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Rooting media and application of auxins in the production of strawberry seedlings

Medios de enraizamiento y aplicación de auxinas en la producción de plántulas de fresa

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Abstract

The production of strawberry with root ball is a somewhat spread propagation technology with which vigorous, healthy, and productive plants can be obtained. The purpose of this research was to evaluate the effect of three rooting media and the application of auxins in strawberry seedling propagation. The experiment was carried out under mesh shade conditions, establishing primary stolons of the Chandler strawberry cultivar in polystyrene foam containers with alveolus of 118 mL, and one stolon per container. The treatments comprised three types of substrates: perlite, pumice and peat, and water with and without the application of indol-3-butyric acid (IBA). Three destructive samplings were carried out at 42, 58, and 72 days after stolon establishment. The experimental design was completely randomized with three replicates, each comprising ten plants in a factorial arrangement. The variables assessed were the following: mortality rate, plant height, crown diameter, number of leaves, leaf area, fresh and dry weight of stem and root, and root quality. The results showed that the type of substrate affects root growth and plant canopy, being pumice the substrate in which higher-quality seedlings were obtained. In general, the exogenous application of auxin did not favor the development of seedlings during the evaluated period.

Keywords: auxins, *Fragaria x ananassa*, plant propagation, seedlings, substrates

Resumen

La producción de plántulas de fresa con cepellón es una tecnología de propagación poco difundida con la que se pueden obtener plantas vigorosas, sanas y productivas. La presente investigación tuvo como objetivo evaluar el efecto de tres sustratos y la aplicación de auxinas en la propagación de plántulas de fresa. El experimento se realizó en condiciones de malla sombra, estableciendo estolones primarios de fresa cultivar Chandler en recipientes de poliestireno con alveolos de 118 mL; se colocó un estolón por recipiente. Los tratamientos consistieron en la evaluación de tres tipos de sustratos: perlita, pumita, turba y agua con y sin aplicación de AIB. Se realizaron tres muestreos destructivos a los 42, 58 y 72 días después del establecimiento. Se utilizó un diseño completamente al azar con tres repeticiones, cada una de las cuales consistió en diez plantas, en arreglo factorial. Las variables evaluadas fueron las siguientes: porcentaje de mortandad, altura de planta, diámetro de corona, número de hojas, área foliar, peso fresco y seco del vástago y raíz, calidad del cepellón. Se determinó que el tipo de sustrato influye en el crecimiento de raíz y dosel vegetal, siendo en el sustrato pumita en el que se obtuvieron plántulas de mayor calidad. En general, la aplicación exógena de la auxina no favoreció el desarrollo de las plántulas durante el periodo evaluado.

Palabras clave: auxinas, *Fragaria* × *ananassa*, plántulas, propagación de plantas, sustratos de cultivo

Introduction

Strawberry plants (*Fragaria × ananassa* Duch.) belong to the Rosaceae family and are clones that propagate through stolons or daughter plants and are transplanted bare rooted or with a root ball (Ruan, Yoon, Yeouna, Larson, & Ponce, 2009). Nonetheless, obtaining quality seedlings requires clones with genetic and physiological quality and a favorable environment for root and stem development (Giménez, Andriolo, Janisch, & Godoi, 2008). Currently, in many production areas, strawberry plant propagation technologies are poorly developed, and the seedlings that are obtained are often of low quality, low in carbohydrates, with infections due to root pathogens and crown fungi, what causes that after the transplant, problems occur in the successful establishment of strawberry plants (Ruan et al., 2009).

The production of strawberry seedlings with root ball is not widespread despite the advantages it has, such as better health, ease for mechanized planting (López-Pérez, Cárdenas-Navarro, Lobit, Martínez-Castro & Escalante-Linares, 2005), reduction of water consumption at the time of transplantation, increase in the survival percentage either in open field or greenhouse conditions (Durner, Barclay, & Mass, 2002), and promptness in the entry into production (Giménez et al., 2008).

Stolons for rooting are classified according to their initial size and weight since these factors influence the final diameter of the crown. Takeda and Newell (2006) recommend choosing stolons that weigh more than 1 g and have a minimum crown diameter of 5 mm. Seedlings for greenhouse transplantation should be propagated in containers with a cell volume of 280-300 cm^3, and they must remain in these until four months before the definitive transplant (Bish, Cantliffe, & Chandler, 2002). However, time cannot be decisive since the growth and development of plants also depend on other conditions, such as temperature and radiation.

In the production of seedlings, the use of substrates and growth regulators is widespread. The substrate favors an adequate distribution of roots and guarantees the supply of water, air, and nutrients at low humidity tensions; these factors facilitate rapid growth and establishment of plants (Pire & Pereira, 2003). The use of growth regulators is a common practice to induce the formation of adventitious roots in vegetative propagation by cuttings (Taiz & Zeiger, 2006). In this sense, mainly auxins, indole-3-acetic acid (IAA), and indole-3-butyric acid (IBA) are used (Medhi, 2002).

