ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 67 42 Number 2, 2019

THE USE OF PHYTOHORMONES IN PRODUCTION OF FRUIT TREE ROOTSTOCKS IN NURSERY WITHOUT IRRIGATION

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To link to this article: https://doi.org/10.11118/actaun201967020463
Received: 5. 9. 2018, Accepted: 25. 1. 2019

To cite this article: WOLF JAN, KISS TOMÁŠ, VENUTA ROSTISLAV, NEČAS TOMÁŠ. 2019. The Use of Phytohormones in Production of Fruit Tree Rootstocks in Nursery Without Irrigation. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis*, 67(2): 463–470.

Abstract

The most commonly used phytohormone to reduce the impact of drought is 24-epibrassinolide (EBR). In this study, EBR was applied over two years on young rootstocks of peach (B-VA-1) and myrobalan (Vanovice myrobalan) seedlings. Young rootstocks were treated by EBR at different concentrations, IAA and a mixture of EBR with IAA. The best results in seedling height of Vanovice myrobalan in both years had the treatment of 0.05% IAA, 549.69 mm (2016) and 553.77 mm (2017). On the other hand, on the crown root thickness was affected the most in 2016 by treatment of EBR 0.06 ppm (5.5 mm) and in 2017 by EBR 0.01 ppm (7.5mm). At B-VA-1 rootstock, the highest seedlings in 2016 were measured after EBR + IAA treatment (1573.18 mm) and in 2017 after IAA treatment (682.75 mm). The highest thickness of the crown root was recorded in 2016 after EBR and IAA (17.76 mm) and in 2017 after EBR 0.1 ppm (8.08 mm) treatments. At more than half of the treatments the control variant was evaluated as a variant with the least satisfying results. According to the results EBR and IAA are possibly increasing the quality of the rootstocks in conditions of a nursery without an irrigation.

Keywords: *Prunus persica, Prunus cerasifera*, fruit nursery, brassinosteroids, drought, 24-epibrassinolide, IAA

INTRODUCTION

Brassinosteroids (BRs) are a class of plant polyhydroxysteroids that are ubiquitously distributed in the plant kingdom (Müssig and Altmann, 1999). Their structure is similar to that of steroid hormones in mammals and arthropods (Clouse and Sasse, 1998). Brassinosteroids have been found in almost all parts of a plant – pollen, flower buds, fruits, seeds, vascular bundles of vascular cambium, leaves, shoots and roots (Bajguz and Tretyn, 2003). Plants need BRs to ensure normal development. Some studies carried out on higher plants describe that BRs play a key role in a wide range of growth processes, e.g. growth of trunk and roots, initiation of flowering, development of fruits and flowers, differentiation of xylem or regulation of gene expression (Hayat and Ahmad, 2003; Sasse, 2003; Mandava 1988; Clouse and Sasse, 1998). Many studies note that BRs increase plants' capacity to endure stress conditions like heavy metal stress, water stress, salt stress, high and low temperature stress and pathogen attacks (Ali *et al.*, 2008; Bajguz and Hayat, 2009; Hayat *et al.*, 2010).

