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MANAGEMENT OF *HIBISCUS ROSA SIENSIS* L. PROPAGATION BY OPTIMIZING THE GROWTH SUBSTRATES AND BIOSTIMULATORS COMBINATION

Maria BĂLA[1], Florin SALA[2]

[1]Banat University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Horticulture, Timișoara, 300645, Romania; Email: mariabalamonicabala@yahoo.com
[2]Banat University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" from Timisoara, Soil Science and Plant Nutrition, Timișoara, 300645, Romania;
Email: florin_sala@usab-tm.ro

Corresponding author: florin_sala@usab-tm.ro

Abstract

The aim of the study was to evaluate the influence of biostimulators and growth substrates on rooting of cuttings, in order to improve the vegetative propagation through cuttings in Hibiscus rosa sinensis L.. Three varieties of Hibiscus (red flowers - R, yellow flowers - Y, and orange flowers - O), two growth biostimulators (Radistim - R, and Atonic - A) and four rooting substrates were used (sand - S1, sand:perlite - S2, sand:peat - S3, sand:peat: perlite - S4). From the combination of the three variables (biological material, biostimulators, and rooting substrates) resulted in 24 experimental variants. Cluster analysis facilitated for each studied hibiscus genotype obtaining a dendrogram in statistical safety conditions, depending on the favorable response given in quality indices of vegetative propagation, as rooting and root length. In the case of the red hibiscus variety, there was a high affinity between R.S2.R (LSD0.5%) and R.S4.A (LSD5%), and between R.S3.R (LSD5%) and R.S3.A (LSD5%) variants, under conditions of Coph.corr = 0.899. In the case of the yellow hibiscus variety, a high affinity was found between Y.S4.R (LSD5%) and Y.S4.A (0.1%), and between Y.S2.R (LSD0.5%) and Y.S2.A (LSD5%) variants, in conditions of Coph.corr = 0.809. In the case of the orange hibiscus variety, there was a high affinity between O.S4.R (LSD0.5%) and O.S4.A (0.5%), respectively between O.S2.R ad O.S2.A (LSD0.5%) variants, under conditions of Coph.corr = 0.859. By PCA analisys, PC1 explained 76.122% of variance and PC2 explained 23.878% of variance, and was obtained a distribution chart and a breakdown of variants in relation to the quality indices of the rooting process.

Key words: *biostimulator, cluster analysis, hibiscus, PCA, vegetative propagation*

INTRODUCTION

The species *Hibiscus rosa sinensis* L. belongs to the genus *Hibiscus* L., Family Malvaceae, order Malvales. It is a perennial species and grows widely as a shrub in tropical areas and warm climate [31]. Taxonomy studies [6] and recent genomic sequencing studies in Hibiscus have aimed to clarify of some phylogenetic discrepancies, and have also evaluated the genetic diversity and variability of different cultivars in relation to their importance [26], [1].
Due to its high content of substances and active principles (alkaloids, anthraquinones, carbohydrates, cardiac glycosides, essential oils, flavanoides, free amino acids, mucilage, protein, phenols, quinines, reducing sugars, saponins, steroids, tannins, terpenoids), *Hibiscus rosa sinensis* L. are multiple pharmaceutical and medicinal uses [49], [5], [32, 35]. Red-flowered hibiscus varieties are most commonly used in the medical and pharmaceutical fields, due to the high content of active principles contained therein [25], [33], [7].
Based on the antioxidant, antibacterial properties, the content of pigments and minerals in flowers, but also of some important active principles in the herb, hibiscus has found utility and in the food industry for different functional foods [42], [31], [12]. Different cultivars have been studied in relation to biomass active principles content, the antioxidant capacity [50], and *H. rosa sinensis* flowers are used in some areas for the treatment of diabetes; some studies has evaluated the potential for secondary,

toxicological effects [3].

Hibiscus rosa sinensis L. occupies an important position within the species of ornamental plants, being studied in relation to the area and the pedo-climatic conditions. Various studies and researches have evaluated horticultural characteristics as ornamental and functional role, and also studied different Hibiscus cultivated genotypes, and their growing conditions [28], [30], [36], [54].

