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STAKES ROOTING OF 'Biloxi' BLUEBERRY (*Vaccinium corymbosum* L.) PLANTS

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ABSTRACT

Blueberry (*Vaccinium corymbosum* L.), native to North America, has become one of the most important fruit crops in Mexico and the world due to its hardiness, flavor, and health benefits. The objective of this study was to evaluate the inductive capacity of naphthaleneacetic acid (NAA), salicylic acid (SAL), their combination, a *Plantago* spp. extract, and indolebutyric acid (IBA) in the rooting of semi-woody cuttings of 'Biloxi' blueberry. In a rooting chamber, 5 cm long cuttings with two apical leaves cut transversely were established and treated with different concentrations of NAA (0, 15, and 20 mg L^{-1}), SAL (0, 50, and 75 mg L^{-1}), and their combination, with three additional controls of *Plantago* spp. extract and IBA (4000 and 3000 mg L^{-1}). A completely randomized design with treatments in a factorial arrangement and three additional controls was used. No significant individual effects of NAA or SAL were detected. However, individual applications of 20 mg L^{-1} of NAA and 75 mg L^{-1} of SAL showed the best results for rooting percentage (93.75 %), number of first- (8.88 and 10.25) and second-order roots (72.38 and 65.13), length of first- (33.92 and 32.44 mm) and second-order roots (17.6 and 19.67 mm), and root volume (81.88 and 85.63 mm^3). These results were statistically equivalent to the 4000 and 3000 mg L^{-1} concentrations of IBA. In contrast, the combination of NAA and SAL, as well as the plant extract, resulted in low values in all the variables evaluated.

Keywords: indolebutyric acid, naphthaleneacetic acid, salicylic acid, plant extract.

INTRODUCTION

Blueberry (*Vaccinium corymbosum* L.) is a plant native to North America. Because of its hardiness, pleasant flavor, antioxidant, and anti-inflammatory properties, the fruit has captured the attention of many producers and consumers. Over the years, the number of hectares dedicated to its cultivation has increased (Tinoco-Plasencia *et al*., 2023). In Mexico, this crop has had an important impact; from 2012 to 2022, the harvested area and production increased from 885 to 5887 ha and from 7191 to 67 304 Mg, respectively (FAO, 2024). This crop can be propagated in two ways: sexually

through seeds and asexually through meristematic tissue. Rooting cuttings is an asexual propagation technique that focuses on multiplying plants from stem segments of a specific individual, which are subjected to induction for root development under specific environmental conditions, which guarantees a high percentage of genetic resemblance to the mother plant (López-Corona *et al*., 2019).

According to Ikeuchi *et al*. (2016) and Druege *et al*. (2019), the rooting process is divided into three stages: induction, initiation, and expression. The first stage involves molecular and biochemical processes that do not produce visible changes in the cutting, including cell reprogramming, during which competent cells from the vascular tissue give rise to root primordia. In some cases, cells must first be reprogrammed to perceive and respond to rooting signals. During the second stage, qualitative changes occur in cell structure, accompanied by cell division and the organization of dome-shaped root primordia resulting from the differentiation of new cell groups. Finally, in the third stage, the root primordia undergo further differentiation and growth, developing into root structures with distinct vascular bundles connected to the vascular cylinder of the cutting, ultimately leading to root emergence.

The time and percentage of rooting depend on the type of stake, as its regenerative capacity is determined by endogenous factors, such as the levels of carbohydrates, mineral salts, and plant hormones available, auxins being considered the most important (Hu *et al*., 2020). Similarly, the substrate plays a determining role, as it is responsible for supporting the cutting of the stake, providing a dark environment, and supplying water and air in the metabolic process of root induction and elongation (Hartmann *et al*., 1997). Similarly, external conditions such as temperature and light must be considered, as they regulate respiration, vegetative shoot development, and stake reserve conservation, whereas relative humidity influences leaf temperature and gas exchange. Microorganisms anchored to the plant material are also an external factor that increases the risk of contamination and death of the cuttings (Owen and Maynard, 2007). To reduce time and increase the percentage of rooting, it is necessary to add exogenous hormones (growth regulators), where auxins stand out, which play an important role in the induction and development of roots, such as indolebutyric acid and naphthaleneacetic acid (López-Corona *et al*., 2019).