In the past century considerable attention was paid to characteristics of endogenous BRs. Their physiological activity was proven to be effective in even very low concentrations (Yokota and Mori, 1992; Khripach *et al.*, 1993). Pustovoitova *et al.* (2001) studied the effect of a synthetic BRs, namely 24-epibrassinolide (EBR), on plants of *Cucumber sativus* L. when applied in a water solution. Their study indicates that plants treated with EBR showed an increased resistance to dehydration. Also, higher survival rate was demonstrated in seedlings of *Arabidopsis thaliana* (L.) Heynh. and *Brassica napus* L. that were treated with EBR and subjected to drought afterwards (Krishna, 2003; Kagale *et al.*, 2007). Farooq *et al.* (2009) applied EBR to seeds and leaves of young plants of *Oryza sativa* L. The plants treated with EBR showed better resistance to drought thanks to their better leaf water economy and CO_2 assimilation as compared to plants cultivated from EBR treated seeds. Li *et al.* (2012) revealed in their work that young plants (Chorispora bungeana Fisch. *et* C. A. Mey.) treated with EBR grow better under water deficiency. The conclusion was supported by an analysis of metabolite content in the treated and untreated plants. Hnilička *et al.* (2007) recorded a lower negative effect of water stress after treatment with EBR in all tested varieties of *Triticum* L., although the effect differed in degree in each variety. Application of EBR to *Solanum lycopersicum* L. plants lead to a higher content of water and abscisic acid in leaves and the EBR treated plants displayed increased resistance to drought (Yuan *et al.*, 2010). The abscisic acid (ABA) belongs among terpenoid phytohormones. It contributes to many aspects of plant growth and development, such as plant dormancy, leaf fall and development of seeds. ABA is also a key hormone that confers tolerance to environmental stresses, mainly to drought and salinity (Wasilewska *et al.*, 2008). EBR applied to *Capsicum annuum* L. plants mitigated significantly inhibition of photosynthesis evoked by drought (Hu *et al.*, 2013), which indicates that EBR reduces the negative effects of drought on plants.

Some reports declare that apart from BRs, there are also other phytohormones that help the plants to adapt to drought, e.g. auxins. Xie *et al.* (2003) state that water deficiency leads to a decreased content of indole-3-acetic acid (IAA) in the leaves of *Triticum* L. Veselov *et al.* (2008) point out that exogenous IAA may stimulate leaf growth in corn during droughts. It is highly probable that IAA had a positive effect on the growth of *Triticum aestivum* L. plants under water stress, yet its effect on the resistance to drought itself could not be proved (Zhao *et al.*, 2012).

These findings led us to the decision to employ 24-epibrassinolide and indole-3-acetic acid in nursery production, namely during the final phase of rootstock cultivation. In the area of southern Moravia and the Czech Republic generally, rainfall has been moving into dormancy period in the last few years. In the worst cases the spring and summer rainfall has been so low that fruit tree nurseries are forced to irrigate their plants. Not all of them have the chance to do so however and that is where the application of EBR comes in, especially because it facilitates better water management in plants. The aim of this study was to evaluate an application of EBR and IAA on increase of the quality of generatively propagated rootstocks in a nursery without irrigation.

MATERIAL AND METHODS

Attributes of the location

The experiment was established in a fruit tree nursery Venuta, located in the municipality of Kadov (277 meters above sea level) in southern Moravia. The area of the Venuta nursery is 12.5 ha, the soil is medium heavy of a clay-sand to clay type. The nursery is permanent, cultivated plants are rotated in small portions of the ground. There is no irrigation system, as there is no water source nearby. Wheat and oilseed rape is being used as precursor crops. Meteorological data are given in Tab. I.

Attributes of the plant material

Young seedlings of Vanovice myrobalan (*Prunus cerasifera* Ehrh.) and peach seedling (*Prunus persica*

I: *Meteorological data from a meteorological station in the municipality of Bohutice (5 km from Kadov).*

Recorded data	2016	2017
Annual rainfall	518.8 mm	309.6 mm
Overall rainfall in the growth period (01.04. to 31.09.)	324.5 mm	203.2 mm
Annual average temperature	10.6°	10.8°
Minimum temperature	−11.2°	−13.7°
Maximum temperature	36.2°	37.8°

(L) Batsch) B-VA-1 were used as test material. The Vanovice myrobalan seeds are produced by the Venuta nursery itself, their purity is 98% and the germinability 99%. The B-VA-1 peach seedlings were purchased from SEVA-FLORA s.r.o. Valtice, a plant breeding company, their purity being 97% and germinability 95%. The seeds are sown in autumn, in the course of October.

