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Cuttings rooting of some *Echeveria* genotypes in relation to biostimulating substances

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Abstract
The study evaluated the rooting of cuttings of five Echeveria species in relation to biostimulating substances. Leaf cuttings from the following genotypes were used: *Echeveria lutea* Rose (G1), *Echeveria amoena* De Smet (G2), *Echeveria shaviana* Walther (G3), *Echeveria longissima* var. *longissima* Walther (G4), and *Echeveria setosa* var. *ciliata* Moran (G5). Three biostimulants were used, Adam LQD (T1), Rizocyn (T2) and Kinactiv root (T3), compared to a control variant (Ct). Twenty experimental variants resulted, organized in repetitions. Fine sand was used as a rooting substrate, in alveolar trays. The experiment was in a protected space, a greenhouse. The G3 genotype, followed by the G2 genotype, was more receptive to the applied treatments and led to better rooting. The T3 biostimulator followed by T2 had a better rooting effect. The combination of the G3 genotype with the T3 biostimulator represented the best rooting option under the study conditions.

Keywords: *Echeveria*; leaf cuttings; rooting biostimulators; vegetative multiplication

Introduction

Vegetative propagation is a frequently used method for the multiplication of some horticultural plant species, with a number of considerable advantages [1], [19], [6].

Multiplication by cuttings (leaf cuttings, stem cuttings or shoots) is an accessible method, which under rigorous technical conditions (healthy biological material, adequate rooting substrate, biostimulating substances, etc.) ensures the obtaining of new plants in large numbers, identical to the source material, of quality, at affordable costs [17], [21], [11], [7], [22].

Vegetative propagation is an important method of plant reproduction, which has been frequently used in various forest and ornamental species [15], [8].

Some studies have developed simple and accessible vegetative propagation protocols for ornamental plant species (e.g. Ilex aquifolium), which have shown high interest in the ornamental plant market [23]. A simple, accessible and efficient method of propagating some medicinal plants (e.g. *Phytolacca acinosa* Roxb.) has been communicated, with economic advantages for the multiplication and profitable growth of the species of interest [16].

The quality of the biological material (cuttings), the type of substrate, and substances with a biostimulating effect, played an important role in clonal propagation in plants [2], [19], [5].

Echeveria (*Crassulaceae* family) includes about 140 species of plants [9]. *Echeverias* are drought-resistant plants, relatively easy to maintain, and have decorative importance due to their appearance, but also importance for the pharmaceutical field [12]. The Echeveria genus has been studied and appreciated for its ornamental potential [3].

The present study evaluated the vegetative propagation based on leaf cuttings of five Echeveria genotypes in relation to different rooting biostimulators, under experimental conditions in a protected space.

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Material and Method

Obtaining seedlings of five Echeveria genotypes through leaf cuttings required organizing the experiment appropriately, starting from substrate, biological material, and rooting biostimulators.

The experiment was conducted between 2023 and 2024, in protected space conditions (greenhouse). The rooting substrate was represented by fine sand.

The biological material was represented by five genotypes of Echeveria, mature plants, aged between 3 and 4 years. These plants represented the source of leaf cuttings. The following genotypes were considered in the study: *Echeveria lutea* Rose (G1), *Echeveria amoena* De Smet (G2), *Echeveria shaviana* Walther (G3), *Echeveria longissima* var. *longissima* Walther (G4), and *Echeveria setosa* var. *ciliata* Moran (G5). Mature leaves were taken from each species, which represented the leaf cuttings for the experiment, Fig. 1.

G1 G2 G3 G4 G5

Figure 1. Leaf cuttings of the Echeveria genotypes used in the study

After callus formation, ten leaf cuttings were selected for each genotype (cuttings of uniform size and high firmness). The cuttings were inserted into the sand with the basal part, about 2 mm. The rooting process was done in alveolar trays, Fig. 2. The experiment began on November 1, 2023, by planting the cuttings.