Each species has a certain propagation capacity, so specific protocols are required to achieve the highest percentage of multiplication (Le *et al*., 2023). Ligarreto *et al*. (2013), Kim *et al*. (2014), and Leiva *et al*. (2023) obtained a higher rooting percentage when applying naphthaleneacetic acid in *Vaccinium meridionale* Swartz, *V. corymbosum* 'Bluecrop' and 'Sunrise', and *V. corymbosum* 'Biloxi', respectively. Salicylic acid, a phenolic compound, can function as an auxin cofactor (Pacholczak and Nowakowska, 2015). By using this compound, Sardoei *et al*. (2014) and Pacholczak and Nowakowska (2015) reported higher rooting percentages in *Euphorbia pulcherrima* Willd. and *V. corymbosum* 'Bluecrop' and 'Duke.'

Extracts of *Plantago major* L. and *P. lanceolata* L. possess antimicrobial, anti-inflammatory, and wound-healing properties, and contain several secondary metabolites (phenols,

flavonoids, saponins, and alkaloids) (Mazzutti *et al*., 2017), in addition to carbohydrates, indoleacetic acid, vitamins, and traces of salicylic acid (Berit, 2000). However, little is known about their use in agricultural practices. Because of their properties, they may be able to induce cutting rooting. A commonly used growth regulator is indolebutyric acid, such as in *V. corymbosum* 'Biloxi' (An *et al*., 2018), 'Powderblue' (Colombo *et al*., 2018), 'Woodard' (Higuchi *et al*., 2021), and wild blueberry (Tejada-Alvarado *et al*., 2021). However, information on growth regulators in rooting cuttings of this crop is scarce and ambiguous. Therefore, the objective of this research was to evaluate the inductive capacity of naphthaleneacetic acid, salicylic acid, the combination of both compounds, *Plantago* spp. extract, and indolebutyric acid on the rooting of 'Biloxi' blueberry cuttings.

MATERIALS AND METHODS

Location of the study area and plant material

This study was conducted in an experimental greenhouse of the Plant Nutrition area of the Postgraduate College Campus Montecillo in Texcoco, State of Mexico, Mexico. Eight-year-old *Vaccinium corymbosum* 'Biloxi' plants were fed a Steiner nutrient solution at half of its original concentration (Steiner, 1984), pH 5.5, and an ammonium:nitrate ratio of 75:25. A rooting chamber was constructed with white greenhouse plastic, where substrate temperature (30 ± 2 °C) and relative humidity (≥ 75 %) were controlled by circulating warm water (50 ± 3 °C) below the seedlings and water misting, respectively.

Experimental design and compounds evaluated

A completely randomized design with treatments in an increased factorial arrangement was used, where naphthaleneacetic acid (NAA) and salicylic acid (SAL) were evaluated at three levels (0, 15, and 20, and 0, 50, and 75 mg L^{-1}, respectively), with three additional controls corresponding to a plant extract from *Plantago* spp. and two concentrations of indolebutyric acid (IBA) (4000 and 3000 mg L^{-1}). Each treatment was carried out with four replicates, where the experimental units consisted of four cells (5 × 5 cm) of a seedbed with a blueberry stake.

Treatments and substrate preparation

NAA (Pacific Growers, Mexico) and SAL (Fagalab, Mexico) with 98 % purity were dissolved in 5 mL of 96 % alcohol at the specified concentration for each treatment and mixed with 95 mL of deionized water. A total of 100 mL of *Plantago* spp. extract was used, which was obtained by boiling 1 L of deionized water with 200 g of aerial parts of plants collected for 15 min and dried in the shade for 30 d. Similarly, the concentrations of 4000 and 3000 mg L^{-1} of IBA were prepared on a 4:6 and 3:7 volume basis from a commercial product containing 10 000 mg of IBA (Intercontinental Import Export S.A. de C.V., Mexico) and hypoallergenic talc (Mennen, USA).

The substrate was prepared in a 1:1 ratio of perlite and peat and saturated with deionized water. Before distribution, the substrate was sterilized three times in pressure pots (Presto, Mexico) with pressures ranging from 0.1 to 0.13 MPa for 3 h at 24-h intervals.

Stakes establishment

Two apical leaves were cut transversely from the apical portion of vegetative shoots of plants with a semi-lignified consistency to create multiple 5-cm-long cuttings, which were disinfested twice in agitation for 1 min in metalaxyl-M (1.7 mL L^{-1}) (Syngenta, Switzerland) and captan (2 g L^{-1}) (Adama, Israel). After 3 s of immersion in each solution, 1 cm of the stake stem was inserted into the designated seedbed alveolus. During the entire experiment (70 d), six fungicide applications were made at the base of the stem of the cuttings at 10-d intervals.

