

THE RESPONSE OF SEMI-HARDWOOD CUTTINGS OF 'FORTIVAL' ROOTSTOCK TO THE ACTION OF SOME ROOTING BIOSTIMULATORS

Nicoleta MUTU (SIMA)¹, Crăișor MAZILU², Silvia NICOLAE², Gheorghe ACHIM³,
Mariana IONESCU⁴, Valeriu ZANFIR¹, Sina Niculina COSMULESCU³

¹University of Craiova, Faculty of Horticulture, Doctoral School of Plant and Animal Resources Engineering, 13 A.I. Cuza Street, 200585, Craiova, Romania

²Research Institut for Fruit Growing, Pitești - Mărăcineni, 402 Mărului Street, Argeș County, PO Box Pitești OP-1, CP-73, Cod 110006, Romania

³University of Craiova, Faculty of Horticulture, Department of Horticulture and Food Science, 13 A.I. Cuza Street, 200585, Craiova, Romania

⁴High School Bechet, 33 A.I. Cuza Street, 207060, Bechet, Romania

Corresponding authors emails: sima.nico@yahoo.com; sinacosmulescu@hotmail.com

Abstract

This study was conducted to investigate the response of the plum rootstock 'Fortival' to the action of rooting biostimulators, using semi-hardwood cuttings. The experience was located in a solarium equipped with artificial mist installation. The cuttings were treated with different hormones (IBA, NAA and Radistim no.2) and reported to control. The results show that among the biostimulators applied to 'Fortival' rootstock cuttings, Radistim no. 2 had a superior influence on the development of the root system, compared to both the control and the other used biostimulators, determining the highest rooting percentage, the highest number of roots, the highest average length of roots/cutting, the highest average length of the rooted segment and the largest volume of roots. The F-Test Two-Sample for Variances shows that the variance of the average length of roots/cutting is higher in the case of chemically treated cuttings, regardless of the used biostimulator, which shows the favorable response of 'Fortival' rootstock to the application of rooting biostimulators used in the study.

Key words: plum rootstock, semi-hardwood cuttings, rooting biostimulators.

INTRODUCTION

The discovery of new technologies, simple and modern, through which to obtain large quantities of fruit planting material, in a short time and with low costs, represents a necessity for the development of romanian fruit growing. An effective method of obtaining fruit seedlings is to root the cuttings to obtain uniform and quality rootstocks (Markovski et al., 2015). Green pruning requires a more laborious harvesting and shaping technique, as well as installations to ensure the microclimate needed to root the rootstock (Sumedrea & Sumedrea, 2011). "Green" pruning made in good conditions, succeeds in virtually all fruit species, because rhizogenesis occurs more easily in young tissues, leaves ensuring both a longer trophic autonomy to rooting and hormonal equipment, especially auxins, which migrates basipetally and facilitates rooting (Drăgănescu, 1998). The rootstock improves plant vigor, prolongs the vegetation period (Lee et al., 2010), productivity and fruit quality

(Tsaballa et al., 2013), prolongs fruit quality after harvest (Zhao et al., 2011), increases tolerance to low and high temperatures (Li et al., 2016), reduces stress caused by salinity and heavy metals (Penella et al., 2016), increases flood resistance (Bhatt et al., 2015), manages resistance to soil pathogens (Arwiyanto et al., 2015), manages nematode resistance (Lee et al., 2010), controls weeds and plant propagation (Fuentes et al., 2014). Rootstocks have an important role in modernizing plum culture (Achim et al., 2015). In current literature there are many studies, in different species, that approaches the rooting process, *in vivo*, but also the influence of growth regulators on this process. Botu et al. (2002) undertook a study on new selections of plum rootstocks with low vigor and high propagation capacity and reported a high propagation capacity through cuttings, with variation limits between 64-72% rooted cuttings. Based on these considerations, the aim of the paper is to evaluate the response of semi-hardwood cuttings 'Fortival' rootstock to the action of rooting biostimulators.

