Influence of Culture Substrates and Biostimulators on *Passiflora* Rooting

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Abstract

Passiflora quadrangularis and *Passiflora caerulea* commonly known as passion flower are two voluble species appreciated for their impressive flowers and delicious fruits. The aim of the presented experiment was to compare the effect of different rooting substrates (vermiculite, peat+vermiculite, peat+perlite, peat+sand) and biostimulators (Radistim-2; Incit-8, IBA 1000 ppm and control - untreated cuttings) on the rooting of stem cuttings from *P. caerulea* and *P. quadrangularis*. Stem cuttings of approximately 15 cm length were harvested from the medial portions of the shoots from mature plants. Regarding the rooting rate by species, it has been found that *P. caerulea* obtained a 79.78% cuttings rooting average compared to *P. quadrangularis* which had a rooting average of 74.57%. Vermiculite and the treatment with IBA 1000 ppm highly influence the length and number of roots. Establishing the most suitable measures for vegetative propagation by cuttings of *Passiflora* species studied could lead to the expansion of their culture in our country.

Keywords: cuttings, *Passiflora caerulea*, *Passiflora quadrangularis*, vegetative propagation

Introduction

Recently, the import of ornamental flowering plants had been increasing, with the need to improve the quality of life and to acquire a general well-being. Among the plants sold on the market in Romania there is also the passion flower.

Passiflora is the general name of plants in the *Passifloraceae* family, which is mainly noted for the *Passiflora* genus, which comprises more than 560 species (Shrestha *et al.*, 2019).

The genus *Passiflora* is native to tropical areas of the South America, and many species grow in Brazil, Colombia, Peru, even in Asia, Australia and China. *Passiflora* is appreciated for the production of fruits that are suitable for fresh or processed

consumption, for flowers of an unusual beauty, with a huge ornamental potential and not least for the presence of phytoconstituents found in various organs of the plant that can be used in alternative medicine (Faleiro *et al.*, 2018, Bernacci *et al.*, 2015).

From the aforementioned considerations, the need arises for the cultivation and propagation of the genus *Passiflora* in Romania, especially for the climate which is changing continuously making the conditions favorable for the introduction of this species as crop of flowering plants from protected areas. The propagation of *Passifloraceae* family is generative propagation by seeds (Meletti *et al.*, 2007), but it can also be successfully

propagated by cuttings, marking or grafting (Leonel and Pedrosso, 2005). Therefore, comes the need to produce vigorous plants for good adaptability and resistance to the changing climate conditions. The use of suitable substrates and the application of different treatments to increase the production efficiency are necessary to reduce the final costs and to obtain high quality planting material. However, to obtain a suitable substrate it is recommended to mix different components such as sand, soil, coconut fiber or commercial substrates (Faleiro *et al.*, 2018).

Propagation by cuttings from *Passiflora* is mainly used for commercial purposes. In Brazil, high number of rooted plants of the species *Passiflora edulis* Sims and *Passiflora alata* Curtis are obtained, with the rooting rate being over 90% (Salomão *et al.*, 2002).

The determining factors for cutting plant propagation are: humidity, temperature, substrate, hormonal balance, the type of cutting and physiological state of the mother plant (Gomes *et al.*, 2018). Root biostimulators can have positive effects on cuttings, increase rooting percentage and root proliferation, representing a trend for sustainable agriculture (Nardi *et al.*, 2016).

Thus, the present research aims to evaluate the effect of rooting substrates and biostimulators on *Passiflora caerulea* and *Passiflora quadrangularis* species in order to develop an optimal vegetative propagation protocol by cuttings.

Materials and methods

The experience regarding the possibilities of vegetative propagation by cuttings for the *Passiflora caerulea* and *Passiflora quadrangularis* species was placed in the greenhouse of the Department of Ornamental Plants, belonging to the Institute of Advanced Horticultural Research of Transylvania, of the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca. From the climatic point of view, the area in which the greenhouse is located, according to the W. Köppen system is the climatic province Df, defined by the boreal climate with cold and humid winters and the lowest temperature recorded during winter below -30°C and the highest above 10°C (Bunescu *et al.*, 2005).

The biological material used to carry out the experiment was represented by cuttings approximately 15 cm long, respectively 2-3 nodes,

harvested from mature plants in the vegetative growth stage, from the middle part of the shoot. The cuttings were trimmed by removing the basal leaves and reducing the leaf surface for *Passiflora quadrangularis*. In case of *P. caerulea* the leaves were kept entirely.