Preparation and application

24-epibrassinolide (EBR) (Sigma-Aldrich) was dissolved in a 70% ethanol while constantly stirring and heating. The stock solution was then used to prepare final solutions with concentrations of 0.01 ppm, 0.06 ppm and 0.1 ppm.

Indole-3-acetic acid (IAA) (Duchefa Biochemie B. V., Netherlands) was dissolved in a 70% ethanol while constantly stirring. The stock solution was then used to prepare a final solution with a concentration of 0.05%.

The final EBR and IAA solutions were prepared from the stock solution by mixing it with demineralized H_2O. Wetting agent Tween®20 (0.5 ml/l) was added to the final solution. The solutions were then first applied to young seedlings of a height of approximately 15 cm. The application was carried out using a manual sprayer in a windless weather in three subsequent dates (in the year 2016: 25.5., 8.6. and 22.6.; in the year 2017: 23.5., 8.6. and 23.6.) with a time interval of 14 days between the applications. Each spraying variant was applied to three randomly selected blocks of 100 plants in such way that the blocks would not adjoin. Each block was marked carefully to avoid confusion.

During the vegetation period merely hoeing was carried out in the area with the treated and untreated plants.

Evaluation

At the end of the vegetation period (end of October) the myrobalan and peach seedling plants were taken out of ground and tied into bundles. From the total of 100 plants 60 were randomly selected from the middle of the block as a prevention of accidental influence of another spraying variant adjoining the block. At selected plants 3 parameters were evaluated: their total height, crown root thickness and number of feathers were measured by caliper and meter.

Obtained data were subjected to statistical analyses in program STATISTICA 12 using a single and multi-factor analysis of variance (ANOVA). Subsequently, Duncan's test was used to calculate differences ($p = 0.05$) between the treatments. Correlation coefficient was calculated between selected parameters.

RESULTS

Number of feathers

In 2016 at Vanovice myrobalan rootstocks, the number of feathers at variants ranged from 3.61 (EBR + IAA) to 6.93 (IAA). Only IAA (6.93) treatment showed significantly higher number of feathers than other variants including control. In 2017, number of feathers ranged from 3.71 (control) to 4.12 (EBR + IAA, EBR 0.06) with no significant differences between variants (see Tab. II).

At B-VA-1, the average number of feathers ranged in 2016 from 11.22 (IAA) to 23.21 (EBR + IAA). Only EBR + IAA variant reached significantly higher feather values than the control. Significantly lower numbers of feathers compared to control were found in the IAA, EBR 0.01 and EBR 0.06 variants. In 2017, the number of feathers ranged from 3.15 (EBR 0.01) to 5.36 (EBR 0.1). Higher numbers of feathers than at the control variant were evaluated at all variants except of EBR 0.01. At EBR 0.1 and EBR 0.06 (Tab. II) the values were significantly higher compared to the control.

Crown root diameter

In 2016, the crown root diameters of Vanovice myrobalan ranged from 4.3 mm (EBR 0.1) to 5.5 mm (EBR 0.06). Variants IAA, EBR 0.06 and EBR + IAA had higher crown root diameter than the control where only EBR 0.06 reached significantly higher values compared to control. The EBR 0.1 variant was the only variant reaching significantly lower values of crown root diameter compared to control. In 2017, crown root diameter ranged from 5.78 mm (control) to 7.5 mm (EBR 0.01). All variants had higher values than control, and all except of EBR + IAA had these values significantly higher than of the control variant (Fig. 1 and Tab. II).

At B-VA-1 rootstocks in 2016 the average crown root diameter ranged from 11.36 mm (IAA) to 17.94 mm (control). Variants IAA, EBR 0.01, EBR 0.1 and EBR 0.06 had significantly lower values of crown root diameters than the control. In 2017, crown root diameter values ranged from 4.8 mm (EBR + IAA) to 8.08 (EBR 0.1). Variants IAA, EBR 0.06 and EBR 0.1 had significantly higher values of crown root diameter than control, whereas variants EBR + IAA and EBR 0.01 had these values significantly lower compared to the control (Fig. 1 and Tab. II).