Figure 2. Alveolar trays with Echeveria cuttings at rooting

To stimulate the rooting process, three biostimulants were used, Adam LQD (T1), Rizocyn (T2) and Kinactiv root (T3), compared with a control (Ct). The biostimulants were applied by spraying on the cuttings (T1, T2, T3) and the control (Ct) was sprayed with water. From the combination of genotypes with the applied treatments, 20 experimental variants resulted, in repetitions. The temperature in the protected space (greenhouse) was between 15 – 25 °C.

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In relation to the purpose of the study, in mid-January 2024, the number of roots (Rn) was noted on each cutting and experimental variant.

The recorded data were analyzed mathematically and statistically, to evaluate the statistical reliability of the data, the presence of variance, the differences and the degree of similarity between the experimental variants. The calculation module in EXCEL and the PAST v 4.17 software [13] were used for the analysis of the experimental data and the generation of graphs.

Results and Discussion

The experimental data regarding the number of roots in Echeveria seedlings under experimental conditions are presented in the box plot format in Fig. 3. The ANOVA test showed the reliability of the experimental data, and the presence of variance in the data set, with statistically significant differences between the median and mean values, Table 1.

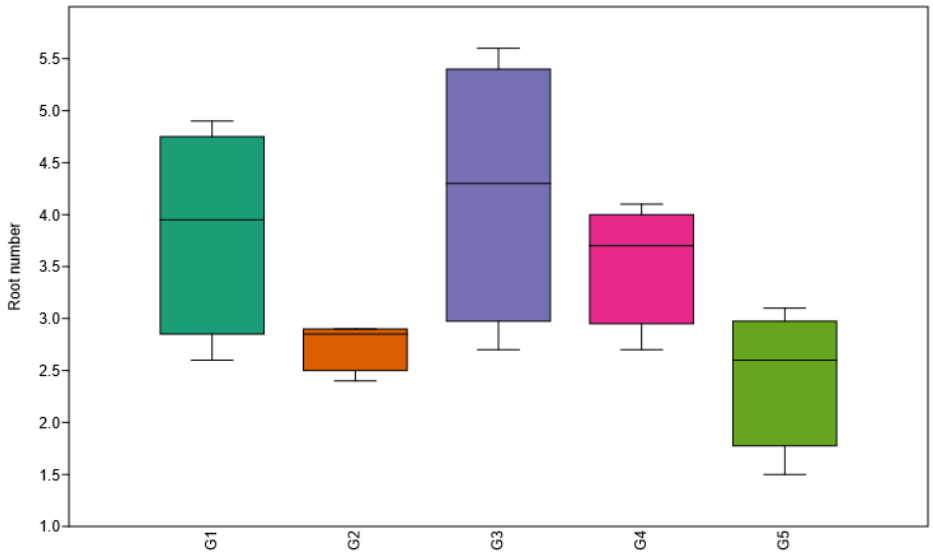

Figure 3. Graphical representation of data series for the number of roots in Echeveria cuttings

Table 1. ANOVA Test results

Statistical Parameters	Sum of sqrs	df	Mean square	F	p (same)
Between groups	88.9800	4	22.2450	7.539	1.15E-05
Within groups	575.3750	195	2.9506	Permutation p (n=99999)	
Total	664.3550	199	1.00E-05		
Components of variance (only for random effects)					
Var(group)	0.4824	Var(error)	2.9506	ICC:	0.1405
omega2	0.1157				
Levene´s test for homogeneity of variance, from means	p (same):	2.54E-05			
Levene´s test, from medians	p (same):	0.0003			
Welch F test in the case of unequal variances	F=8.048, df=92.61, p=1.295E-05				
Bayes factor	716.3 (decisive evidence for unequal means)				

According to the Kruskal-Wallis test for equal medians, it resulted that there was a significant difference between the median values of the sample ($H(chi^2) = 27.80$, H_c tie corrected = 29.15, $p < 0.001$). Based on the Kruskal-Wallis test result, the Mann-Whitney test was applied, with the results in Table 2. The fields colored in blue in the matrix table indicate the statistical safety for the genotypes tested comparatively.