MATERIALS AND METHODS

The 'Fortival' rootstock was created by the Vâlcea Research-Development Station for Fruit Growing. The material was harvested from the mother plantation producer of cutting shoots. At the time of harvest, the mother plants were in good condition and did not have significant diseases and pests. The shoots were harvested at the end of June and the experiment was placed at Pitești - Mărăcineni Research-Development Institute, in plastic green houses provided with artificial mist. The cuttings were made from annual branches, the length of the cuttings being 25 cm and 6-7 leaves/cutting were left at the top. The cuttings were planted 0-4 mm with the base in river washed sand substrate, its height being 15 cm. The distance between rows was 8 cm and 5 cm between cuttings per row. The conditions in the plastic green houses with artificial mist were of 80-90% humidity in the air and the temperature of about 30°C. It is important that until root formation, a film of water is permanently maintained on the leaves during the day. After the roots have formed, the watering interval has been increased only to keep the soil moist. The experimental variants were the following: V1 (CT) - without stimulation; V2 - chemical stimulation (Indole-3-butyric acid, IBA 2000 ppm, solution); V3 - chemical stimulation (1-Naphthylacetic acid, NAA 2000 ppm, solution); V4 - chemical stimulation (Radistim no.2, powder). In the NAA and IBA stimulators, the cuttings were kept for 4-5 seconds and in Radistim biostimulator, the cuttings were passed through powder up to 1-2 cm, after having previously been passed through water. For each variant, a number of 100 cuttings were made with a total of 400 rootstocks. At the end of November, the following determinations were made on the rooted cuttings: the number of roots/cutting; root length/cutting (cm); length of the rooted segment/cutting (cm); root volume (cm³), number of anticipated shoots and length of anticipated shoots (cm). The data was statistically processed using Microsoft Excel, Data Analysis and F-Test Two-Sample for Variances to test the variance of the average root length/ cutting depending on the applied biostimulator compared to the control. In order

to achieve the distribution of cuttings according to the number of roots and the average length of roots/cutting for each biostimulator, the histogram was used and in order to highlight the connection between different characteristics of the root system and different growth characteristics, correlations were used.

RESULTS AND DISCUSSIONS

The use of rootstocks is an important tool used by horticulturists to improve crop adaptation to certain stresses, especially those related to ecological plasticity (Mondragón-Valero et al., 2017). Rootstocks can change the ecological adaptability of varieties, so an accurate characterization of them is essential to obtain high quality crops (Mondragón-Valero et al., 2019). Table 1 shows the characteristics of the root system of the 'Fortival' rootstock, depending on the biostimulator applied exogenous on the cuttings. The highest percentage of rooted cuttings (98%) was obtained when applying Radistim no. 2. By applying NAA 2000 ppm a rooting percentage of 77% was obtained and by applying IBA 2000 ppm a rooting percentage of 64% was obtained. Control cuttings, not chemically stimulated, recorded a rooting percentage of 90%, which may explain the dependence of the rooting capacity on the genotype and the quality of the propagating material. Johnson et al. (2020) claim that in *Prunus* the rooting capacity is genotype dependent. Also Mutu et al. (2020) support the influence of genotype on rooting capacity. Ancu et al. (2008) showed that the percentage of rooting of herbaceous cuttings treated with Radistim 2, varied between 82.83% and 95.96% in the analyzed plum rootstock selections. Markovski et al. (2015) reported a rooting rate of rootstocks for plum and peach of 31.3%. Edizer & Demirel (2012) reported a rooting percentage of herbaceous cuttings treated with 3000 ppm IBA of 90.00% at 'St. Julien', 'Marianna GF 8-1' and 'SL-64' rootstocks. On the other hand, 86.67% rooting percentage was obtained at the 'Garnem' clonal rootstock at 4000 ppm IBA concentration. Markovski et al. (2015) recorded at 'St. Julian Orleans' cuttings a negative influence of auxin treatments on the rooting percentage. Regarding the number of roots/

cutting, the highest average value was also obtained for cuttings stimulated with Radistim no. 2 (21.41). Stimulation of cuttings with NAA 2000 ppm determined a number of 11.17 roots/cutting and stimulation of cuttings with IBA 2000 ppm determined a number of 9.69 roots/cutting. Chemically unstimulated cuttings showed the lowest number of roots/cutting, respectively 9.14, which shows that biostimulators determine the development of rootstocks root system. The highest average number of roots/cutting obtained by Markovski et al. (2015) was 13.2 cm and the influence of IBA auxin 2% was crucial in the formation of large number of roots, respectively 27.3 cm while auxin NAA did not have a positive influence on root formation compared to the control. Also, the best results regarding the average length of roots/cutting (6.16 cm) were obtained for cuttings treated with Radistim no. 2, followed by cuttings treated with NAA 2000 ppm (6 cm). The lowest average root length/cutting (5.62 cm) was obtained for cuttings treated with IBA 2000 ppm while chemically untreated cuttings recorded an average root length/cutting even lower, 5.20 cm. The results obtained by Szecskó et al. (2006) showed a relatively weak link between rooting and physiological factors. Rooting biostimulators have also led to an increase in the rooted segment. Its average value varied depending on the biostimulator. For cuttings treated with Radistim no. 2, the average length of the rooted segment was 2.13 cm, for those treated with NAA 2000 ppm it was 1.48 cm, for those treated with IBA 2000 ppm it was 1.18 cm and for those not chemically treated 1.18 cm. Also, the largest volume of roots/cutting (4.26 cm³) was obtained for cuttings treated with Radistim no. 2. It is found that among the biostimulators applied to the cuttings of 'Fortival' rootstock, Radistim no. 2 had a superior influence on the development of the root system, compared to both the control and the other used biostimulators. Johnson et al. (2020) analyzed the influence of K-IBA (potassium salt of IBA) on herbaceous cuttings on *Prunus* genotypes; in plum cuttings the highest number of roots was obtained at 2000-4000 mg/L K-IBA, and rooting was different in *Prunus* depending on the genotype. Shoots and roots are autotrophic and heterotrophic organs