Total height

At Vanovice myrobalan in 2016 the average height ranged from 443.73 mm (EBR 0.1) to 549.64 mm (IAA). No significant differences were observed between the variants. Similar values were measured also in 2017, where the average height ranged from 450.16 mm (control) to 553.77 mm (IAA). However, this year all variants had significantly higher values of total height than control (Fig. 2 and Tab. II).

In 2016 B-VA-1 rootstocks reached height ranging from 905.11 mm (IAA) to 1573.18 mm (EBR + IAA). Compared to control, higher values, however not significantly, were recorded at EBR + IAA treated plants. Significantly lower values than of the control were measured only at IAA and EBR 0.01 variants, which also significantly differed between each other. In 2017, height values ranged from 398.44 mm (EBR 0.01) to 682.75 mm (IAA). Significantly higher plants were measured in EBR 0.06, EBR 0.1 and IAA variants compared to the control. On the other hand, EBR 0.01 and EBR + IAA variants had significantly lower heights than control (Fig. 2 and Tab. II).

Correlation coefficient

Based on the data obtained, we created a correlation matrix. The matrix was used to calculate a correlation coefficient between the total height and the crown root thickness. For Vanovice myrobalan the correlation coefficient was calculated to be r = 0.86476 in 2016 and r = 0.60231 in 2017. For the B-VA-1 rootstock the correlation coefficient was r = 0.67849 in 2016 and r = 0.77792 in 2017 (see Fig. 3).

DISCUSSION

The requirements nurseries must fulfil nowadays are very demanding. Nursery trees must exhibit a distinct and well-branched crown, sufficiently thick trunk and a good and prolific root system (Sazo and Robinson, 2011). In nursery trees it is the rootstock what forms the root system. The rootstock itself affects both the growth characteristic of the grafted variety and the quality of fruits (Al-Hinai and Roper, 2004). Just as the growers demand high quality nursery trees, the nurseries demand high quality rootstocks. The rootstocks must be sufficiently branched and prolific in the root system and must have a sufficient crown root thickness.

Drought is one of the main abiotic factors influencing negatively production of foodstuff. Severe water shortage is probably the most detrimental environmental problem predicted fort the 21st century (Yuan *et al.*, 2010). In last years the summer months have been significantly warmer and rainfall distribution uneven, especially in the south of Moravia. The results we present here indicate that exploitation of phytohormones, especially EBR, for production

II: *Average measured data with ± SE for the studied characteristics of the rootstocks in 2016 and 2017.*

Myrobalan	Number of shoots		Crown root thickness (mm)		Total height (mm)	
	2016	2017	2016	2017	2016	2017
EBR + IAA 0.06ppm + 0.05%	3.61 ± 0.39a	4.12 ± 0.33a	5.12 ± 0.25bc	6.49 ± 0.21ab	456.11 ± 19.6a	516.75 ± 16.4b
EBR 0.1ppm	3.94 ± 0.42a	4.11 ± 0.33a	4.3 ± 0.25a	7.11 ± 0.21bc	443.73 ± 19.6a	533.83 ± 16.4b
EBR 0.06ppm	3.75 ± 0.42a	4.12 ± 0.32a	5.5 ± 0.25c	7.27 ±0.21bc	540.15 ± 19.6b	540.5 ± 16.4b
EBR 0.01ppm	4.26 ± 0.45a	3.94 ± 0.32a	4.84 ± 0.25b	7.5 ± 0.21c	515.87 19.6b	541.22 ± 16.4b
IAA 0.05%	6.93 ± 0.48b	3.93 ± 0.31a	5.12 ±0.3bc	6.88 ± 0.21bc	549.64 ± 24b	553.77 ± 16.4b
Control	4.13 ± 0.35a	3.71 ±0.6a	5.11 ± 0.22bc	5.78 ± 0.36a	493.92 ± 17ab	450.16 ± 28.4a
B-VA-1						
EBR + IAA 0.06ppm + 0.05%	23.21 ± 0.65d	3.97 ± 0.62ab	17.76 ± 0.33cd	4.80 ± 0.21a	1573.18 ± 21.05d	423.83 ± 13.56a
EBR 0.1ppm	19.46 ±0.6c	5.36 ± 0.42d	16.59 ± 0.3b	8.08 ± 0.2d	1508.98 ± 18.24.	671.89 ± 13.56a
EBR 0.06ppm	16.70 ± 0.59b	5 ± 0.41cd	16.72 ± 0.3bc	7.86 ± 0.2d	1468.27 ± 19c	656.5 ± 12.74c
EBR 0.01ppm	16.16 ± 0.51b	3.15 ± 0.55a	16.01 ± 0.26b	4.95 ± 0.2a	1404.95 ± 16.53b	398.44 ± 12.74a
IAA 0.05%	11.22 ± 0.58a	4.28 ± 0.45bc	11.36 ± 0.29a	7.27 ± 0.21c	905.11 ± 18.66a	682.75 ± 13.23c
Control	20.15 ± 0.62c	3.79 ± 0.79ab	17.94 ± 0.32d	6.46 ± 0.35b	1522.08 ± 20.15cd	497.66 ± 22.07b