Acclimatization of rooted cuttings

Seventy days after establishment (DAE), the rooted cuttings were removed from the chamber seedbeds. To avoid poor root development and facilitate transplanting, their root system was pruned. The cuttings were immersed for 1 s in mancozeb (2 g L^{-1}) (Syngenta, Switzerland) before being transplanted in a 1:1 volume of perlite and peat substrate in 60-cavity unicell forest trays (Hydrocultura, Mexico), with cells measuring 182 cm^3. Propamocarb hydrochloride (2.5 mL L^{-1}) (Bayer, Germany) was applied at the base of the stem. Finally, each tray was covered with polypropylene plastic, and the plastic was cut weekly with scissors until the tray was completely free.

Study variables and statistical analysis

At the end of the experiment (70 DAE), the percentage of survival and rooting was obtained by considering the surviving and rooted cuttings over the total number of cuttings per experimental unit. The number of first- and second-order roots was recorded by counting the number of roots in the root system of the rooted cuttings. The diameter and length of first- and second-order roots were evaluated by measuring the diameter of the thickest root and the longest first- and second-order roots, using the digital processing program ImageJ version 1.8.0. Root volume was obtained by submerging the roots of each stake into a 500 μL (mm^3) syringe and comparing the initial water volume against the final volume.

The data obtained were subjected to a normality test (Shapiro-Wilk) ($p \geq 0.05$), homogeneity of variances (Bartlett) ($p \geq 0.05$), and independence of residuals; likewise, an analysis of variance corresponding to a randomized design with treatments in augmented factorial arrangement and a multiple comparison of means by using Fisher's least significant difference test ($p \leq 0.05$) were performed in the statistical program R version 4.3.2 and Microsoft Excel 2021.

RESULTS AND DISCUSSION

Survival and rooting rates

After 70 d of establishment, there were no significant effects on the survival percentage attributable to the additional factors, interactions, and controls (Table 1). In general, the survival percentages were high (87.5 to 100 %), which is attributed to the care given during this experiment, especially the disinfection of the substrate used, which is an important factor when establishing cuttings in an environment with high relative humidity (Badilla-Valverde and Murillo-Gamboa, 2005). In this regard, Li *et al*. (2021) state that in order to maintain live explants of *Vaccinium arboreum* Marshall, a humid environment inside chambers using nebulizers is required, a factor that allowed them to report a 100 % survival rate.

Table 1. Comparison of means using Fisher's least significant difference test for survival and rooting rates in cuttings of *Vaccinium corymbosum* L. 'Biloxi' with the effects of various treatments.

Treatment	Composition (mg L^{-1})	Survival percentage	Rooting percentage
1	0 NAA+ 0 SAL	93.75 a	75.00 ab
2	15 NAA+ 0 SAL	100.00 a	68.75 ab
3	20 NAA+ 0 SAL	100.00 a	93.75 a
4	0 NAA+ 50 SAL	93.75 a	62.50 b
5	0 NAA+ 75 SAL	100.00 a	93.75 a
6	15 NAA+ 50 SAL	87.50 a	62.50 b
7	15 NAA+ 75 SAL	100.00 a	62.50 b
8	20 NAA+ 50 SAL	87.50 a	62.50 b
9	20 NAA+ 75 SAL	87.50 a	56.25 b
10	*Plantago* spp.^{z}	100.00 a	56.25 b
11	4000 IBA^{z}	87.50 a	68.75 ab
12	3000 IBA^{z}	100.00 a	81.25 ab
F-NAA	0	95.83 a	77.08 a
	15	95.83 a	64.58 a
	20	91.67 a	70.83 a
F-SAL	0	97.92 a	79.17 a
	50	89.58 a	62.50 a
	75	95.83 a	70.83 a
Overall average number of treatments		94.79	70.31
CV (%)		12.03	30.93
p-value of treatments		0.4556^{ns}	0.0495*
p-value of F-NAA		0.5912^{ns}	0.5587^{ns}
p-value of F-SAL		0.1911^{ns}	0.4232^{ns}
$LSD_{0.05}$ of treatments		16.36	31.19
$LSD_{0.05}$ of factors		8.55	17.57

NAA: naphthaleneacetic acid; SAL: salicylic acid; IBA: indolebutyric acid; z: additional control; F-NAA: naphthaleneacetic acid factor; F-SAL: salicylic acid factor; CV: coefficient of variation; ^{ns}: not significant; *: significant differences; $LSD_{0.05}$: least significant difference. Values with the same letter are statistically similar according to Fisher's least significant difference test ($p \leq 0.05$).