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Table 2. Mann-Whitney test results

	G1	G2	G3	G4	G5
G1		0.0002	0.5159	0.2266	0.0010
G2	0.0002		5.99E-05	0.0042	0.9475
G3	0.5159	5.99E-05		0.0849	0.0003
G4	0.2266	0.0042	0.0849		0.0137
G5	0.0010	0.9475	0.0003	0.0137	

Experimental trials (data by "genotype x treatment" combination; G1 to G5 with Ct, T1, T2, T3), were analyzed comparatively (190 total combinations), and the results that showed statistical significance are presented in Table 3. One comparative analysis generated a difference at the $p<0.001$ level, nine comparative analyses generated differences at the $p<0.01$ level, and five comparative analyses generated differences at the $p<0.05$ level.

Table 3. Post Hoc Comparisons Test results

Comparison Trial		Mean Difference	95% CI for Mean Difference		Statistical parameters			Significance
		(no)	Lower	Upper	SE	t	p	(Sig)
G1Ct	G3T3	-3.140	-5.601	-0.679	0.650	-4.833	0.003	**
G1T2	G5Ct	2.750	0.289	5.211	0.650	4.233	0.015	*
G1T3	G5Ct	3.250	0.789	5.711	0.650	5.003	0.002	**
G2Ct	G3T3	-3.253	-5.714	-0.792	0.650	-5.008	0.002	**
G2T1	G3T3	-2.810	-5.271	-0.349	0.650	-4.325	0.012	*
G2T2	G3T3	-2.863	-5.324	-0.402	0.650	-4.407	0.010	**
G2T3	G3T3	-2.780	-5.241	-0.319	0.650	-4.279	0.014	*
G3Ct	G3T3	-2.920	-5.381	-0.459	0.650	-4.495	0.007	**
G3T2	G5Ct	3.280	0.819	5.741	0.650	5.049	0.001	**
G3T3	G4Ct	2.977	0.516	5.438	0.650	4.582	0.006	**
G3T3	G5Ct	4.087	1.626	6.548	0.650	6.290	< .001	***
G3T3	G5T1	3.113	0.652	5.574	0.650	4.792	0.003	**
G3T3	G5T2	3.113	0.652	5.574	0.650	4.792	0.003	**
G3T3	G5T3	2.560	0.099	5.021	0.650	3.940	0.034	*
G4T3	G5Ct	2.500	0.039	4.961	0.650	3.848	0.043	*

Multivariate analysis (PCA) facilitated the correlated distribution of genotypes with treatment variants. The first two components explained 98.923% of total variance. Genotype G4 was positioned correlated with the control variant (Ct). Genotypes G1 and G3 were positioned correlated with treatments T2 and T3. Genotypes G2 and G5 presented an independent position.

The factor loadings in the principal components, mode and intensity of action, are presented in Table 4. According to the recorded values, for each factor, positive or negative action was recorded, with higher intensity within a component (e.g. r = 0.761 in PC2 for Ct; r = -0.835 in PC3 for T1; r = -0.722 in PC4 for T2; r = 0.665 in PC4 for T3).

The graphical distribution of the PCA diagram is shown in Fig. 4(a), and the interaction relationship between Component and Eigenvalue is shown in Fig. 4(b).

Table 4. Factor loadings in the Principal Components

Factors	PC 1	PC 2	PC 3	PC 4
Ct	0.461	0.761	0.421	0.175
T1	0.520	0.164	-0.835	-0.072
T2	0.518	-0.326	0.322	-0.722

