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Giberelic acid does not promote positive effect on seedlings of 'Okinawa' peach rootstock

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Abstract: The quality of seedlings is essential for a successful crop cycle, since they are the basis of the orchard; and directly linked to its future performance. In peaches, obtaining rootstock is one step process for the production of the plants, which can be often increased by using plant growth regulators. In this way, this study aimed to evaluate the response of gibberellic acid to grow 'Okinawa' peach seedlings rootstock. The experimental design was a randomized complete block, consisting of four treatments (0, 25, 50 and 75 mg L^{-1} GA_3); three subplots (30, 60 and 90 days after transplanting the seedlings) in 10 repetitions with 30 plants per treatment. All the experiment was conducted in the nursery with shade cloth (70%). Evaluations consisted of plant height (cm); root length (cm); stem diameter (mm); leaf area; specific area and leaf number; leaf matter; fresh and dry stem matter (g); fresh and dry root matter (g). Thereby, doses of gibberellic acid did not present any statistical significance on the growth of 'Okinawa' peach seedlings rootstock; thus it should be considered future evaluation of this phytoregulator at higher doses.

Keywords: *Prunus persica*; plant regulator; vegetative propagation

Uso de ácido giberélico no desenvolvimento de mudas do porta-enxerto de pessegueiro 'Okinawa'

Resumo: A produção de mudas de qualidade é importante para o ciclo da cultura, uma vez que são a base do pomar, e estão diretamente ligadas ao seu desempenho futuro. No pessegueiro, uma das etapas da produção das mudas é a obtenção do porta-enxerto, que pode ser potencializada com o uso de reguladores vegetais. Neste sentido, objetivou-se com o trabalho avaliar o uso do ácido giberélico no desenvolvimento de mudas de porta-enxerto de pessegueiro 'Okinawa'. O experimento foi realizado em viveiro com tela de 70% de sombreamento em delineamento de blocos casualizados com parcelas subdivididas no tempo. Sendo quatro tratamentos (0, 25, 50 e 75 mg L^{-1} de GA_3) e três subparcelas (30, 60 e 90 dias após o transplantio das mudas) distribuídos em 10 repetições de 30 plantas. Avaliou-se a altura da planta (cm), o comprimento da raiz (cm), o diâmetro do caule (mm), a área foliar, a área foliar específica, o número e a massa das folhas, caule e raízes frescas e secas (g). Com base nos resultados, verificou-se que as doses de GA_3 utilizadas não promoveram efeito positivo no desenvolvimento das

mudas do porta-enxerto de pessegueiro 'Okinawa', sendo indicado o estudo com doses mais altas deste fitorregulador.

Palavras-chave: *Prunus persica*, regulador vegetal, propagação vegetativa

Introduction

In recent years there has been a increase in the cultivation of many temperate fruits in Brazil. Among these fruits, there are the stone fruits (FACHINELLO et al., 2011), such as peach (i.e. *Prunus persica*) that has a great importance in the southern and southeastern states of Brazil. In 2014, the largest producers of peach in Brazil were Rio Grande do Sul, Santa Catarina and Sao Paulo, with a production estimated in 127.9; 31.3 and 31.2 thousand tons, respectively (IBGE, 2017).

However, Brazilian domestic production is still insufficient to fulfil the domestic demand; consequently, imports of peach fruits have increased; thus becoming crucial the need to adopt new technologies to improve local production (RAMBOLÀ et al., 2012).

In Brazil, there are a few factors that influence growth of peach crops; however, the quality of seedlings has stood out (WAGNER JÚNIOR et al., 2008). The use of rootstock is a modern practice, commonly used with fruiting plants (PICOLOTTO et al., 2009); and vegetative propagation is based on grafting onto rootstocks derived mainly from seeds (LEONEL et al., 2013).

Seedling quality is an important factor for fruit species, since they are perennial plants and produce for a long period (TOMAZ et al., 2014). The production of high quality fruits, as well as the productivity, indirectly depends from using good techniques for seedlings formation, accounting for roughly 60% of both successful implementation and crop establishment (ZACCHEO et al., 2013).

Several agricultural practices can be used to produce quality seedlings, e.g. by using plant growth regulators (PGRs), which has become a common practice in many countries (TECCHIO et al., 2015). According to these authors, gibberellic acid (GA_3) is PGRs that has been extensively used on the production process and fruit seedlings formation.