Anatomical and morphological studies were performed on numerous spontaneous and cultivated genotypes for the characterization and evaluation of diversity in Hibiscus [2], [40], [17], [51].

Studies on the plant leaves have been performed in different species in relation to the nutrients, the chlorophyll content and the quantitative and qualitative modeling of the useful biomass [23], [41], the leaf surface and models for its determination [44], [15], classification of genotypes based on the fractal geometry of the leaves [46], or to determining the degree of attack at foliar level of some pathogens [16]. Such researches can also be adapted and extended to Hibiscus.

Various factors have been studied and tested to stimulate propagation, in relation to the species, the base material, the propagation methods and techniques, such as: magnetic nanoparticles treatments [43], mechanical r chemical scarification [13], [18], stimulation by microwave or electromagnetic field [4], [52], ultrasound treatments [27], and bio-stimulatory substances [34], [56].

Propagation of hibiscus genotypes, as ornamental plants, has been approached by different methods, vegetative, generative, germination of pollen in vitro, but more common is the vegetative method [29], [47]. Some research has focused on the evaluation of the quality of the biological material source (cuttings) and of biostimulatory substances or rooting media in the vegetative propagation in Hibiscus [48], [22].

Even though most studies have aimed at optimizing cereal production and the agri-food market [14], [20], [21], [45], [11], [37], [38], [39], the market of the ornamental plant is just as important in the economic balance of a country [9], [53], [57], [24].

In the presented context, this study evaluated the influence of biostimulators and rooting substrates on vegetative propagation in *Hibiscus rosa sinensis* L.

MATERIALS AND METHODS

The objective of the study was to optimize the combination of biological material, growth biostimulators and rooting substrate for vegetative propagation in *Hibiscus rosa sinensis* L.

Three varieties of *Hibiscus rosa sinensis* L. (H.rs) were used: red - R, yellow - Y, and orange - O (R, Y and O symbols are used in experimental variant codes). Substrates for rooting were represented by: sand, as substrate 1 – S1; sand:perlite as substrate 2 – S2; sand:peat, as substrate 3 – S3; sand:peat:perlite, as substrate 4 – S4, (S1, S2, S3 and S4 symbols are used in experimental variant codes). Two biostimulators were used to treat cuttings for rooting stimulation, Radistim - R, and Atonik - A (R and A symbols were used for experimental variant code).

From the combination of the three varieties of hibiscus (R, Y, O), four rooting substrates (S1, S2, S3 and S4) and two biostimulators (R and A) resulted 24 experimental variants: R.S1.R; R.S2.R; R.S3.R; R.S4.R; R.S1.A; R.S2.A; R.S3.A; R.S4.A; Y.S1.R; Y.S2.R; Y.S3.R; Y.S4.R; Y.S1.A; Y.S2.A; Y.S3.A; Y.S4.A; O.S1.R; O.S2.R; O.S3.R; O.S4.R; O.S1.A; O.S2.A; O.S3.A; O.S4.A.

The degree of rooting of the cuttings and the length of the roots in the rooted cuttings were evaluated, under the influence of biostimulators and of the rooting and growth substrate. The experimental data set was analyzed by analysis of variance, cluster analysis and PCA [19].

RESULTS AND DISCUSSIONS

Radistim and Atonic biostimulators, and rooting substrates (S1, S2, S3 and S4) differentially influenced the rooting of cuttings in the three varieties of *Hibiscus rosa sinensis* L., Tables 1 - 6.

For *Hibiscus rosa sinensis* L., red variety, positive results were obtained, with statistically significant differences for R.S2.R (LSD0.5%), R.S4.A (LSD5%) for rooting cuttings, and R.S3.R (LSD5%), respectively R.S3.A (LSD5%) and R.S4.A (LSD5%) for the root length, the results being presented in tables 1 and 2.