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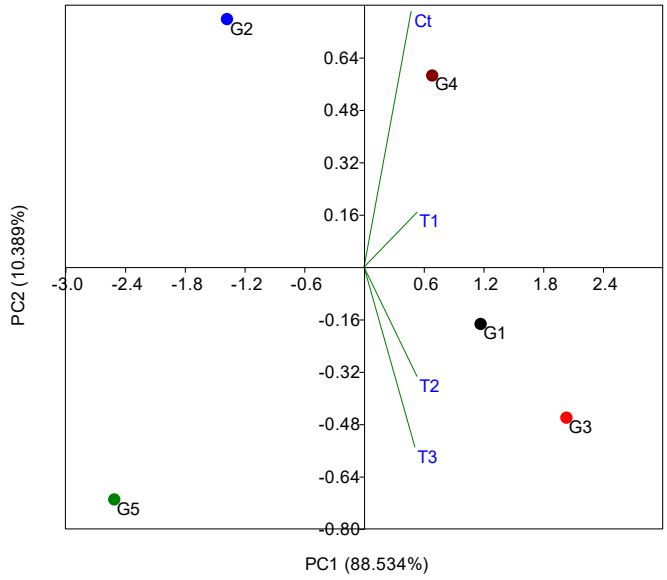
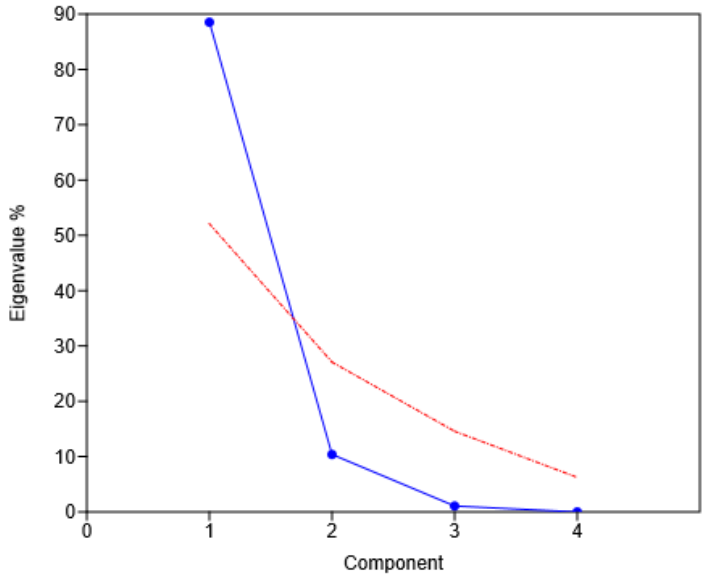
T3	0.498	-0.536	0.148	0.665



(a)



(b)

Figure 4. Graphical distribution of the PCA diagram (a), and the Component – Eigenvalue interaction relationship (b)

The control variant (Ct) as an influencing factor showed positive action in all principal components. Treatment T1 showed positive action in PC1 and PC2, and negative action in PC3 and PC8. Treatment T2 showed positive action in PC1 and PC3, and negative action in PC2 and PC4. Treatment T3 showed positive action in PC1, PC3 and PC4 and negative action in PC2.

Cluster analysis led to the grouping of experimental trials (genotype x treatment) based on similarity, Fig. 5, in relation to the number of roots at the propagation of cuttings (Coph.corr. – 0.741).

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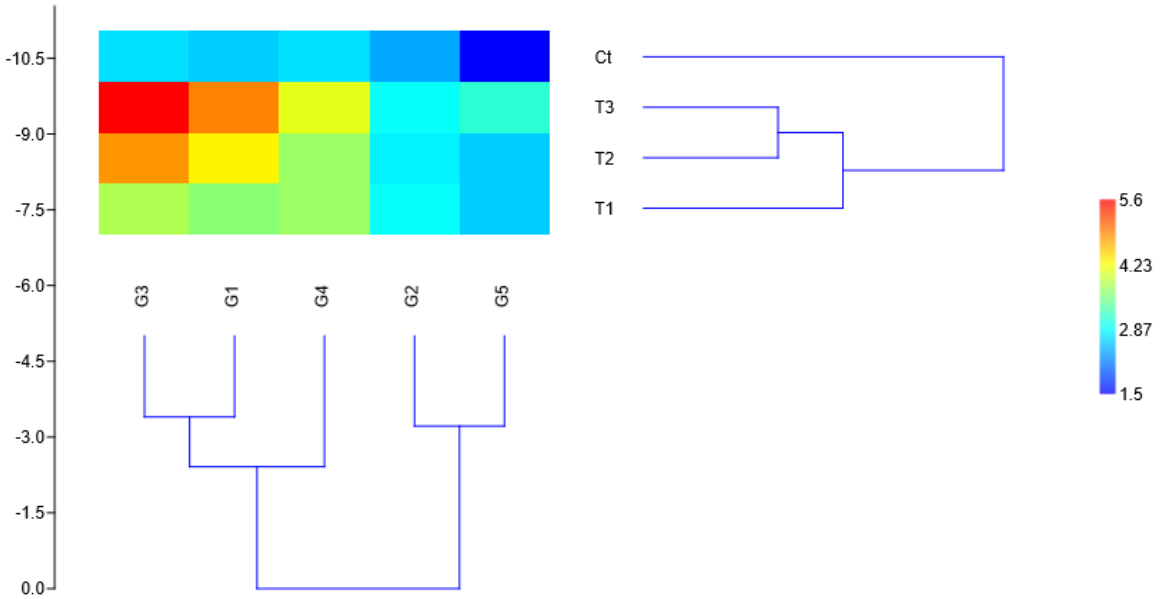