Exogenous gibberellic acid strongly promotes stem elongation and cell division, due to the increase in the length and the number of cells promoted by this growth regulator (TAIZ and ZEIGER, 2017).

By using gibberellic acid on the growth of *Mentha arvensis* L., Bose et al. (2013) obtained greater average in the length of the branches; internodes; leaf width; leaf area; and number of branches. Also, Wani et al. (2014) observed that gibberellic acid (at 450, 500 and 550 ppm) promoted a better growth of the apple seedlings, presenting the highest average for seedling length; leaf width; leaf length; number of leaves; and stem diameter. Additionally, Santos et al. (2013) reported that the influence of gibberellic acid on passion fruit seeds, favoured seedlings vigour and shoots length.

Yet it is unsure the influence of this plant regulator on peach production, because there have been very few studies in this crop, mainly, when a large number of cultivars have been considered. In a single study, Wagner Júnior et al. (2008) recommended 200 mg L^{-1} GA_3 to promote a better growth on the peach seedlings rootstock of variety Campinas-1. While Wagner Júnior et al. (2012) reported that the dose of 400 mg L^{-1} GA_3, as being the best dose to develop peach seedlings of progeny 290. However, Peche et al. (2016) while conducted an experiment with persimmon rootstock of Pomelo cultivar, found a negative effect of seedlings length; seedling shoots and root dry matter by using GA_3 in seeds.

Given all the above, it is clear that the use of gibberellic acid on the growth of seedlings may even differ according to each cultivar and species. Therefore, this study aimed to evaluate the response of gibberellic acid on the early development of 'Okinawa' peach seedlings rootstock, which is commonly used for seedlings formation in the state of São Paulo.

Material and Methods

Firstly, all peach fruits were 'Okinawa' rootstock from the Fruit Center of the Agronomic Institute of Campinas (IAC/ APTA, Jundiai, SP, Brazil). The stones were removed from the fruits at the Fruticulture Laboratory, Department of Horticulture, School of Agronomy, São Paulo State University (UNESP / FCA).

A manual lathe was used to remove the core from the fruits. Therein, seeds were washed in running water; dried in the shade; stored in plastic trays covered with plastic bags; and remained in cold stratification for 60 days (at 3 ° C). After stratification, the seeds were placed on sheets of paper 'germitest' dampened with distilled water and wrapped in plastic to maintain the moisture. Afterward, these rolls were placed in biochemical oxygen demand (BOD) at 4°C.

The rolls were weekly moistened with fungicidal solution comprising dicarboximide (Sialex 500®) and bactericide (Agrimicina®), consisted of oxytetracycline; hydrochloride; and streptomycin sulphate containing 200; 300; and 100 g L^{-1} H_2O, respectively.

All the material used was sterilized with sodium hypochlorite (1.5% NaClO) and alcohol (70%), according to the methodology described by Picolotto et al. (2007); i.e. manual lathe; trays; forceps; gloves; stands for handling materials; and germinator were sterilized.

The seeds began to germinate about 20 days later. Once there were a significant number of germinated seeds; they were transferred into plastic bags filled with substrate (350g of "Tropstrato"). Then, these bags were packed in a protected environment with shade cloth (70%) at the Experimental Area of the Department of Forest Science (FCA/ UNESP).

By the time the young plants presented an average of four leaves (i.e. 15 days after transplanting), the first application of gibberellic acid (GA_3) was carried out; i.e. Progibb® at 0 (control), 25, 50 and 75 mg L^{-1}. After 15 days, a second application with the same doses was performed. The product was foliar applied by spraying with a 5 L knapsack and a volume of 250 ml young plant $^{-1}$.

The experimental design was a randomized complete block, consisting of four treatments (4 doses of GA_3); three subplots (3 evaluation times) with 10 seedlings each, which was evaluated on the 30th, 60th and

90th days after transplanting the germinated seeds.

From this moment, evaluations consisted of plant height and root length with a graduated ruler expressed in centimetres (cm); the stem diameter was obtained by using a digital calliper (mm). For leaf area, specific leaf area and number of leaves, it was only considered the expanded ones.

For leaf area (LA), it was used the area meter (model: LI 3100). They measured total leaves of seedlings per repetition, i.e. the definite value is the arithmetic mean of the five repetitions. To determine the leaf fresh and dry matter, stems and roots from all 10 repetitions, samples were placed in paper bags, identified and taken to dry into forced-air-circulation oven at 65°C for 48 hour; and re-weighed for dry matter. Finally, the specific leaf area (SLA) is the ratio of leaf area (cm^2) and leaf weight (g).