The research regarding the vegetative propagation by cuttings was carried out as a trifactorial experiment of the type $4 \times 4 \times 2$, in a randomized block design, in three repetitions (Ardelean *et al.*, 2007) as follows:

Factor A - rooting substrate:

- a1 - vermiculite (V);
- a2 - peat + vermiculite in 1:1 mixture (P + V);
- a3 - peat + perlite in 1: 1 mixture (P + P);
- a4 - peat + sand in 1: 1 mixture (P + S).

Factor B - the rooting biostimulator:

- b1 - Incit - 8 (I8);
- b2 - Radistim - 2 (R2);
- b3 - IBA 1000 ppm (IBA);
- b4 - no treatment (Nt).

Factor C - the species:

- c1 - *Passiflora caerulea*;
- c2 - *Passiflora quadrangularis*.

By combining the three experimental factors, 32 experimental variants resulted.

At 60 days from the date of planting the cuttings, observations were carried out regarding: the number of roots emerged by each plant; the length of the roots emerged by each plant; the length of the main stem emitted by each plant; the number of shoots developed by each plant and the number of leaves. After the measurements were made, the rooted plants were planted in pots containing a mixture of peat + vermiculite (70% + 30%) where they were kept for 90 days, after which they were planted in the field.

The experimental data obtained regarding the variability of the morphological characters of the rooted cuttings were statistically processed using the analysis of variance (ANOVA) followed by Duncan's multiple comparison test.

Results and discussions

Based on the research carried out on the two species, *Passiflora caerulea* and *Passiflora quadrangularis*, the influence of the rooting substrate and rooting biostimulator on the rooting rate of the cuttings and on morphological characters was analyzed.

Figure 1. The rate of rooting for *Passiflora* cuttings depending on substrate and rooting biostimulators

Regarding the rooting rate by species (Fig. 1), it was found that *Passiflora caerulea* obtained an average rooting rate of 79.78%, compared with *Passiflora quadrangularis*, which obtained an average percentage of 74.57% rooted cuttings. The cuttings of *Passiflora caerulea* (Fig. 2) recorded the highest rooting percentage (96.7%) on peat + vermiculite mixing substrates following treatment with Radistim - 2 and IBA 1000 ppm. The lowest rooting rate of this species being obtained on the peat + sand substrate in the case of cuttings without biostimulator treatment. The cuttings of *Passiflora quadrangularis* (Fig. 3) recorded 93.3% cuttings rooting on the vermiculite substrate and the peat + perlite mixture associated with IBA treatment, respectively the lowest rooting rate of the species of 53.3% was obtained on the peat + sand substrate in the case of cuttings not treated with biostimulator.

According to a study that investigated the effect of some types of cuttings and the concentration of IBA (0; 1000 mg kg⁻¹, 1500 mg kg⁻¹, and 2000 mg kg⁻¹) on rooting in *Passiflora mucronata*, Sobreira *et al.* (2016), found that the effect of IBA is dispensable for the production of plants from cuttings in this species.

a. The average root length

Root length at rooted cuttings (Tab. 1) varied between 3.73 cm (T₁₆) and 8.59 cm (T₃) for *P. caerulea* and between 4.77 cm (T₈) and 8.63 cm (T₁) for *P. quadrangularis*. Regarding the species *P. caerulea*, statistically significant differences were noted for the T₁, T₇, T₁₀, T₁₂, T₁₄, T₁₅, and T₁₆ variants and the *P. quadrangularis* cuttings obtained statistically assured differences for

the T₃, T₄, T₉, T₁₀, T₁₂ and T₁₄ variants, the rest of the variants showed no statistically significant differences.

Treatment with IBA 1000 ppm (T₃ and T₇) determined the absolute lengths of the upper root for the *P. caerulea* compared to *P. quadrangularis* species, the differences were statistically significant.

b. The average number of roots

The average number of roots (Tab. 1) varied between 4.16 (T₁₆) and 11.95 (T₃) for *P. quadrangularis* and between 4.84 (T₁₆) and 10.85 (T₃) for *P. caerulea*. Regarding the *P. caerulea* species, statistically significant differences were obtained for the T₂, T₃, and T₁₁ treatment variants and the *P. quadrangularis* cuttings presented statistically significant differences for the T₂, T₃, T₅, T₉, T₁₀, T₁₂ and T₁₆ treatment variants, the rest of the variants showed no statistically significant differences.