Table 1. Effect of rooting substrates, Radistim and Atonic biostimulators on the rooting at *Hybiscus rosa sinensis* L., red variety

Experimental variants	Mean values	Relative values (%)	Differences and significance
R.S1.R	5.66	74.72	-1.91
R.S2.R	11.33	149.45	3.75**
R.S3.R	3.66	48.35	-3.91^{00}
R.S4.R	9.66	127.47	2.08
Average of experience	7.58	100.00	-
LSD values	$LSD_{5\%}$=2.53; $LSD_{1\%}$=3.69; $LSD_{0.1\%}$=5.54		
R.S1.A	5.66	64.76	-3.08
R.S2.A	11.00	125.71	2.25
R.S3.A	5.66	64.76	-3.08
R.S4.A	12.66	144.76	3.91*
Average of experience	8.75	100.00	-
LSD values	$LSD_{5\%}$=3.36; $LSD_{1\%}$=4.89; $LSD_{0.1\%}$=7.34		

Source: original data, the 2018 experiment.

Table 2. Effect of rooting substrates, Radistim and Atonic biostimulators on root length at *Hybiscus rosa sinensis* L., red variety

Experimental variants	Mean values	Relative values (%)	Differences and significance
R.S1.R	16.60	89.83	-1.88
R.S2.R	17.00	91.99	-1.48
R.S3.R	21.00	113.64	2.52*
R.S4.R	19.33	104.60	0.85
Average of experience	18.48	100.00	-
LSD values	$LSD_{5\%}$=2.47; $LSD_{1\%}$=3.43; $LSD_{0.1\%}$=5.49		
R.S1.A	7.33	48.67	-7.73^{000}
R.S2.A	14.33	95.15	-0.73
R.S3.A	19.60	130.14	4.54*
R.S4.A	19.00	126.16	3.94*
Average of experience	15.06	100.00	-
LSD values	$LSD_{5\%}$=3.69; $LSD_{1\%}$=4.82; $LSD_{0.1\%}$=6.28		

Source: original data, the 2018 experiment.

Cluster analysis of the experimental data set regarding rooting of the cuttings and the root length, of the red variety of *Hibiscus rosa sinensis*, led to the grouping of variants into two distinct clusters, under statistical safety conditions (Coph.corr = 0.899), as presented in Fig.1.

There was a high affinity between the variants R.S2.R with R.S4.A, respectively R.S3.R with R.S3.A, and on the separate position was placed the variant R.S1.A, with the lowest values for evaluated parameters.

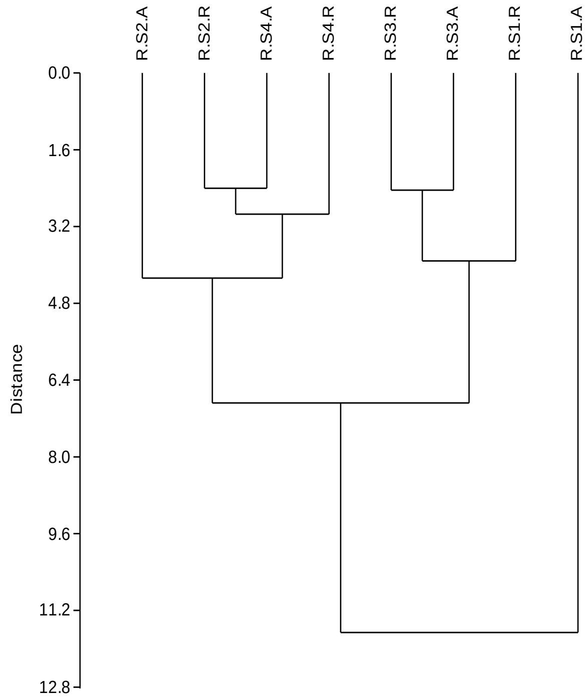

Fig. 1. Clustering of variants in the case of *Hibiscus rosa sinensis* L. - red variety
Source: original graph, based on own experimental data.