of plants with different physiological functions, with different metabolism, which respond differently to environmental changes. Plants have complex regulatory mechanisms that coordinate physiological activity, growth and development (Hibberd & Quick, 2002). Based on this idea, the percentage of cuttings that showed early shoots, the number of early shoots/cutting and the average length of early shoots/cutting were analyzed and the results are shown in Table 2. It can be seen that the cuttings that recorded the highest number of roots, the largest average length of the root/cutting, the largest average length of the rooted segment and the largest volume of roots/cutting, meaning those stimulated with Radistim no. 2, recorded the lowest percentage of anticipated shoots (56.12%). The explanation lies in the fact that nutrients are allocated differently to optimize the efficiency of their use. Plants under different available resources allocate differently the available resources for shoots and roots to optimize the efficiency of their use. The results obtained by Gargallo-Garriga et al. (2014) provide clear evidence that plants have a high capacity to modulate and vary the nutrient allocation and relative activities of different metabolic pathways for biomass production in both shoots and roots. Table 3 shows the correlations established between the characteristics of the root system and the anticipated shoots depending on the used biostimulator. Significantly positive correlations were established between the length of the rooted segment and the number of roots/cutting, regardless of the applied biostimulator to the cuttings ($r = 0.65$ for cuttings stimulated with Radistim no. 2; $r = 0.50$ for cuttings stimulated with IBA and $r = 0.31$ for cuttings stimulated with NAA). Also, significant positive correlations were established between the volume of roots and the number of roots/cutting ($r = 0.82$; $r = 0.66$; $r = 0.48$), between the volume of roots and the length of the rooted segment, between the number of roots/cutting and their average length. Negative correlations were established between the number of anticipated shoots and the average length of the root/cutting, between the average number of roots/cutting and the length of the rooted segment. These can be explained by the fact

that the anticipated shoots negatively influence the development of the root system, using the nutrients needed by the rootstock for rooting. To test the variance of the average length of roots/cutting according to the applied biostimulator compared to the control, the F-Test Two-Sample for Variances test was applied and the results are shown in Table 4. It can be seen that in the case of all three biostimulators applied to cuttings compared to control cuttings, not chemically stimulated, the statistical value of F is higher than Fcrit, so the null hypothesis that the two variables, meaning chemically treated and untreated cuttings, would have equal variances, is rejected, so the variances of the two analyzed areas are uneven, which shows that there are variations in the average length of the roots/cutting depending on the biostimulator applied, compared to the

control cuttings. It can also be seen that the variance of the average length of roots/cutting is higher in the case of cuttings treated with biostimulators compared to those not chemically treated. The P value is higher than the specified alpha level of 0.05, so the probability of obtaining an F greater than F critical is between 0.18 and 0.38, which strengthens the rejection of the null hypothesis that the two variables would be equal. In conclusion, the variance of the average root length/cutting is higher in the case of chemically treated cuttings, regardless of the used biostimulator, which shows the favorable response of semi-hardwood cuttings 'Fortival' rootstock to the application of rooting biostimulators used in the study.