c. The average length of main shoot

Regarding the length of main shoot (Tab. 1), the average recorded was between 1.35 cm (T₄) and 3.47 cm (T₁₃) for *P. quadrangularis* and between 1.43 cm (T₁₂) and 4.22 cm (T₂) for *P. caerulea*. In the case of the *P. caerulea* species, statistically significant differences were recorded for T₂, T₃, T₄, T₆, T₈, T₉, T₁₂, T₁₄, T₁₅ and T₁₆ variants and *P. quadrangularis* cuttings obtained statistically significant differences for the T₁, T₂, T₄, T₇, T₈, T₁₀ and T₁₅ variants, the rest of the variants showed no statistically significant differences. Analyzing the combinations between factors, it was found that *P. caerulea* recorded higher average values regarding the length of the shoot, compared to

Figure 2. *P. caerulea* cuttings rooted in:
a - vermiculite; b - peat + vermiculite;
c - peat + perlite; d - peat + sand

Figure 3. *P. quadrangularis* cuttings rooted in:
a - vermiculite; b - peat + vermiculite;
c - peat + perlite; d - peat + sand

P. quadrangularis, except for T_{13} (3.47) where *P. quadrangularis* recorded a significantly higher value for average length of main shoot, the rest of variants presenting no statistically significant differences.

d. The average number of shoot /cuttings

Regarding the comparative analysis of the number of shoots emitted (Tab. 1), the average recorded varied between 0.77 (T_{16}) and 0.94 (T_7) for *P. caerulea* and between 1.19 (T_{10}) and 2.10 (T_2) for *P. quadrangularis*.

In case of *P. caerulea* cuttings, the T_7 , T_{11} , T_{13} and T_{16} treatment variants, registered statistically significant differences, the rest of the variants had no statistically significant differences. As for *P. quadrangularis* cuttings, the T_2 , T_3 , T_6 , T_7 , T_{10} , T_{12} and T_{13} treatment variants obtained statistically significant differences, the rest of the variants showed no statistically significant differences.

e. The average number of leaves

Regarding the number of leaves (Tab. 1), the average recorded was between 1.46 (T_5) and 3.55 (T_{14}) for *P. quadrangularis* and between 2.00 (T_{13}) and 4.09 (T_{10}) for *P. caerulea*.

Analyzing the combinations between factors, it was found that *P. caerulea* recorded higher average values regarding the number of leaves, compared to *P. quadrangularis*, except for T_{13} (3.41) and T_{15} (3.37) where *P. quadrangularis* recorded a statistically significant higher number of leaves. In the case of *P. caerulea*, the treatment variants T_2 , T_3 , T_8 , T_9 , T_{10} , T_{11} and T_{13} obtained statistically significant differences and *P. quadrangularis* cuttings obtained statistically significant differences for the treatment variants

T_5 , T_7 , T_{13} and T_{15} , the rest of the variants showed no statistically significant differences.

The results obtained from correlations between each of the morphological characters was presented as a correlation matrix. Regarding *P. caerulea* species (Fig. 4), using Pearson correlation coefficients, direct links evidenced by highly positive correlated values ($p < 0.001$) between the average length of the main root and the average number of roots ($r = 0.94$) respectively between the average length of the main shoot and the average number of leaves ($r = 0.78$). The average root length correlated with the average length of the main shoots ($r = 0.5$), with the average number of shoots ($r = 0.56$) and with the average number of leaves ($r = 0.5$) obtained a moderate correlation ($p < 0.05$). It appeared that with the growth of the root, the aerial part of the plants also grows. Also, between the average number of roots and the average number of shoots ($r = 0.51$), there was a moderate correlation.

In the case of *P. quadrangularis* species (Fig. 5), statistically significant correlation coefficient values were obtained only between the average root length and the average number of roots ($r = 0.81$), indicating variables highly correlated.

In contrast to the values obtained in the case of the correlation between the morphological characters in *P. caerulea*, in *P. quadrangularis* are identified negatively correlated values, no statistically significant, between the average root length and the average number of leaves ($r = -0.09$) and between the average number of roots and the average leaf number ($r = -0.17$). No

Table 1. Results on the interaction of substrate, rooting biostimulator and species on the morphological characters of *Passiflora* plants