The effect due to the application of auxins in plants depends on the concentration (Rastogi et al., 2013). The rooting process consists of two stages: primordial root formation and root growth; both stages require auxins, and their needs depend on the species (Acosta, Sánchez, & Bañon, 2000). In this context, the rooting of many species is very fast in a great diversity of substrates such as soil, sand, peat, vermiculite, among others. However, there are no reports about an appropriate growth medium for the production of strawberry seedlings by adding auxins.

Materials and methods

The experiment was carried out from June to August 2016 under shade mesh conditions with 35% shade, in the Agricultural Academic Unit of the Universidad Autónoma de Nayarit, located at the following coordinates: latitude 21°25'36" N and longitude 104°53'28" W, at an altitude of 922 m above the sea level. Primary strawberry stolons of the Chandler cultivar of 5 cm of height obtained from mother plants with 0.5 cm of roots were used. During the evaluated period, the minimum temperature recorded was 17 °C and the maximum temperature was 41 °C. The minimum relative humidity was 30 %, and the maximum was 80 %. The light intensity during the period evaluated was 746.9 nmol m2.

A completely randomized experimental design with a 4 × 2 treatment design was used. The factors evaluated were three substrates and a nutritive solution. These four growth media were evaluated with and without the application of a synthetic auxin, resulting in a total of eight treatments. The substrates were the following: perlite, pumice (3-7 mm in diameter), and peat. Indole-3-butyric acid (IBA) at 0.15 % was used, prepared in agricultural talc in a homogeneous mixture, and the stolons were impregnated at the base and planted in the growth medium.

The experimental unit consisted of a stolon placed in a polystyrene container with a capacity of 118 cm^3, with three repetitions per treatment, each consisting of ten plants. Irrigation with the Steiner (1984) nutritive solution at a concentration of 75 % was applied daily in the morning hours on the substrates. Seedlings were kept under shade mesh conditions throughout the experimental period. Some physical and chemical characteristics of the substrates, which were established before starting the test according to the methodology indicated by Pire and Pereira (2003) are shown in table 1.

Table 1. Physical and chemical characteristics of the substrates used for the production of strawberry seedlings

Sustratos	Total porosity (%)	Aeration capacity (%)	Moisture retention capacity (%)	pH	CE$^{\&}$ dS.m^{-1}
Perlite	94.20	37.20	50.78	7.70	1.51
Pumice	90.21	29.10	59.44	7.35	1.32
Peat	94.22	30.36	58.34	7.00	2.62
Nutritious solution	---	---	---	7.34	0.22

EC$^{\&}$: electrical conductivity.
Source: Elaborated by the authors

The substrate selection was made based on the ideal substrate proposed by Cabrera (1999), who indicated that the ideal substrate must have a minimum total porosity of 70 %, 50 % moisture retention capacity, and 20 % aeration.

Evaluation stages and quantified variables

The first stage corresponds to the first 15 days after the establishment (DAE) of the stolons, where the seedling mortality was evaluated since it represents a critical period for rooting or death. Seedlings that looked healthy and did not show signs of imminent death were found.

The second stage included three evaluation periods at 42, 58, and 72 DAE, and the following variables were quantified: seedling height and root length using a flexometer; the number of leaves per stem counting fully expanded leaves; crown diameter of each seedling using a digital vernier. The leaf area was established by destructive sampling and using a portable leaf area meter CI-202 CID (BioScience Inc.). Fresh stem weight (g) and fresh root weight (g) were quantified on a digital scale Ohaus® CS2000; for dry stem and root weight, the material was dried in an oven at 70 °C until a constant weight was obtained. The quality of the root ball was established when the seedling was removed from the container using the visual evaluation scale proposed by Quesada and Méndez (2005) as follows: 1, 100 % of the root ball is intact; 2, 90 % of the root ball is intact; 3, 75 % of the root ball is intact; 4, 50 % of the root ball is intact, and 5, less than 50 % of the root ball is intact. All the statistical analyses were performed with the SAS statistical software version 9.4 (Statistical Analysis System, 2004), in which an analysis of variance and a multiple comparison of means test were performed employing Tukey's procedure ($\alpha \leq 0.05$).

Results and discussion

Seedling mortality (15 DAE) depending on the substrate was as follows: peat, 7.5 %; water, 12.5 %; pumice, 15 %, and finally, perlite, 25 %. This indicates that the type of substrate has an effect on survival, which is mainly due to the physical and chemical characteristics of the substrates.