After collecting the data, analysis of variance of the same was performed; in case of significance, means were subjected to regression analysis by adopting a 5 and 1% level of significance, through SISVAR statistical analysis program (FERREIRA, 2011).

Results and Discussion

With regards to plant height; leaf number; root length; leaf fresh matter; root fresh matter; stem dry matter; and root dry matter, the interaction between doses and days after transplanting the seedlings was statically significant. By measuring in isolation an effect of other variables (i.e. variables that did not show an interaction), it was verified that the dose factor was only significant for leaf dry matter. However, results were significant for stem diameter; leaf area; specific leaf area; stem fresh matter; and leaf dry matter on the days after transplanting the seedlings (Table 1).

Table 1. F test values and coefficients of variation (CV) for plant heigh (PH); stem diameter (SD); number of leaves (NL); leaf area (LA); specific leaf area (SLA); roots length (RL); leaf fresh matter (LFM); stem fresh matter (SFM); roots fresh matter (RFM); leaf dry matter (LDM); stem dry matter (SDM) and roots dry matter (RDM) of 'Okinawa' peach seedlings rootstock

S.V.[1]	F TEST VALUES											
	PH	SD	NL	LA	SLA	RL	LFM	SFM	RFM	LDM	SDM	RDM
Block	0.9^{ns}	1.2^{ns}	1.2^{ns}	0.9^{ns}	1.6^{ns}	1.3^{ns}	0.4^{ns}	0.5^{ns}	0.5^{ns}	0.6^{ns}	1.0^{ns}	0.5^{ns}
Doses	0.4^{ns}	0.1^{ns}	5.9^{**}	1.4^{ns}	2.4^{ns}	2.3^{ns}	2.1^{ns}	1.9^{ns}	3.0^{*}	4.6^{**}	2.6^{ns}	6.2^{**}
Days	46.9^{**}	57.4^{**}	58.5^{**}	17.0^{**}	51.4^{**}	17.8^{**}	37.1^{**}	23.8^{**}	29.0^{**}	54.6^{**}	43.4^{**}	54.1^{**}
Int.[2]	3.6^{**}	1.5^{ns}	6.5^{**}	0.6^{ns}	1.6^{ns}	2.1^{*}	2.3^{*}	2.9^{ns}	3.9^{**}	1.8^{ns}	2.5^{*}	4.3^{**}
CV1(%)[3]	22.9	23.2	38.8	56.0	70.8	30.1	53.8	57.9	54.7	81.7	84.1	80.7
CV2 (%)	22.0	22.0	39.6	51.6	68.4	29.2	46.0	56.6	46.6	81.4	89.4	87.8

**Significant at 5%, **Significant at 1%, ^{ns}not significant. [1]Source of variation. [2]Interaction between doses of GA_3 x Days after transplanting.[3]Coefficient of variation.

Regards to the plant height and number of leaves, the interaction was only significant at 90 days after transplanting the seedlings. The means of these characteristics at 30 and 60 days did not adjust the linear and quadratic models. In 90 days, it was found that the doses negatively affected the aforementioned variables, i.e. negative quadratic effect; therefore, the higher the doses were, the lower the averages till the curve inflection point were; whilst averages started to increase again at the curve inflection point. The maximum function points were estimated at 41.5 and 38.0 mg GA_3 L^{-1} for plant height and leaf number, respectively (Figure 1A, B). Peche et al. (2016) also reported a negative linear effect for the length of the aerial part of 'Pomelo' persimmon seedling rootstock by using 100, 200 and 300 mg L^{-1} GA_3.

Among the gibberellins, GA_3 is the most widely used plant growth regulator, since it promotes growth by stimulating cell enlargement; consequently, causing elongation of stems and apical dominance (TAIZ and ZEIGER, 2017). However, this effect was not observed in this study, which may be related to the lower doses. Besides that, this study presented a delayed effect of the GA_3, i.e. only after 90 days (Figure 1A).

Only when the dose of 25 mg L^{-1} were used the plants evaluated at 60 and 90 days were statistically equal to 30 days in height (Figure 1A). While for the number of leaves, only in the absence of the product or when the 75 mg L^{-1} dose was used averages greater than 90 days were observed (Fifure 1B).

