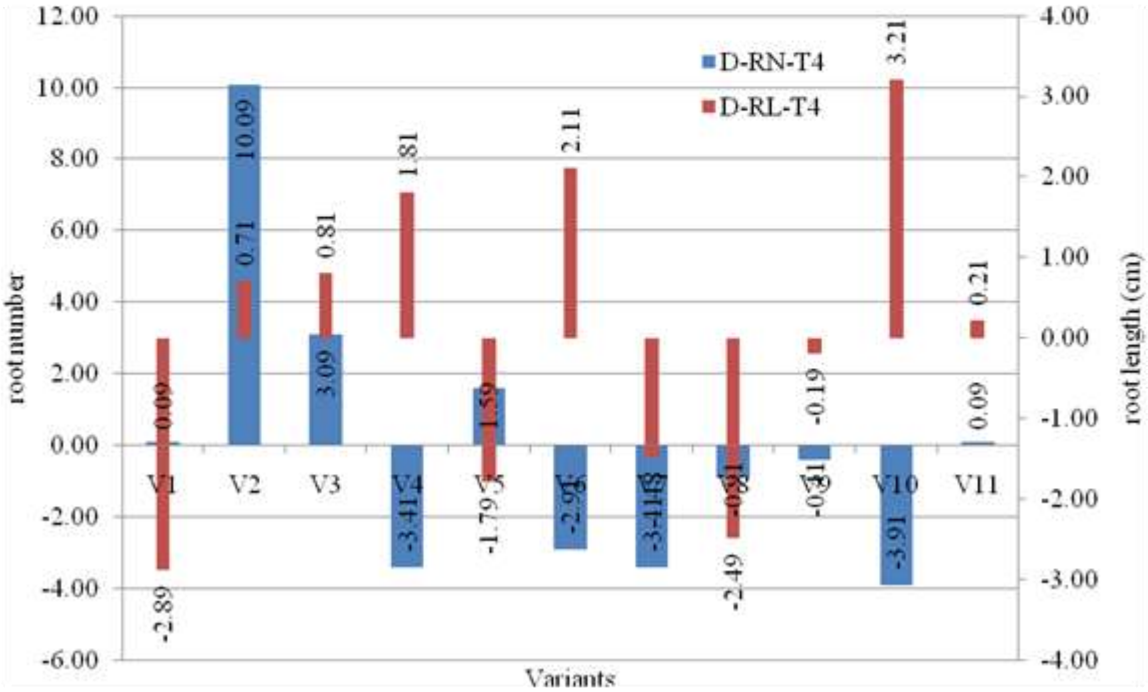

Fig. 9. Graphic distribution of differences regarding RN and RL on experimental variants, T4 moment, in relation to the average value of the experiment, vegetative propagation in geranium
Source: original graph generated based on experimental data.

In the context of environmental protection, some studies have used organic waste (eg compost of tea, leaf residues, sand, organomineral sediment, coco peat etc.) to make substrates for vegetative propagation of geranium (*Pelargonium peltatum*), with good results for multiplication and plant growth [1]. Plant growth, as a whole process, as well as the sequential variation of some vegetative or floral parameters in geranium, in relation to certain influencing factors, have been described by different linear models (eg dry mass of flowers), logarithmic models (eg dry mass of roots and shoots) in relation to phosphorus, or of linear models (eg biomass of roots, stems, leaves) in relation to integral daily light - DLI [8, 36]. The allocation of morphometric or biomass indicators, according to the plants development status, are important to understand and describe plants relationship with different habitat elements or growing conditions, and can generate model patterns of behaviour and plant response, or different intervention measures [10].

The results and models obtained, based on this study, described the geranium seedlings evolution (RN, RL parameters) according to the time under the influence of different biostimulating substances, but under the same peat-based substrate conditions.

CONCLUSIONS

The biostimulating substances tested differentially influenced the rooting process of geranium seedlings, *Pelargonium peltatum*, in the vegetative propagation. In terms of the roots number (RN), variants V2 (18.5 ± 1.21) and V3 (11.5 ± 1.21) were highlighted, and in terms of root length (RL), variants V6 (16.10 ± 0.60 cm) and V10 (17.20 ± 0.60 cm) were highlighted.

Strong correlations were recorded between RN-T2 and RN-T3, RN-T3 and RN-T4, which shows the importance of the processes that determine the emission of roots as quickly as possible in the rooting process of geranium cuttings, *Pelargonium peltatum*. Also, strong correlations were recorded between RL-T2 and RL-T3.

Mathematical models described the variation of the RN and RL parameters, at the end of the multiplication process (T4), in relation to different intermediate stages, in statistical safety conditions.

Based on PCA and Cluster analyses, the grouping and association diagrams of the studied variants were obtained, according to the affinity degree, and the similarity level to the evaluated RN and RL parameters.

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