Table 1. Characteristics of the root system of 'Fortival' rootstock depending on the growth stimulator

Characteristics	Descriptive statistics	Radistim	IBA	NAA	Control
Rooting	%	98%	64%	77%	90%
No. of roots/cutting	Mean±SD	21,41±11.33	9.69±4.47	11.17±5.85	9.14±6.41
	CV%	52.94	46.20	52.36	70.06
Average root length/cutting (cm)	Mean±SD	6.16±1.99	5.62±1.92	6.00±2.08	5.20±2.15
	CV%	32.30	34.25	34.57	41.33
Length of rooted segment (cm)	Mean±SD	2.13±1.35	1.19±0.99	1.48±1.02	1.18±1.21
	CV%	62.97	82.97	69.03	103.24
Roots volume/cutting (cm ³)	Mean±SD	4.26±2.96	1.31±0.77	2.06±1.66	1.61±1.24
	CV%	69.76	58.98	80.25	77.11

Table 2. Characteristics of the anticipated shoots of 'Fortival' rootstock depending on the growth stimulator

Characteristics	Descriptive statistics	Radistim	IBA	NAA	Control
Cuttings with anticipated shoots	%	56.12%	67.19	74.02	64.44%
Number of anticipated shoots	Mean ±SD	2.15±1.13	1.77±0.87	2.67±1.33	2.38±1.82
	CV%	52.63	49.13	49.87	49.69
Average length of anticipated shoots/cutting (cm)	Mean±SD	8.72±5.39	6.15±4.69	5.80±4.00	6.48±4.31
	CV%	61.76	76.16	69.03	66.57

Table 3. Correlations between the growth characteristics of 'Fortival' rootstock cuttings depending on the chemical stimulator

	L				N				LS				V				I			
	RAD	NAA	IBA	CT	RAD	NAA	IBA	CT	RAD	NAA	IBA	CT	RAD	NAA	IBA	CT	RAD	NAA	IBA	CT
L	1	1	1	1																
N	0.30	0.43	0.32	0.29	1	1	1	1												
LS	0.22	0.19	0.26	0.28	0.65	0.31	0.50	0.47	1	1	1	1								
V	0.47	0.37	0.23	0.42	0.82	0.66	0.48	0.78	0.61	0.38	0.36	0.49	1	1	1	1				
I	-0.17	-0.08	0.13	-0.15	0.19	0.14	0.08	0.03	0.13	0.11	0.40	0.01	0.16	0.05	-0.01	-0.01	1	1	1	1
n*	0.27	0.09	-0.03	-0.12	-0.02	0.00	-0.02	-0.03	0.16	-0.15	-0.19	-0.27	0.10	0.16	0.03	-0.09	-0.37	-0.25	-0.28	-0.33

L= Average root length/cutting (cm); N= Number of roots/cutting; LS= Length of the rooted segment (cm); V= Roots volume/cutting; I= Average length of the anticipated shoots/cutting; CT= control

Table 4. Variation of the average length of roots/cutting depending on the chemical stimulator (F-Test Two-Sample for Variances) (cm)

	Radistim 2	Control	NAA	Control	IBA	Control
	<i>Variable 1</i>	<i>Variable 2</i>	<i>Variable 1</i>	<i>Variable 2</i>	<i>Variable 1</i>	<i>Variable 2</i>
Mean	6.16	5.20	6.00	5.20	5.62	5.20
Variance	3.99	4.61	4.31	4.61	3.71	4.61
Observations	98	90	77	90	64	90
df	97	89	76	89	63	89
F	0.87		0.93		0.80	
P(F<=f) one-tail	0.24		0.38		0.18	
F Critical one-tail	0.71		0.69		0.68	

CONCLUSIONS

It was found that among the biostimulators applied to the cuttings of 'Fortival' rootstock, Radistim no. 2 had a superior influence on the development of the root system, compared to both the control and the other biostimulators used, determining the highest percentage of rooting, the highest number of roots, the highest average length of roots/cutting, the highest average length of the rooted segment and the largest volume of roots.

ACKNOWLEDGEMENTS

This research work was carried out with the support of Pitești - Mărăcineni Research-Development Institute.

REFERENCES

Achim, G., Botu, I., Botu, M., Duțu, I., Stănică, F., & Mazilu, C. (2015). The rootstock's role in modernizing plum culture. *Acta Horticulturae*, 1175, 49-54.

Ancu, S., Dutu, I., Mazilu, C., & Plopa, C. (2008). Promising selections for new vegetative rootstocks. *Bulletin UASVM*, 65, 1.

Arwiyanto, T., Lwin, K., Maryudani, Y., & Purwatoro, A. (2015). Evaluation of local *Solanum torvum* as a rootstock to control of *Ralstonia solanacearum* in Indonesia. *Acta Horticulturae*, 1086, 101–106.