in fruit tree nurseries may reduce the impact of drought on the growth and quality of the cultivated rootstocks.

The positive effect on growth and resistance to drought was obvious for both tested rootstocks in year 2017. This can be attributed to the extremely dry weather occurring during the vegetation months. The results we obtained lead us to the conclusion that EBR affect plants positively mainly in the period with lack of precipitation. The difference between annual precipitation in 2016 and 2017 is shown in Tab. I, where in 2017 precipitation during vegetation period and annual precipitation were by 111 mm and 209 mm, respectively, lower than in 2016.

The number of feathers does not constitute an important parameter for nurseries nor for growers, because in the year following the budding all plant mass above the bud is removed in early spring. Yet, the number of feathers reflects the amount of biomass. Branching was the most abundant in rootstock B-VA-1 in 2016, with the variants treated with EBR and IAA having 23 branches. The variant treated with IAA 0.05%, on the other hand, grew only 11 branches, with the control having 20 branches. Still, the data must be correlated to the total height. The IAA belongs to auxins that support apical dominance. This is evident from the obtained results where, in most variants the IAA prolonged growth and, in most cases, inhibited the lateral branches of the rootstocks.

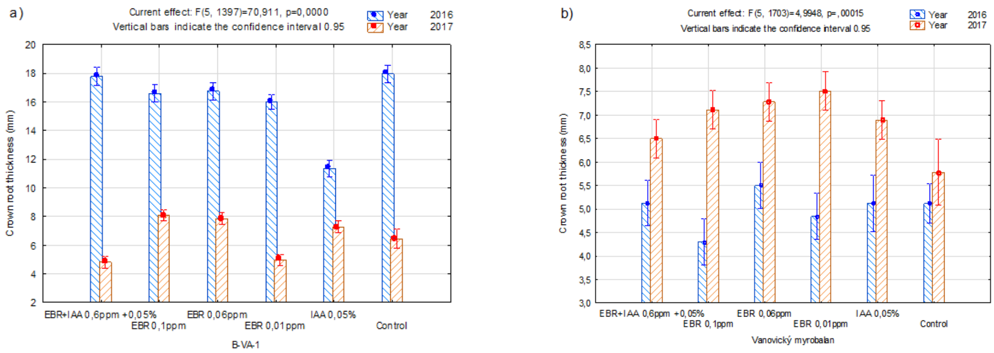
Crown root thickness is a very important parameter in rootstocks. If a rootstock's diameter in crown root is below 7–8 mm, the rootstock is not suitable for budding in its first year and must remain in the nursery till the next year. In 2016, the B-VA-1 rootstock reached a diameter larger than 16 mm in all variants with the exception of the variant treated with IAA 0.05%, where the measured crown root diameter was 11 mm. Yet even this value is sufficient. In 2017, only the variants treated with EBR 0.1 ppm, EBR 0.06 ppm and IAA 0.05% reached values higher than 7 mm. For Vanovice myrobalan rootstock none of the variants achieved the required value of 7 mm in the crown root in 2016. In 2017, the variants treated with EBR 0.1 ppm, EBR 0.06 ppm and EBR 0.01 ppm exceeded the thickness of 7 mm. The results we obtained show that the effect of EBR is different for each rootstock and it even differs in the individual years.