Another important factor was the consistency of the stake. In general, a semi-lignified consistency stake was used, which resulted in greater resistance to dehydration (Badilla-Valverde and Murillo-Gamboa, 2005). On the other hand, the exposure time of the cuttings in the solution can affect their survival, since prolonged times can cause phytotoxicity and death of the cuttings. Similarly, this can be aggravated depending on the concentration and type of plant material used (Coimbra *et al*., 2016).

In this study, it was demonstrated that 3 seconds of exposure with NAA, SAL, and IBA at the concentrations used did not affect the survival of semi-lignified 'Biloxi' blueberry cuttings, as opposed to deionized water and *Plantago* spp. The survival rates were similar to those shared by Colombo *et al*. (2018), with 100 % survival in 5 cm cuttings of 'Powderblueberry' blueberry when applying 3000 mg L^{-1} of IBA in talc. On the other hand, other studies report high survival percentages with long exposure times, such as Sardoei *et al*. (2014) on cuttings with 300 mg L^{-1} of NAA for 24 h and Leiva *et al*. (2023) on 'Biloxi' blueberry nodal segments for 15 min. This shows that exposure time will depend on cultivar, stake consistency, and type of compound (Coimbra *et al*., 2016).

At the end of the experiment, there were no significant differences in the rooting rates between the NAA and SAL factors (Table 1). On the other hand, for the interactions and controls, the comparison of means indicated that the rooting rates reported when using 20 and 75 mg L^{-1} of NAA and SAL (93.75 %) were statistically equal to those achieved with deionized water (75 %), 15 mg L^{-1} of NAA (68.75 %), 4000 mg L^{-1} of IBA (68.75 %), and 3000 mg L^{-1} of IBA (81.25 %). However, these values were higher than those obtained with other compounds, with values between 56.25 and 62.5 % (Figure 1). The best rooting percentages in this experiment were considerably high (68.75 to 93.75 %), corresponding to the individual use of NAA and SAL, IBA, and deionized water.

In other studies, it was reported that the highest rooting percentage was achieved in 'Biloxi' blueberry cuttings with 100 mg L^{-1} of NAA (Leiva *et al*., 2023). With 300 mg L^{-1} of SAL, a 75 % rooting rate was obtained in semi-lignified cuttings of *Euphorbia pulcherrima* (Sardoei *et al*., 2014), and with various concentrations of IBA in blueberry 'Biloxi' (3000 mg L^{-1}) (An *et al*., 2018), 'Powderblue' (3000 mg L^{-1} IBA in talc) (Colombo *et al*., 2018), 'Woodard' (1000 mg L^{-1}) (Higuchi *et al*., 2021), and wild blueberry (2000 mg L^{-1}) (Tejada-Alvarado *et al*., 2021). Likewise, Isfendiyaroğlu and Özeker (2008) and Pacholczak and Nowakowska (2015), by combining IBA and SAL, achieved high rooting percentages in cuttings of *Olea europaea* L. 'Domat' and blueberry 'Bluecrop' and 'Duke,' respectively.

The positive effects of NAA, SAL, and IBA on the rooting of plant material are due to the versatility of these compounds in the accumulation of indoleacetic acid, which has an impact on the rooting speed of cuttings and root development (Hopkins and Hüner, 2008; Damodaran and Strader, 2019; Dong *et al*., 2020). However, in this research, the percentage of rooting obtained was statistically equal to the control with deionized water, which can be attributed to a greater production of endogenous auxins in stakes