Variant Treatment (Substrate+Biostimulator)	Morphological characters									
	The average root length (cm)		The average number of roots		The average length of main shoot (cm)		The average number of shoots/ cuttings		The average number of leaves	
	P.c.	P.q.	P.c.	P.q.	P.c.	P.q.	P.c.	P.q.	P.c.	P.q.
T ₁ (V - I8)	6.50 ^{h-k}	8.63 ⁿ	8.96 ⁱ	10.58 ^{kl}	2.41 ^{e-i}	1.93 ^{b-e}	0.89 ^{a-c}	1.26 ^{de}	2.74 ^{g-i}	2.30 ^{d-g}
T ₂ (V - R2)	7.14 ^{j-l}	7.67 ^{lm}	9.42 ^{ij}	9.86 ^{jk}	4.22 ⁿ	3.44 ^{lm}	0.88 ^{a-c}	2.10 ^k	3.81 ^{no}	2.71 ^{g-i}
T ₃ (V - AIB)	8.59 ⁿ	8.46 ^{mn}	10.85 ^l	11.95 ^m	2.92 ^{i-l}	2.58 ^{f-i}	0.91 ^{a-c}	1.60 ⁱ	2.92 ^{h-j}	1.89 ^{a-d}
T ₄ (V - Nt)	5.77 ^{e-h}	7.55 ^l	7.5 ^h	7.87 ^h	2.16 ^{c-h}	1.35 ^a	0.82 ^{a-c}	1.34 ^{ef}	2.68 ^{g-i}	1.62 ^{ab}
T ₅ (T+V - I8)	5.61 ^{d-h}	5.32 ^{c-f}	7.55 ^h	5.06 ^{bc}	2.71 ^{h-j}	1.69 ^{a-c}	0.87 ^{a-c}	1.29 ^{de}	2.31 ^{d-g}	1.46 ^a
T ₆ (T+V - R2)	7.13 ^{j-l}	5.63 ^{d-h}	8.74 ⁱ	4.71 ^{a-c}	2.54 ^{f-i}	1.76 ^{a-c}	0.83 ^{a-c}	1.47 ^{f-i}	2.54 ^{f-i}	1.72 ^{a-c}
T ₇ (T+V - AIB)	6.87 ^{i-l}	5.80 ^{e-h}	10.22 ^{kl}	6.62 ^{fg}	3.68 ^m	2.13 ^{c-g}	0.94 ^c	1.51 ^{hi}	3.39 ^{k-n}	2.94 ^{i-k}
T ₈ (T+V - Nt)	6.39 ^{g-k}	4.77 ^{b-d}	6.06 ^{ef}	5.18 ^{b-d}	2.68 ^{g-j}	1.85 ^{a-d}	0.81 ^{a-c}	1.34 ^{ef}	2.45 ^{e-h}	1.53 ^{ab}
T ₉ (T+P - I8)	4.79 ^{b-d}	5.69 ^{d-h}	7.80 ^h	5.49 ^{c-e}	3.15 ^{j-m}	1.42 ^{ab}	0.84 ^{a-c}	1.93 ^j	3.25 ^{j-m}	2.18 ^{c-f}
T ₁₀ (T+P - R2)	7.26 ^{kl}	6.26 ^{f-j}	6.41 ^{fg}	5.87 ^{d-f}	3.38 ^{k-m}	2.37 ^{d-i}	0.82 ^{a-c}	1.19 ^d	4.09 ^o	2.33 ^{d-g}
T ₁₁ (T+P - AIB)	5.87 ^{e-h}	7.61 ^{lm}	7.08 ^{gh}	5.16 ^{b-d}	2.43 ^{e-i}	1.71 ^{a-c}	0.92 ^{bc}	1.5 ^{g-i}	2.99 ^{i-l}	1.82 ^{a-c}
T ₁₂ (T+P - Nt)	4.27 ^{ab}	5.43 ^{c-g}	5.15 ^{b-d}	4.53 ^{ab}	1.43 ^{ab}	1.50 ^{ab}	0.89 ^{a-c}	1.37 ^{e-g}	2.61 ^{f-i}	1.58 ^{ab}
T ₁₃ (T+N - I8)	4.76 ^{b-d}	5.78 ^{e-h}	6.24 ^{ef}	4.74 ^{a-c}	2.59 ^{f-i}	3.47 ^m	0.78 ^{ab}	1.40 ^{e-h}	2.00 ^{b-e}	3.41 ⁱ⁻ⁿ
T ₁₄ (T+N - R2)	4.53 ^{a-c}	5.48 ^{d-g}	5.27 ^{b-d}	4.75 ^{a-c}	3.3 ^{k-m}	1.75 ^{a-c}	0.88 ^{a-c}	1.49 ^{g-i}	3.48 ^{mn}	3.55 ^{mn}
T ₁₅ (T+N - AIB)	5.27 ^{c-e}	6.08 ^{e-i}	6.51 ^{fg}	4.71 ^{a-c}	2.85 ^{i-k}	2.12 ^{c-f}	0.87 ^{a-c}	1.81 ^j	2.78 ^{g-i}	3.37 ^{j-n}
T ₁₆ (T+N - Nt)	3.73 ^a	6.07 ^{e-i}	4.84 ^{a-c}	4.16 ^a	2.65 ^{f-j}	1.72 ^{a-c}	0.77 ^a	1.29 ^e	2.16 ^{c-f}	1.92 ^{a-d}
DS 5%	0.82-0.99		0.71-0.86		0.48-0.58		0.13-0.15		0.42-0.50	