Figure 5. Cluster diagram for genotypes and treatments (two-way representation)

Treatment T3 (Kinactiv root) generated the best rooting effects of cuttings (genotype G3, followed by genotype G1), compared to the other treatments. Treatment T3 generated average results in genotype G4.

Treatment T2 (Rizocyn) followed, with high values of root number in cuttings from genotype G3, and average values in genotypes G1 and G4. Treatment T1 (Adam LQD) generated average results in genotypes G3, G1 and G4.

Genotype G3 was the most responsive to treatments (T3, followed by T2, and T1, respectively). It was followed by genotype G1 (T3, T2, and T1). It was followed by genotype G4 with intermediate values (T3, T2, and T1). Genotypes G2 and G3 showed low responsiveness to the treatments used.

Echeveria, through the diversity of species, has shown high interest in the ornamental plant category, with a series of advantages regarding tolerance to arid conditions, relatively low requirements for the growing substrate, and the particular ornamental appearance [14], [12].

Plant breeding and propagation of different *Echeveria* species have been the subject of numerous studies, with the aim of obtaining quality biological material with better ornamental value [4].

Substantele biostimulatoare sunt importante pentru plante incepand de la primele stadii de vegetatie si pe tot parcursul activitatilor metabolice, cu avantaje pentru germinarea semintelor, multiplicarea plantelor, toleranta la diferiti factori de stress, randamente, indici de calitate florala si comerciala [20], [24], [10], [18].

In the present study, the results recorded regarding the rooting of leaf cuttings presented statistical reliability, with obvious differentiation of median and mean values, according to the applied tests. Mann-Whitney test showed significant differences between genotypes, based on mean values, with some exceptions.

Combining the five Echeveria genotypes with four treatment variants (Ct, three biostimulators) resulted in 20 experimental variants, and the Post hoc comparisons analysis generated 190 combinations. Among these combinations of comparative analyses, in 15 cases differences with statistical certainty were recorded.

Multivariate analysis showed the correlation of genotypes with the treatments applied, and showed the positioning of the treatments in relation to the principal components. Cluster analysis facilitated the grouping of genotypes and treatments based on similarity, in relation to the number of roots recorded in leaf cuttings in the vegetative propagation process. Overall, the results confirmed the vegetative propagation method for the tested Echeveria genotypes, in relation to the treatments applied, and promote the propagation method for research studies and practical applicability.

Conclusions

The five *Echeveria* genotypes used in the present study had different responses in the process of vegetative propagation by leaf cuttings. In the control variant (Ct), without rooting biostimulants, genotypes G3 and G4 showed better rooting compared to the other genotypes, and the worst rooting was shown by genotype G5. In all genotypes, rooting was improved by treatments with biostimulants.

The best effect was shown by the biostimulator T3, followed by T2 and T1. Three genotypes (G3, G1,

G4) gave better results under the influence of the treatments, and two genotypes (G5, G2) had worse results. Genotype G3 showed the best rooting process (Rn) under the influence of the T3 treatment. They were followed by genotype G1 with T3, and genotype G3 with T2.

The results recorded are important for the process of vegetative propagation of Echeveria by leaf cuttings, and offer the favorable combinations "genotype x biostimulator" that generated the best results, under the study conditions. The results also recommend continuing studies to include other *Echeveria* species, respectively other biostimulator products.

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