In studies conducted with substrates for the production of seedlings of horticultural species, the physical characteristics of the peat helped the seedlings remain moistened, favoring the development of the leaves and the accumulation of dry matter (Ortega-Martínez, Sánchez-Olarte, Díaz-Ruíz, & Ocampo-Mendoza, 2010). Other factors that could have influenced the development of the leaves are the aeration and the temperature of the growth medium (Calderón, Angulo, Rodríguez, Grijalba, & Pérez, 2013). At 72 DAE, statistical differences were found in the eight variables evaluated (table 2).

Table 2. Mean squares of the analysis of variance for the variables evaluated in the production of strawberry seedlings at 42, 58 and 72 days after establishment (DAE)

Variable	Source of variation	df§	MSE	42 DAE	MSE	DAE	MSE	72 DAE
Seedling height	Substrate (S)	3	0.89	0.40	5.38	0.001	26.53	< 0.0001
	Auxin (±A)		0.03	0.84	0.13	0.64	60.34	< 0.0001
	S x ±A	1	3.39	0.03	2.99	0.01	7.18	< 0.0001
	MSE§§			0.85		0.58		0.7170
	Pr > F§§§	3		NS		NS		**
Number of leaves	Substrate (S)	3	0.50	0.02	1.00	0.09	4.27	0.0002
	Auxin (±A)		0.00	1.00	0.66	0.21	8.16	0.0002
	S x ±A	1	0.33	0.08	1.88	0.01	4.27	0.0002
	MSE			0.12		0.38		0.3154
	Pr > F	3		NS		NS		*

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(Continuation of table 2)

Variable	Source of variation	df[§]	MSE	42 DAE	MSE	DAE	MSE	72 DAE
Root length	Substrate (S)	3	40.27	<0.0001	4.21	0.27	63.62	< 0.0001
	Auxin (±A)		0.58	0.47	5.27	0.20	108.35	< 0.0001
	S x ±A	1	12.08	0.00	8.08	0.08	100.12	< 0.0001
	MSE			1,08		2.95		0.5535
	Pr > F	3		NS		NS		**
Crown diameter	Substrate (S)	3	2.37	0.05	2.59	0.10	18.98	< 0.0001
	Auxin (±A)		0.94	0.27	4.27	0.06	55.95	< 0.0001
	S x ±A	1	5.38	0.003	4.29	0.02	33.66	< 0.0001
	MSE			0.72		1.05		1.1476
	Pr > F	3		NS		NS		**
Leaf area	Substrate (S)	3	773.87	< 0.0001	2884.30	0.004	12495.13	< 0.0001
	Auxin (±A)		147.38	0.04	44.14	0.75	25325.63	< 0.0001
	S x ±A	1	645.80	< 0.0001	1685.12	0.03	20825.07	< 0.0001
	MSE			29.67		427.29		106.4954
	Pr > F	3		*		NS		**
Fresh stem weight	Substrate (S)	3	0.79	0.01	1.02	0.13	28.0762	< 0.0001
	Auxin (±A)		0.41	0.13	0.18	0.53	58.5937	< 0.0001
	S x ±A	1	0.80	0.01	2.13	0.01	44.9762	< 0.0001
	MSE			0.16		0.45		1.1542
	Pr > F	3		NS		NS		**

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(Continuation of table 2)

Variable	Source of variation	df[§]	MSE	42 DAE	MSE	DAE	MSE	72 DAE
Dry stem weight	Substrate (S)	3	0.01	0.29	0.11	0.09	1.9225	< 0.0001
	Auxin (±A)		0.11	0.01	0.01	0.57	2.3437	< 0.0001
	S x ±A	1	0.07	0.01	0.21	0.01	1.8862	< 0.0001
	MSE			0.01		0.04		0.0650
	Pr > F	3		NS		*		**
Dry root weight	Substrate (S)	3	0.03	< 0.0001	0.01	0.21	0.9184	< 0.0001
	Auxin (±A)		0.01	0.0004	0.02	0.16	0.5859	0.0007
	S x ±A	1	0.01	< 0.0001	0.09	0.001	0.1909	0.0069
	MSE			0.0007		0.01		0.0310
	Pr > F	3		*		NS		*

[§] Degrees of freedom, [§§] MSE = Mean square of the error, [§§§] Probability, * significance at 5 %, ** significance at 1 %, NS: not significant.
Source: Elaborated by the authors

The seedlings took 72 days to acquire the features required for transplantation stated by Houchmuth et al. (2006); however, this period contrasts with what Bish et al. (2002) found, as these authors indicated a time of four months, with a maximum and minimum temperature of 25/15 °C, while those recorded in the conditions under which the current study was conducted (40/17 °C), could have accelerated seedling growth.

Seedling height

The highest seedling height was obtained in the peat substrate at 72 DAE, with a value of 10.33 cm per plant (table 3). These results are similar to those obtained by Tehranifar, Poostchi, Arooei and Nematti (2007) in the strawberry cultivars Camarosa and Gaviota established in a mixture of peat and coconut fiber, where the vegetative growth was higher compared to the plants grown in 100 % sand and perlite. The application of IBA did not favor seedling height, an effect reported by Medhi (2002) and Rastogui et al. (2013), who found that the exogenous application of auxins around the roots inhibited growth.