Total height of rootstock is not really significant for nurseries, but it signals the amount of biomass just as the feathers do. With B-VA-1 rootstock all the variants reached more than 1,400 mm of total height in 2016 with the exception of the variant treated with IAA 0.05% (905 mm). In 2017, only in variants treated with EBR 0.1 ppm, EBR 0.06 ppm and IAA 0.05% reached the height exceeded 500 mm (497 mm for the control). In 2016, Vanovice myrobalan grew up to 500 mm and higher only in the case of the variants treated with EBR 0.1 ppm, EBR 0.06 ppm and IAA 0.05% (493 mm for the control). In 2017, all the treated variants exceeded the height of 500 mm, only the control did not (450 mm). Yet, with Vanovice myrobalan rootstock the differences recorded in 2016 and in 2017 were not as considerable as with rootstock B-VA-1.

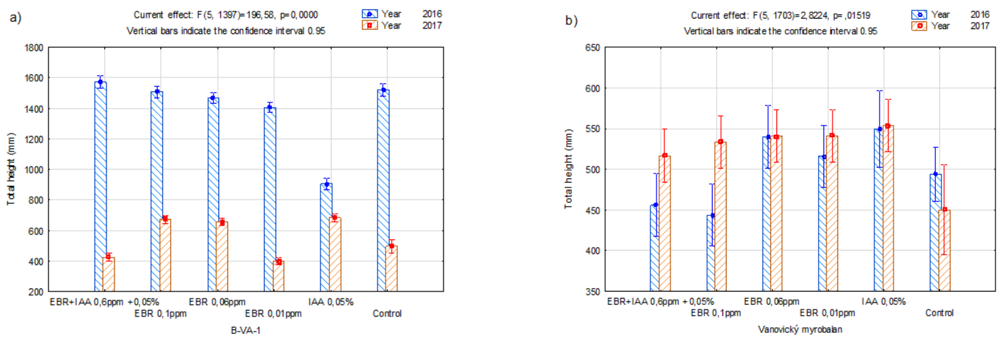
IAA (native auxin) positively affects prolongation growth and strengthens apical dominance (Teale *et al*., 2006). This fact is apparent on the total height of Vanovice myrobalan, where in both years the variants treated with 0.05 % IAA were the highest, in 2016 549.64 mm and 553.77 mm in 2017. Similarly, it was in 2017 for the B-VA-1 rootstock where the variant treated with IAA reached 682.75 mm. Increased prolongation growth after IAA treatment has been reported in Zhao *et al*. (2012). However, enhancement of growth prolongation resulted in a decrease in stem thickness. This effect is most apparent in the Vanovice myrobalan rootstock in 2017, where the crown root thickness values of all variants treated with EBR exceeded the value measured in the variant treated by the IAA.

24-epibrassinolide did not support prolongation growth as IAA, but positively affected the thickness of the crown root. The combination of IAA and EBR would be ideal in such concentrations, which could positively affect both properties. However, the combination of EBR and IAA (0.06 ppm and 0.05%) did not prove to be very effective. It had a very different effect on both types of rootstocks and in individual years. Therefore, it would be advisable to test multiple concentrations of both phytohormones in mixtures.