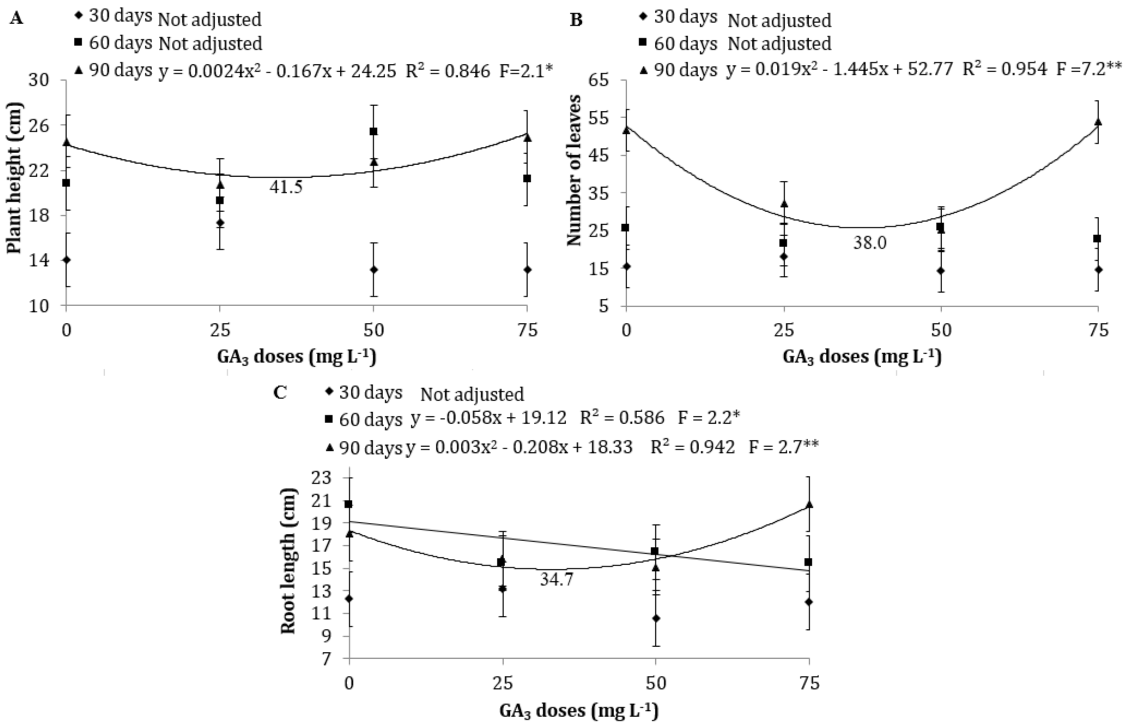

Figure 1. Plant height (A), number of leaves (B) and root length (C) of 'Okinawa' peach seedlings rootstock under gibberellic acid doses.

In a single study, Wagner Júnior et al. (2008) also observed a significant interaction between GA_3 doses and evaluation days, which reflected in a greater plant length. It is worth mentioning that these authors used higher doses (i.e. 0, 50, 100, 150 and 200 mg L^{-1} GA_3) than this study.

Regarding to the roots length, a significant effect of doses was observed at 60 and 90 days after transplanting the seedlings. At 60 days after transplanting, the averages were adjusted to linear model, with an average decrease until 75 mg L^{-1} GA_3. Likewise, Peche et al. (2016) reported a negative linear effect of roots length for "Pomelo" persimmon rootstock by using GA_3. However, at 90 days after transplanting, a quadratic adjustment occurred for the average, whose effect was negative until the estimated dose of 34.7 mg L^{-1} GA_3. At this point, averages increased, being the highest value at 75 mg L^{-1} GA_3 (Figure 1C). Consequently, suggesting that higher doses may promote greater root growth. The root length averages at 30 days did not fit the evaluated models (Figure 1C).

For fruit seedlings, it is desirable that the plant growth regulators promote further root growth, such as the greater the shoots growth are, the more compromise the roots growth will be, whose adaptation will be quite limited in the field (TECCHIO et al., 2015). According to Timm et al. (2015), the issue of roots is related to both endogenous and environmental conditions.

The adverse effect of GA_3 in this experiment could be explained by the highest ratio gibberellin/auxin; according to Das and Prasad (2014), auxin is the hormone responsible for increasing roots production. Thus, the application of exogenous synthetic auxin promoted a hormonal balance; consequently, rooting and roots uniformity (DUTRA et al., 2012).