Note: The difference between any two values followed by at least one common letter is insignificant.

Legend: V - I8 = Vermiculite + Incit - 8; V - R2 = Vermiculite + Radistim - 2; V - IBA = Vermiculite + IBA 1000 ppm; V - Nt = Vermiculite - without treatment;

P+V - I8 = Peat + vermiculite - Incit - 8; P+V - R2 = Peat + vermiculite - Radistim - 2; P+V - IBA = Peat + vermiculite - IBA 1000 ppm; P+V - Nt = Peat + vermiculite - without treatment; P+P - I8 = Peat + perlite - Incit - 8; P+P - R2 = Peat + perlite - Radistim - 2; P+P - IBA = Peat + perlite - IBA 1000 ppm; P+P - Nt = Peat + perlite - without treatment; P+S - I8 = Peat + sand - Incit - 8; P+S - R2 = Peat + sand - Radistim - 2; P+S - IBA = Peat + sand - IBA 1000 ppm; P+S - Nt = Peat + sand - without treatment.

statistically significant correlations between the morphological characters indicated the lack of influence between the analyzed variables.

In order to determine the influence of some substances on the possibilities of rooting and growing the cuttings of passion fruit, Parse *et al.* (2018), applied three different treatments using IBA, ANA, salicylic acid, ethanol and control. The results of the study showed that there were significant differences between the T₁ treatment (IBA 500 ppm) and the T₂ treatment (IBA 750 ppm) regarding the root length, diameter and weight. However, T₃ treatment (IBA 1000 ppm) was the most effective results. Jin *et al.* (2010) studied the influence of some types of rooting substrates and the type of cutting on rooting for *Passiflora caerulea*. The results obtained from this experiment showed that peat and vermiculite substrates were most effective, and in the perlite the rooting was deficient.

Conclusion

Regarding the rooting rate, the highest percentage of rooting for *Passiflora* species was obtained in cuttings treated with IBA 1000 ppm. The lowest percentage of rooting was obtained in cuttings with no treatment with rooting biostimulator in the peat + sand mixed substrate (1: 1).

Regarding the influence of the rooting substrates, vermiculite showed statistically higher values, compared to other substrates for the root length and their number. Regarding the average length of the main shoot, the average number of shoots/cuttings and the number of leaves, it was found that the rooting substrates did not have a particular influence.

From the interaction of the biostimulator with the rooting substrate, resulted that the untreated cuttings with the biostimulator (T₂, T₄, T₆ and T₈) recorded the lowest values of the length of the shoot, from which it was concluded the importance of using the biostimulator both for facilitating

Figure 4. Correlation matrix with Pearson significance levels, variables distribution on diagonal and graphical representation of the dispersion of each pair of variables in *Passiflora caerulea*

Legend: RL = root length; NoR = number of roots; LS = length of main shoot; NoS = number of shoots / cuttings; NoL = number of leaves

Figure 5. Correlation matrix with Pearson significance levels, variables distribution on diagonal and graphical representation of the dispersion of each pair of variables in *Passiflora quadrangularis*

Legend: RL = root length; NoR = number of roots; LS = length of main shoot; NoS = number of shoots / cuttings; NoL = number of leaves

the rooting, as well as for development of newly formed plants.

In the case of the *P. quadrangularis* species, the recorded data indicate significantly higher values for the number of developed shoots, compared to *P. caerulea*, which indicated a high capacity to shoot development for the species.

Based on the results obtained in this study, it can be concluded that the propagation of *Passifloraceae* by cuttings is the most efficient and it is recommended to use the vermiculite substrate, respectively the peat + vermiculite 1: 1 mixture and the treatment of the cuttings with IBA 1000 ppm.

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