Table 3. Effect of rooting substrates and Radistim and Atonic biostimulators on the rooting at *Hybiscus rosa sinensis* L., yellow variety

Experimental variants	Mean values	Relative values (%)	Differences and significance
Y.S1.R	7.00	83.16	-1.41
Y.S2.R	11.33	134.65	2.92**
Y.S3.R	4.33	51.48	-4.08^{00}
Y.S4.R	11.00	130.69	2.59*
Average of experience	8.41	100.00	-
LSD values	$DL_{5\%}$=1.96; $DL_{1\%}$=2.85; $DL_{0.1\%}$=4.28		
Y.S1.A	6.00	59.01	-4.36^{0}
Y.S2.A	13.66	134.42	3.5*
Y.S3.A	10.00	98.36	-0.16
Y.S4.A	11.00	108.19	0.83
Average of experience	10.16	100.00	-
LSD values	$LSD_{5\%}$=2.92; $LSD_{1\%}$=4.28; $LSD_{0.1\%}$=6.42		

Source: original data, the 2018 experiment.

In the case of *H. rosa sinensis*, yellow variety, positive results were obtained, with statistically significant differences for variants

Y.S2.R (LSD0.5%), Y.S4.R (LSD5%) and Y.S2.A (LSD5%) for rootings, and Y.S4.R (LSD5%), respectively Y.S4.A (LSD0.1%) for root length (Tables 3 and 4).

Table 4. Effect of rooting substrates, Radistim and Atonic biostimulators on root length at *Hybiscus rosa sinensis* L., yellow variety

Experimental variants	Mean values	Relative values (%)	Differences and significance
Y.S1.R	5.33	63.98	-3.00
Y.S2.R	7.33	87.99	-1.00
Y.S3.R	9.00	108.04	0.67
Y.S4.R	11.66	139.97	3.33*
Average of experience	8.33	100.00	-
LSD values	$LSD_{5\%}$=3.11; $LSD_{1\%}$=4.53; $LSD_{0.1\%}$=6.79		
Y.S1.A	4.66	58.94	-3.25^{0}
Y.S2.A	9.33	117.89	1.41
Y.S3.A	4.33	54.73	-3.58^{00}
Y.S4.A	13.33	168.42	5.41***
Average of experience	7.91	100.00	-
LSD values	$LSD_{5\%}$=2.31; $LSD_{1\%}$=3.36; $LSD_{0.1\%}$=5.04		

Source: original data, the 2018 experiment.

Cluster analysis led to the grouping of variants, based on Euclidean distances, under statistical safety conditions, Coph.corr = 0.809, (Fig. 2).

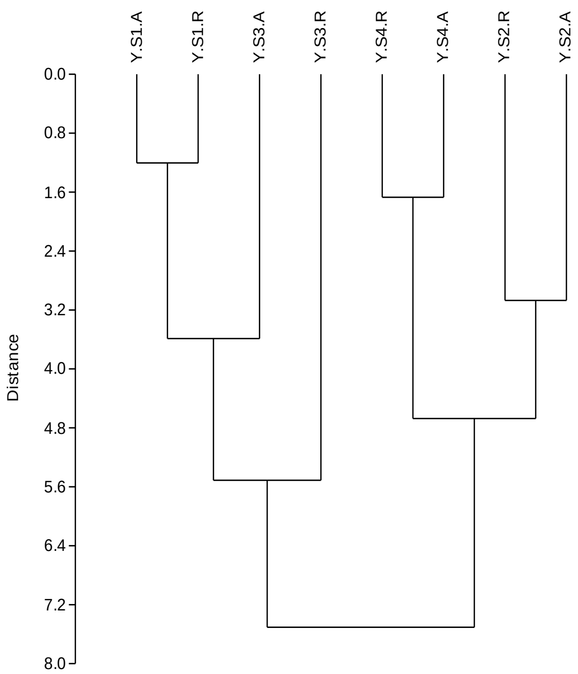

Fig. 2. Clustering of variants in the case of *Hibiscus rosa sinensis* L., yellow variety
Source: original graph, based on own experimental data.