The use of the 75 mg L^{-1} dose allowed to obtain plants with greater root length after 90 days, justifying their indication, since the other doses did not result in higher plants after this same period (Figure 1C).

For the leaf matter and root fresh matter, averages were adjusted to the quadratic model. For leaves matter, the effect was significant at 90 days after transplanting; but for roots matter at 60 days. Both presented a negative effect of the doses, being the lower averages estimated at 42.9 and 62.5 mg L^{-1} GA_3 for leaf and root fresh matter, respectively (Figure 2A and 2B). At 30 and 60 days of evaluation there was no effect of the doses for the leaf fresh matter, as well as the averages at 30 and 90 days for the root fresh matter (Figure 2A and 2B).

When GA_3 was not used, the leaf fresh matter differed statistically at each evaluation time. However, when the dose of 75 mg L^{-1} was used, the plants evaluated at 60 and 90 days did not differ among them, but were higher than those evaluated at 30 days (Figure 2A). For the dry matter of roots, after 90 days only the dose of 50 mg L^{-1} resulted in averages statistically superior to the averages of 30 and 60 days (Figure 2B).

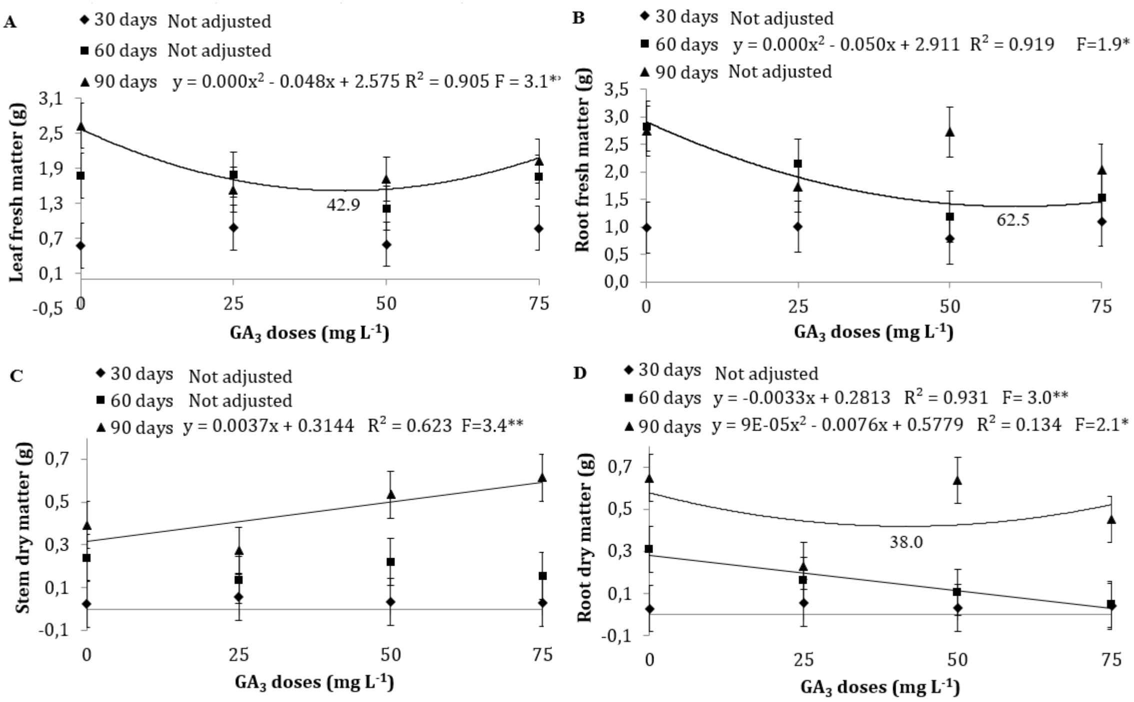

Figure 2. Leaf fresh matter (A); Root fresh matter (B); Stem dry matter (C) and root dry matter (D) of 'Okinawa' peach seedlings rootstock under gibberellic acid doses.

Unlike the previous variables, a linear model was adjusted for the dry matter of the stem at 90 days, while at 30 and 60 days there was no adjustment of the means to the models evaluated. In addition, the doses of GA_3 had significant effect at 90 days after transplanting, i.e. the greater stem dry matter was obtained at 75 mg L^{-1} GA_3 (Figure 2C). For root dry matter variables, it was obtained a significant effect of GA_3 doses at 60 and 90 days after transplanting. Moreover, a negative linear effect occurred with the doses at 60 days; but quadratic at 90 days, with averages decrease until the estimated dose of 38.0 mg L^{-1} GA_3 (Figure 2D).