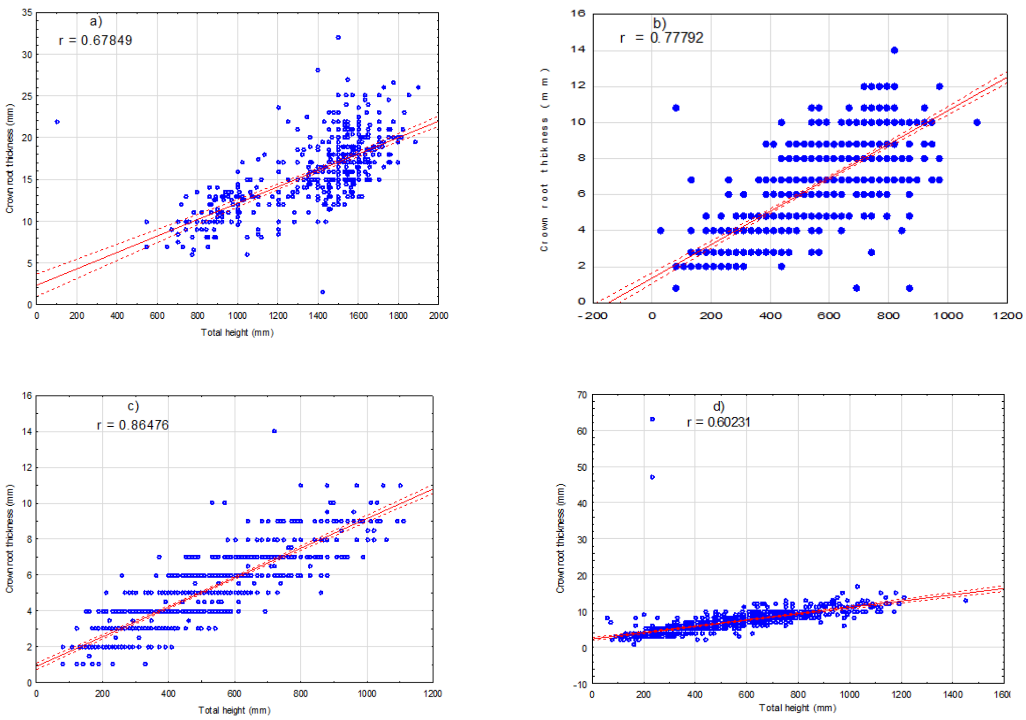
The total height of rootstocks had direct effect on the crown root thickness. The dependence is also supported by our calculated correlation coefficients between the two values. The correlation matrices we created had a correlation coefficient higher than $r = 0.5$, which means the correlation coefficient is highly conclusive. It follows that the higher the rootstock, the wider its crown root diameter, which increases the quality of the rootstock and a potential profit for the nursery.



1: *Differences in crown root thickness in 2016 and 2017: a) B-VA-1 peach seedling; b) Vanovice myrobalan.*



2: *Differences in total height in 2016 and 2017: a) B-VA-1 peach seedling; b) Vanovice myrobalan.*



3: *Correlation coefficients between total height and crown root thickness: a) B-VA-1 in 2016, r = 0.67849; b) B-VA-1 in 2017, r = 0.77792; c) Vanovice myrobalan in 2016, r = 0.86476; d) Vanovice myrobalan in 2017, r = 0.60231.*

CONCLUSION

As the most effective concentration for the both rootstock types appear to be EBR at 0.06 ppm. In 2016 and 2017 this variant showed in most of the evaluated parameters values higher than control and in some cases the highest of all variants.

EBR seems to have the best effect in the periods when the plants are exposed to the conditions with lower water supplies and higher temperatures.

The rootstock height affects the diameter of the crown root, but strong support of apical dominance and prolongation growth with IAA can negatively affect the thickness of the crown root)

Optimal concentrations of EBR and IAA need to be found so they will positively affect both height and crown root thickness.

Acknowledgements

This research was funded by NAZV/KUS project No QJ1510081 by the Ministry of Agriculture of the Czech Republic.

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