From the analysis of the formed clusters, we found a high affinity between the variants Y.S4.R and Y.S4.A, respectively YS2R and Y.S2.A, with favorable results, and between Y.S1.R and Y.S1.A variants, with low results.
In the case of *H. rosa sinensis*, the orange variety, positive results were obtained, with statistically significant differences for O.S4.R (LSD0.5%), O.S4.A (LSD0.5%) for rootings, and O.S4.R (LSD0.5%), respectively O.S2.A (LSD0.5%) and O.S4.A (LSD0.5%) for root length (Tables 5 and 6).

Table 5. Effect of rooting substrates and Radistim and Atonic biostimulators on the rooting at *Hybiscus rosa sinensis* L., orange variety

Experimental variants	Mean values	Relative values (%)	Differences and significance
O.S1.R.	9.66	119.58	1.58
O.S2.R	4.66	57.73	-3.41^{0}
O.S3.R	5.33	65.97	-2.75^{0}
O.S4.R	12.66	156.70	4.58**
Average of experience	8.08	100.00	-
LSD values	$DL_{5\%}$=2.49; $DL_{1\%}$=3.62; $DL_{0.1\%}$=5.44		
O.S1.A	8.00	101.05	0.09
O.S2.A	4.66	58.94	-3.25^{0}
O.S3.A	6.33	80.00	-1.58
O.S4.A	12.66	160.00	4.75**
Average of experience	7.91	100.00	-
LSD values	$LSD_{5\%}$=2.43; $LSD_{1\%}$=3.54; $LSD_{0.1\%}$=5.31		

Source: original data, the 2018 experiment.

Table 6. Effect of rooting substrates, Radistim and Atonic biostimulators on root length at *Hybiscus rosa sinensis* L., orange variety

Experimental variants	Mean values	Relative values (%)	Differences and significance
O.S1.R.	6.00	75.00	-2.25
O.S2.R	10.66	125.00	2.41
O.S3.R	3.33	41.66	-4.92^{00}
O.S4.R	12.99	158.33	4.74**
Average of experience	8.25	100.00	-
LSD values	$LSD_{5\%}$=3.18; $LSD_{1\%}$=4.63; $LSD_{0.1\%}$=6.95		
O.S1.A	4.66	45.82	-5.51^{00}
O.S2.A	15.63	153.69	5.46**
O.S3.A	5.33	52.41	-4.84^{0}
O.S4.A	15.06	148.08	4.89**
Average of experience	10.17	100.00	-
LSD values	$LSD_{5\%}$=3.32; $LSD_{1\%}$=4.85; $LSD_{0.1\%}$=7.25		

Source: original data, the 2018 experiment.

Cluster analysis led to the grouping of variants based on Euclidean distances into two distinct clusters, each with several sub-clusters, under statistical safety conditions, Coph.corr = 0.859 (Fig. 3). From the analysis of the formed clusters, it was found a high affinity between the variants O.S4.R with O.S4.A, and O.S2.R with O.S2.A, in conditions of favorable results, respectively O.S1.A with O.S3.A, to which O.S3.R and O.S1.R are affiliated, with weaker results.

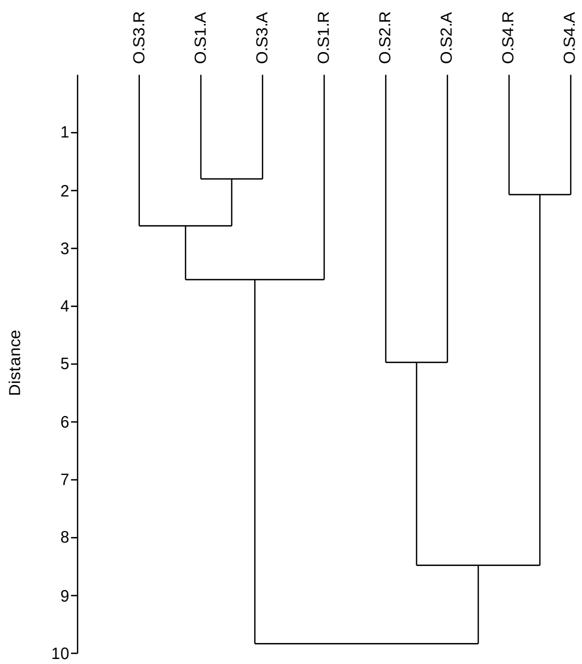

Fig. 3. Clustering of variants in the case of *Hibiscus rosa sinensis* – orange variety
Source: original graph, based on own experimental data.