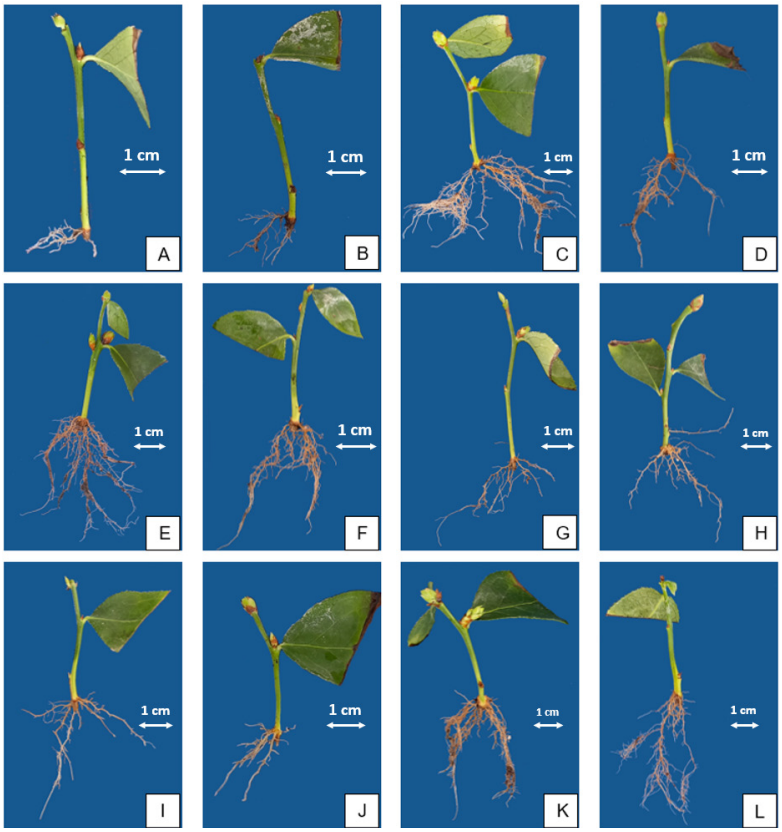

Figure 1. Rooted cuttings of *Vaccinium corymbosum* L. 'Biloxi' after 70 d of establishment with the effects of various treatments. A: deionized water; B: 15 mg L^{-1} naphthaleneacetic acid (NAA); C: 20 mg L^{-1} NAA; D: 50 mg L^{-1} salicylic acid (SAL); E: 75 mg L^{-1} SAL; F: 15 + 50 mg L^{-1} NAA and SAL; G: 15 + 75 mg L^{-1} NAA and SAL; H: 20 + 50 mg L^{-1} NAA and SAL; I: 20 + 75 mg L^{-1} NAA and SAL; J: extract of *Plantago* spp. K: 4000 mg L^{-1} of indolebutyric acid (IBA); L: 3000 mg L^{-1} of IBA.

without complete lignification, so that sometimes it is not necessary to add exogenous auxins to stimulate rooting (Shiembo *et al*., 1997). Therefore, it is possible that the conditions to which the cuttings were exposed were sufficient to stimulate rooting, even without the addition of growth regulators (Owen and Maynard, 2007).

On the other hand, it is likely that the low rooting percentage reported in the treatments where NAA and SAL were combined is due to an endogenous hormonal imbalance caused by the concentrations of these regulators (Haissig, 1970). This agrees with de Klerk *et al*. (1997), who found that SAL inhibits rooting produced by IBA and NAA in apple micro-cuttings. Likewise, Isfendiyaroğlu and Özeker (2008) emphasize that SAL should be applied in conjunction with auxins to avoid inhibiting root formation.

Number of first and second order roots

There are variables that are used as morphological indicators of the quality of root development, such as root number, length, diameter, and volume. These serve to evaluate root development, an important factor that reduces mortality in transplanting and acclimatization of cuttings (Colombo *et al*., 2018). According to the analysis of variance, no differences in the number of first- and second- order roots were reported in any of the factors (Table 2).

For the number of first-order roots, the comparison of means of the interactions and additional controls indicated that when 75 mg L^{-1} of SAL was applied, 10.25 first-order

Table 2. Comparison of means with Fisher's least significant difference test of the number of first and second order roots in cuttings of *Vaccinium corymbosum* L. 'Biloxi' with the effects of various treatments.

Treatment	Composition (mg L^{-1})	Number of roots			
		First order		Second order	
1	0 NAA+ 0 SAL	4.37	bc	16.50	d
2	15 NAA+ 0 SAL	3.25	c	14.50	d
3	20 NAA+ 0 SAL	8.87	ab	72.37	a
4	0 NAA+ 50 SAL	7.00	abc	45.50	abcd
5	0 NAA+ 75 SAL	10.25	a	65.12	ab
6	15 NAA+ 50 SAL	5.25	abc	35.50	bcd
7	15 NAA+ 75 SAL	5.62	abc	23.62	cd
8	20 NAA+ 50 SAL	4.62	bc	24.50	cd
9	20 NAA+ 75 SAL	7.62	abc	39.88	abcd
10	*Plantago* spp.^{z}	4.87	bc	20.25	cd
11	4000 IBA^{z}	9.37	ab	54.12	abc
12	3000 IBA^{z}	7.62	abc	45.62	abcd
F-NAA	0	7.20	a	42.37	a
	15	4.70	a	24.54	a
	20	7.04	a	45.58	a
F-SAL	0	5.50	a	34.46	a
	50	5.62	a	35.17	a
	75	7.83	a	42.87	a
Overall average number of treatments		6.56		38.12	
CV (%)		53.20		66.07	
p-value of treatments		0.0150	*	0.0097	**
p-value of F-NAA		0.1609	ns	0.1022	ns
p-value of F-SAL		0.1978	ns	0.6652	ns
$LSD_{0.05}$ of treatments		5.01		36.12	
$LSD_{0.05}$ of factors		2.83		21.06	