Normally, plant growth regulators is associated with higher means for fresh and dry matter due to the increased number and length of the roots (DAS and PRASAD, 2014), which was not observed in this study probably due to the lower doses of GA_3. However, Peche et al. (2016) evaluated the influence of gibberellic acid on root and shoot dry matter of persimmon seedlings rootstock, found that the higher the dose, the lower the averages for these features. These authors used doses ranging from 100, 200 and 300 mg L^{-1}. Furthermore, the response of plants to plant growth regulators depends on genetic material used and local weather conditions.

Comparing the mean dry matter of stem and roots according to the time of evaluation, it was verified that when the doses of 50 and 75 mg L-1 were used, there were higher mean values at 90 days. These results show the best development of the plants when higher doses are used (Figure 2C and 2D).

Lately, dry matter production has been used to assess seedling quality; however, in order to measure that there is the need to wipe out the whole plant; thus, unfeasible in many nurseries (DODE et al., 2012).

As stated before, there was no interaction between doses of GA_3 and days after transplanting the seedlings for leaf dry matter. However, it was observed a quadratic negative effect of GA_3 on this characteristic with an average decrease until the estimated dose of 44.4 mg L^{-1} (Figure 3). This result also shows that the number of leaves presented a negative effect of GA_3.

Regarding to the leaf dry matter; stem diameter; leaf area; specific leaf area; and stem fresh matter, a significant effect was observed at the days after transplanting the seedlings, as all these average variables increased from 30 days until 90 days, except for specific leaf area, which the highest average was obtained after 30 days. This result is due to the fact that this variable is a ratio between leaf area and leaf dry matter. Thus, one can say that leaf area was relatively greater than the weight of the leaves after 30 days, i.e. the leaves were lighter at that time than at 60 and 90 days.

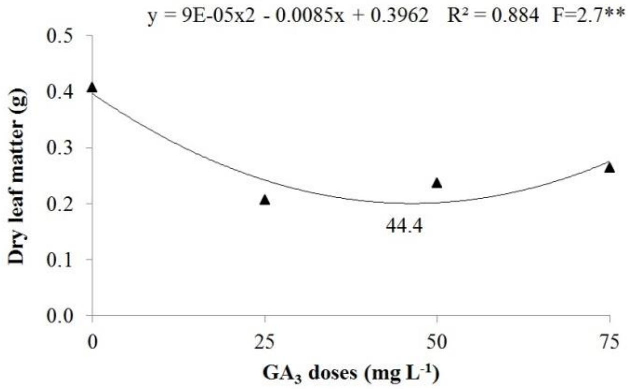

Figure 3. Dry leaf matter of 'Okinawa' peach seedlings rootstock.

The highest average of stem diameter, leaf area, stem fresh matter and leaf dry matter were expected after 90 days, since they are due to the natural growth of the seedlings. Evaluating the effect of GA_3 on peach seedlings of variety Campinas-1, Wagner Júnior et al. (2008) found no significant effect of plant growth regulator for seedlings diameter, even at much higher doses than presented in this study.

With regards to the leaf area and specific leaf area, the absence of GA_3 effects could be related to the fact that the leaf expansion is the result of physiological action of cytokinins (TAIZ and ZEIGER, 2017). However, it is worth emphasizing the importance of evaluating these characteristics, since they are directly related to the photosynthetic capacity of plants.

A literature review associated with the present results make-believe that higher GA_3 doses can promote better development of rootstock seedlings of peach 'Okinawa'. Although, the influence of GA_3 is mainly related to the elongation of plants, it did not promote the growth of the plants length. Moreover, the effect of phytohormones depends on several factors, including the already existing hormonal balance in the plants. Furthermore, the best results could be obtained by using biostimulants (i.e. consisted of multiple plant growth regulators). Additionally, there are studies in literature that already found promising results by using them (ABRECHT et al., 2014; DAN et al., 2014; FERRAZ et al., 2014).

Conclusions

The studied doses of gibberellic acid did not promote any further development of 'Okinawa' peach seedling rootstock. However, it is clearly evident that by increasing the GA_3 doses, it may lead to a better development of the seedlings.

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