PCA test, over the entire experimental data set, led to the graphical distribution in Figure 4. PC1 explained 76.122% of variance, and PC2 explained 23.878% of variance.

From the analysis of the distribution of variants in relation to biplots Rooting and Root length, it was found that the grouping of variants with respect to the response given from the combination of biological material x biostimulator x substrate.

Wang and Andersen (1989) [55] identified a number of interdependence relationships between seedlings, their age, treatment with bioregulatory substances and rooting environment in *Hibiscus rosa sinensis* L. Shadparvar et al. (2011) [48] found the favorable influence of higher concentrations of IBA (Indole Butyric Acid), in the range 0 - 4,000 mg l^{-1} tested, on the vegetative propagation through cuttings in *Hibiscus rosa sinensis* 'yellow double hybrid' variety. They evaluated the callus time of cuttings, beginning of rooting, and percentage of rooting, number of root at cuttings, root length and number of buds, in relation to IBA concentrations.

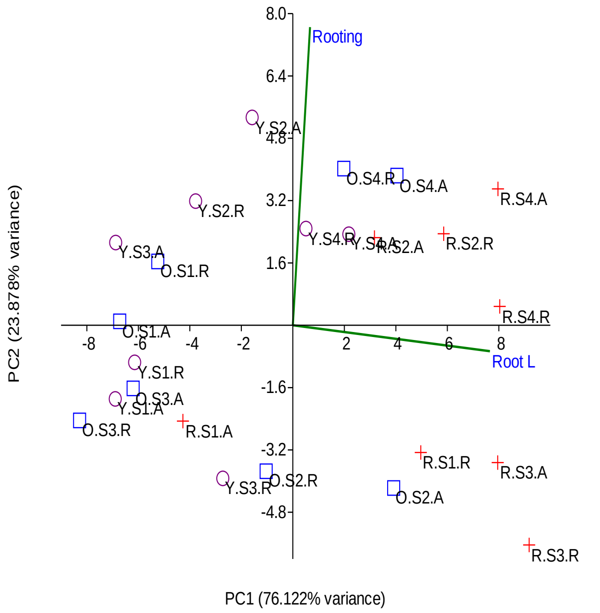

Fig. 4. PCA - grouping of variants as a result of the interaction [genotype x biostimulator x substrate]; R – root, Y – yellow, O – orange; S1, S2, S3, S4 – substrates; R, A – biostimulators
Source: original graph, based on own experimental data.

Baldotto et al. (2012) [8] reported favorable results on the propagation by cuttings in Hibiscus using the biostimulators IBA (indolbutyric acid) and HA (humic acid). Similar results were reported by Izadi and Zarei (2014) [22] in Hibiscus, 'Blue Stain' and 'Jeanne d'Arc' cultivars, in which high concentrations of IBA had favorable effects on vegetative propagation through seedlings. Chowdhuri et al. (2017) [10] reported favorable results by testing three root and growth biostimulators (IAA, IBA, NAA) in *Hibiscus rosa sinensis*, China rose.

The objectives of the present study are in the context of the research and studies to which it was referred. The results were obtained by using biostimulatory substances, accessible to flower producers, Hibiscus species, in conditions of statistical certainty of the differences identified between the experimental variants.

CONCLUSIONS

The independent variables used, such as growth biostimulators (Radistim, Atonic) and rooting media (sand, sand:perlite, sand:peat, sand:peat:perlite) differentially influenced the vegetative propagation, through cuttings, in *Hibiscus rosa sinensis* L., varieties red, yellow, and orange.

Cluster analysis and PCA led to obtaining cluster groups and PCA distribution, under statistical safety conditions, based on which the best variants for obtaining vigorous plants were found, in order to optimize vegetative propagation in Hibiscus.

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