Bhatt, R. M., Upreti, K. K., Divya, M. H., Bhat, S., Pavithra, C. B., & Sadashiva, A. T. (2015). Interspecific grafting to enhance physiological resilience to flooding stress in tomato (*Solanum lycopersicum* L.). *Scientia Horticulturae*, 182, 8-17.

Botu, I., Turcu, E., & Botu, M. (2002). New plum rootstock selections with low vigor and high capacity of propagation. *Acta Horticulturae*, 658, 441-447.

Drăgănescu E., 1998. *Pomicultură*. Ed. Mirton, Timișoara.

Edizer, Y., & Demirel, M. A. (2012). A study on the some characteristics of rooting of green cuttings of the some clonal rootstock in mist propagation. *Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi*, 29(2), 1-8.

Fuentes, I., Stegemann, S., Goleczyk, H., Karcher, D., & Bock, R. (2014). Horizontal genome transfer as an asexual path to the formation of new species. *Nature*, 511(7508), 232-235.

Gargallo-Garriga, A., Sardans, J., Pérez-Trujillo, M., Rivas-Ubach, A., Oravec, M., Vecerova, K., & Penuelas, J. (2014). Opposite metabolic responses of shoots and roots to drought. *Scientific reports*, 4(1), 1-7.

Hibberd, J. M. & Quick, W. P. (2002). Characteristics of C4 photosynthesis in stems and petioles of C3 flowering plants. *Nature* 415, 451–4

Johnson, E. P., Preece, J. E., Aradhya, M., & Gradziel, T. (2020). Rooting response of *Prunus* wild relative semi-hardwood cuttings to indole-3-butyric acid potassium salt (KIBA). *Scientia Horticulturae*, 263, 109-144.

Lee, J. M., Kubota, C., Tsao, S. J., Bie, Z., Echevarria, P. H., Morra, L., & Oda, M. (2010). Current status of vegetable grafting: Diffusion, grafting techniques, automation. *Scientia Horticulturae*, 127(2), 93-105.

Li, H., Wang, Y., Wang, Z., Guo, X., Wang, F., Xia, X. J., & Zhou, Y. H. (2016). Microarray and genetic analysis reveals that csa-miR159b plays a critical role in abscisic acid-mediated heat tolerance in grafted cucumber plants. *Plant, Cell & Environment*, 39(8), 1790-1804.

Markovski, A., Popovska, M., & Gjamovski, V. (2015). Investigation of the possibility for production of some stone fruit rootstocks by rooting cuttings. *Acta Agriculturae Serbica*, 20(39), 75-831.

Mondragón-Valero, A., López-Cortés, I., Salazar, D. M., & de Córdova, P. F. (2017). Physical mechanisms produced in the development of nursery almond trees (*Prunus dulcis* Miller) as a response to the plant adaptation to different substrates. *Rhizosphere*, 3, 44-49.

Mondragón-Valero, A., Malheiro, R., Salazar Hernández, D. M., Pereira, J. A., & López-Cortés, I. (2019). Changes produced by the application of biostimulants on almond rootstocks properties during

- the nursery process. *Advances in Agriculture & Botany*, 11(1), 56-71.
- Mutu, N., Ionescu, M., & Cosmulescu, S. (2020). The influence of certain hormones on the callusing ability in some rootstocks for plums. *Annals of the University of Craiova*, XXV (LXI), 112-117.
- Penella, C., Landi, M., Guidi, L., Nebauer, S. G., Pellegrini, E., San Bautista, A., & Calatayud, A. (2016). Salt-tolerant rootstock increases yield of pepper under salinity through maintenance of photosynthetic performance and sinks strength. *Journal of Plant Physiology*, 193, 1-11.
- Sumedrea D., Sumedrea M. (2011). *Pomicultura generală*, Editura INVEL Multimedia București.
- Szecsó, V., Hrotkó, K., & Stefanovits-Bányai, É. (2006). Physiological factors influencing the rooting of plum rootstocks' hardwood cuttings. *Agronomijas Vestis*, (9), 156-161.
- Tsaballa, A., Athanasiadis, C., Pasentsis, K., Ganopoulos, I., Nianiou-Obeidat, I., & Tsaftaris, A. (2013). Molecular studies of inheritable grafting induced changes in pepper (*Capsicum annuum*) fruit shape. *Scientia Horticulturae*, 149, 2-8.
- Zhao, X., Guo, Y., Huber, D. J., & Lee, J. (2011). Grafting effects on postharvest ripening and quality of 1-methylcyclopropene-treated muskmelon fruit. *Scientia Horticulturae*, 130(3), 581-587.