NAA: naphthaleneacetic acid; SAL: salicylic acid; IBA: indolebutyric acid; ^{z}: additional control; F-NAA: naphthaleneacetic acid factor; F-SAL: salicylic acid factor; CV: coefficient of variation; ^{ns}: not significant; *: significant differences; **: highly significant differences; $LSD_{0.05}$: least significant difference at a significance of 0.05. Values with the same letter are statistically similar according to Fisher's least significant difference test ($p \leq 0.05$).

roots developed, a value that only surpassed the means with deionized water (4.37), 15 mg L^{-1} of NAA (3.25), 20 + 50 mg L^{-1} of NAA and SAL (4.62), and with *Plantago* spp. (4.87) (Table 2). On the other hand, for the number of second-order roots, the analysis of variance showed significant effects in the interactions and the controls, where the comparison of means indicated that the treatment with 20 mg L^{-1} of NAA (72.37) was statistically equal to 50 mg L^{-1} of SAL (45.50), 75 mg L^{-1} of SAL (65.12), 20 + 75 mg L^{-1} of NAA and SAL (39.88), 4000 mg L^{-1} of IBA (54.12), and 3000 mg L^{-1} of IBA (45.62), but greater than the rest.

When speaking of the quality of root development, the rooting percentage usually takes second place, since in some cases there may be high percentages, but the root is slightly developed, i.e., with one or two roots, or *vice versa*. Such was the case of the treatment with deionized water, where its percentage was one of the highest (75 %), but its means were among the lowest in these variables, with 4.37 first-order roots and 16.5 second-order roots. The opposite case was the treatment with 20 + 75 mg L^{-1} of NAA and SAL, in which the rooting percentage was among the lowest (56.25 %), but it had a more developed root, with 7.62 first-order roots and 39.88 second-order roots. All of this was influenced by rooting time, as some treatments had cuttings with first-order roots after only 21 d of establishment, giving them more time to develop. This shows the effect of NAA and SAL applied individually on rooting induction and the rate of indoleacetic acid accumulation in the cuttings (Hopkins and Hüner, 2008; Dong *et al*., 2020).

These results are similar to those obtained by Sardoei *et al*. (2014), who obtained 11.6 roots in *E. pulcherrima* cuttings when applying 100 mg L^{-1} of SAL. Likewise, Coimbra *et al.* (2016) report eight roots in *Bertholletia excelsa* Humb. and Bonpl. cuttings when applying 3000 mg L^{-1} of IBA; An *et al*. (2018) obtained 19.09 roots in 'Biloxi' blueberry cuttings when applying 3000 mg L^{-1} of IBA; Colombo *et al*. (2018) obtained 4.59 roots when using 3000 mg L^{-1} of IBA in alcohol on blueberry cuttings 'Powderblue'; and Tejada-Alvarado *et al*. (2021) reported up to 2.95 roots in wild blueberry cuttings when applying 2000 mg L^{-1} of IBA. On the other hand, when applying 15 mg L^{-1} of NAA, means equal to those of the deionized water treatment were obtained, both in the percentage of rooting and in the number of first- and second-order roots. In this regard, it is likely that the concentration used was not sufficient to stimulate rooting, which unbalanced the endogenous hormone content (Haissig, 1970), inhibiting the rooting of cuttings (Hopkins and Hüner, 2008).

Root diameter and length

For root diameter and root length of first- and second-order of the root system, no statistical differences were found for the factors NAA and SAL, but they were found in the interactions and controls (Table 3). The diameter reported when applying 20 mg L^{-1} of NAA (0.68 mm) alone was larger than the means achieved by deionized water (0.51 mm), 15 mg L^{-1} of NAA (0.52 mm), and 3000 mg L^{-1} of IBA (0.5 mm). The greatest first-order root length was obtained when applying 20 mg L^{-1} of NAA (33.91 mm) in

Table 3. Comparison of means by Fisher's least significant difference test of root diameter and length of longest first and second order root in cuttings of *Vaccinium corymbosum* L. 'Biloxi' with the effects of various treatments.

Treatment	Composition (mg L^{-1})	Root diameter (mm)	Root length (mm)	
			First order	Second order
1	0 NAA+ 0 SAL	0.51 b	18.69 c	8.63 b
2	15 NAA+ 0 SAL	0.52 b	20.21 bc	8.44 b
3	20 NAA+ 0 SAL	0.68 a	33.91 a	17.60 ab
4	0 NAA+ 50 SAL	0.56 ab	29.60 abc	14.35 ab
5	0 NAA+ 75 SAL	0.58 ab	32.44 ab	19.67 a
6	15 NAA+ 50 SAL	0.58 ab	25.96 abc	13.67 ab
7	15 NAA+ 75 SAL	0.57 ab	23.27 abc	13.85 ab
8	20 NAA+ 50 SAL	0.55 ab	25.34 abc	9.51 b
9	20 NAA+ 75 SAL	0.65 ab	27.70 abc	12.42 ab
10	*Plantago* spp.^{z}	0.57 ab	21.65 abc	8.24 b
11	4000 IBA^{z}	0.65 ab	31.19 abc	16.77 ab
12	3000 IBA^{z}	0.50 b	25.52 abc	14.36 ab
F-NAA	0	0.55 a	26.91 a	14.22 a
	15	0.55 a	23.14 a	11.99 a
	20	0.63 a	28.99 a	13.18 a
F-SAL	0	0.57 a	24.27 a	11.56 a
	50	0.56 a	26.97 a	12.51 a
	75	0.60 a	27.81 a	15.32 a
Overall average number of treatments		0.57	26.29	13.12
CV (%)		19.08	34.76	51.73
p-value of treatments		0.0103 *	0.0176*	0.0298 *
p-value of F-NAA		0.1687 ^{ns}	0.2960^{ns}	0.7244 ^{ns}
p-value of F-SAL		0.7068 ^{ns}	0.6170^{ns}	0.3803 ^{ns}
$LSD_{0.05}$ of treatments		0.16	13.11	9.74
$LSD_{0.05}$ of factors		0.08	7.85	5.72

NAA: naphthaleneacetic acid; SAL: salicylic acid; IBA: indolebutyric acid; ^{z}: additional control; F-NAA: naphthaleneacetic acid factor; F-SAL: salicylic acid factor; CV: coefficient of variation; ^{ns}: not significant; *: significant differences; $LSD_{0.05}$: least significant difference at a significance of 0.05. Values with the same letter are statistically similar according to Fisher's least significant difference test ($p \leq 0.05$).

relation to that of deionized water (18.69 mm) and to the concentration of 15 mg L^{-1} of NAA (20.21 mm). On the other hand, the second-order root length reported with 75 mg L^{-1} of SAL alone was greater than that of deionized water, 15 mg L^{-1} of NAA, 20 + 50 mg L^{-1} of NAA and SAL, and the *Plantago* spp. extract (Table 3).

The results are different from those published by Sardoei *et al*. (2014), where SAL application was not needed to obtain the best root length in *E. pulcherrima* (28.1 cm). On the other hand, the results reported for the treatment with 15 mg L^{-1} of NAA differ from Das *et al*. (1997), who indicate that NAA can generate good sources of energy and carbon for root formation. This may be attributed to the concentration used, since

in other studies they applied concentrations of 100 and 4000 mg L^{-1} of NAA with good results in blueberry 'Biloxi' (Leiva *et al*., 2023) and *V. meridionale* (Ligarreto *et al*., 2013). These effects depend on the plant species used (López-Corona *et al*., 2019), which is reflected in the results shared by Kim *et al*. (2014), where the best root lengths were achieved with 500 mg L^{-1} of IBA in blueberry 'Bluecrop' (7.5 cm) and 'Duke' (7.6 cm), while in 'Sunrise', IBA did not present the same effect, since the best value was obtained with 500 mg L^{-1} of NAA (6.0 cm).

In this study, IBA did not stand out for these variables, as opposed to Coimbra *et al*. (2016), An *et al*. (2018), and Colombo *et al*. (2018), who achieved greater lengths when using 3000 mg L^{-1} of IBA on *B. excelsa* (7.0 cm), 'Biloxi' blueberry (4.0 cm), and 'Powderblue' blueberry (6.38 cm) cuttings, respectively. Likewise, Higuchi *et al*. (2021), when applying 1000 mg L^{-1} of IBA, reported a root length of 14.33 cm in 'Woodard' blueberry, while Tejada-Alvarado *et al*. (2021) obtained a root length of 27.51 mm when applying 2000 mg L^{-1} of IBA in wild blueberry.

Root volume

The analysis of variance showed that the factors had no significant effect on root volume (Table 4).

Likewise, for the interactions and controls, there were statistical effects on root volume, where the values reported for the treatments with 20 mg L^{-1} of NAA (81.87 mm^3) and 75 mg L^{-1} of SAL (85.62 mm^3) were statistically identical to the treatments with 50 mg L^{-1} of SAL (46.25 mm^3), 4000 mg L^{-1} of IBA (73.12 mm^3), and 3000 mg L^{-1} of IBA (46.25 mm^3), but evidently greater than the rest of the treatments (Table 4). Since this variable represents the space occupied by the developed root, it is inferred that with 20 mg L^{-1} of NAA, 75 mg L^{-1} of SAL, and 4000 mg L^{-1} of IBA, a greater number of roots was obtained.

The positive effects of NAA on rooting stimulation are due to the fact that NAA tends to accumulate indoleacetic acid faster (Hopkins and Hüner, 2008), coupled with an acceleration in starch hydrolysis, decreasing stake sugars, and generating large sources of energy and carbon for root formation (Das *et al*., 1997). In contrast, IBA is directly converted to indoleacetic acid, mainly by processes similar to β-oxidation of fatty acids (Damodaran and Strader, 2019). Dong *et al*. (2020) note that the use of SAL tends to increase endogenous levels of indoleacetic acid in the rooting zone by reducing its degradation through the intervention and inhibition of auxin-conjugating enzymes, thereby enhancing adventitious root development.

On the other hand, Colombo *et al*. (2018) found that optimal substrate physicochemical properties such as temperature, pH, aeration, and moisture retention promote root growth rate. In this regard, it can be concluded that using perlite and peat in a 1:1 volume ratio is a good option for rooting blueberry 'Biloxi' cuttings, as good root development was demonstrated. Other authors, such as Colombo *et al*. (2018), reported similar results when using 3000 mg L^{-1} IBA in rooting cuttings of blueberry 'Powderblueberry.'

Table 4. Comparison of means with Fisher's least significant difference test of root volume in cuttings of *Vaccinium corymbosum* L. 'Biloxi' with the effects of various treatments.

Treatment	Composition (mg L^{-1})	Root volume (mm^3)	
1	0 NAA+ 0 SAL	16.25	c
2	15 NAA+ 0 SAL	19.00	c
3	20 NAA+ 0 SAL	81.87	a
4	0 NAA+ 50 SAL	46.25	abc
5	0 NAA+ 75 SAL	85.62	a
6	15 NAA+ 50 SAL	35.00	bc
7	15 NAA+ 75 SAL	29.50	bc
8	20 NAA+ 50 SAL	33.75	bc
9	20 NAA+ 75 SAL	36.25	bc
10	*Plantago* spp.^{z}	25.62	c
11	4000 IBA^z	73.12	ab
12	3000 IBA^z	46.25	abc
F-NAA	0	49.37	a
	15	27.83	a
	20	50.62	a
F-SAL	0	39.04	a
	50	38.33	a
	75	50.46	a
Overall average number of treatments		44.04	
CV (%)		70.22	
p-value of treatments		0.0097	**
p-value of F-NAA		0.1422	ns
p-value of F-SAL		0.5642	ns
$LSD_{0.05}$ of treatments		44.35	
$LSD_{0.05}$ of factors		26.22	

NAA: naphthaleneacetic acid; SAL: salicylic acid; IBA: indolebutyric acid; ^{z}: additional control; F-NAA: naphthaleneacetic acid factor; F-SAL: salicylic acid factor; CV: coefficient of variation; ^{ns}: not significant; **: highly significant differences; $LSD_{0.05}$: least significant difference at a significance of 0.05. Values with the same letter are statistically similar according to Fisher's least significant difference test ($p \leq 0.05$).

CONCLUSIONS

The combination of naphthaleneacetic acid, salicylic acid, and the *Plantago* spp. extract decreased the rooting percentage and root volume of semi-lignified 'Biloxi' blueberry cuttings. Therefore, to obtain a greater number of rooted cuttings with good root development and guarantee their survival in transplanting and acclimatization, naphthaleneacetic acid, salicylic acid, and indolebutyric acid should be applied individually.

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