Number of leaves per stem

Seedlings that grew in perlite, pumice, and peat had more leaves compared to those that grew in water. The application of IBA decreased leaf formation in seedlings; in contrast, with the non-application of IBA, a higher number of leaves/stems was obtained. At 72 DAE, an average of 3.67 leaves/stem was obtained compared to the treatment with the application of IBA, in which only 2.50 leaves/stem were obtained (table 3).

Table 3. Comparison of means for the variables evaluated in the production of strawberry seedlings 42, 58 and 72 days after establishment (DAE) depending on the substrate and the exogenous application of indol-3-butyric acid (IBA) for rooting

Variable evaluated	Source of variation	42 DAE	58 DAE	72 DAE
Seedling height (cm)	Perlite	3.25 a	3.41 b	8.66 b
	Pumice	3.00 a	4.25 b	8.91 ab
	Peat	3.83 a	5.58 a	10.33 a
	Water	3.91 a	4.00 b	3.00 c
	With auxin	3.45 a	4.20 a	6.04 b
	Without auxin	3.54 a	4.41 a	9.41 a
Number of leaves	Perlite	2.33 a	2.50 a	3.50 a
	Pumice	2.16 ab	3.00 a	3.33 a
	Peat	2.16 ab	3.00 a	3.66 a
	Water	1.66 b	2.16 a	1.83 b
	With auxin	2.08 a	2.50 a	2.50 b
	Without auxin	2.08 a	2.83 a	3.66 a
Crown diameter (cm)	Perlite	5.42 ab	6.06 a	8.09 a
	Pumice	6.14 a	5.97 a	8.72 a
	Peat	5.55 ab	6.54 a	8.69 a
	Water	4.61 b	7.41 a	4.99 b
	With auxin	5.23 a	6.07 a	6.10 b
	Without auxin	5.63 a	6.92 a	9.15 a
Root length (cm)	Perlite	11.50 a	14.12 a	1.25 a
	Pumice	8.50 b	12.87 a	14.50 a
	Peat	10.50 a	14.50 a	14.75 a
	Water	5.62 c	12.62 a	8.00 b
	With auxin	8.87 a	12.81 a	10.75 b
	Without auxin	9.18 a	13.75 a	15.00 a

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(Continuation of table 3)

Variable evaluated	Source of variation	42 DAE	58 DAE	72 DAE
Fresh stem weight (g)	Perlite	1.40 ab	3.65 a	7.70 b
	Pumice	1.85 a	3.65 a	9.27 ab
	Peat	1.80 a	4.05 a	10.32 a
	Water	1.07 b	3.05 a	5.35 c
	With auxin	1.40 a	3.51 a	6.60 b
	Without auxin	1.66 a	3.68 a	9.72 a
Dry stem weight (g)	Perlite	0.32 a	0.87 a	1.87 a
	Pumice	0.40 a	0.80 a	2.30 a
	Peat	0.37 a	0.95 a	2.25 a
	Water	0.27 a	0.62 a	1.07 b
	With auxin	0.27 b	0.78 a	1.56 b
	Without auxin	0.41 a	0.83 a	2.18 a
Dry root weight (g)	Perlite	0.12 b	0.37 a	0.50 c
	Pumice	0.25 a	0.35 a	0.82 b
	Peat	0.15 b	0.35 a	1.15 a
	Water	0.07 c	0.25 a	0.25 c
	With auxin	0.12 b	0.36 a	0.52 b
	Without auxin	0.17 a	0.30 a	0.83 a

Different letters in columns indicate statistical difference.
Source: Elaborated by the authors

Crown diameter

For the crown diameter, there were no significant differences during the first two samplings. However, in the third stage, the treatments with solid substrates exceeded by 58% the crown diameter values compared to the liquid substrate in which 4.99 mm were obtained.

The results are similar to those obtained by Türkben (2008) in rooting media based on soil, cow dung and sand (1:1:1), obtaining crown diameters of 8.4 mm in strawberry cultivars Brio, Pocahontas, and Selva. However, it is important to note that, in this test, the crown diameters obtained in the seedlings subjected to different treatments at 72 DAE in the culture medium, showed lower values compared to those found by Bartczak, Pietrowska, and Kanaflewski (2007). These authors obtained crown diameters of 18 mm in the Elsanta cultivar rooted in rock wool, and 10 mm crowns for the Honeoye cultivar rooted in a mixture of coal with ground rock wool; it must be noted that these diameters were achieved at 100 DAE. Moreover, these authors mention that the crown diameter is a good indicator of plant growth and yield intensity. Durner et al. (2002) stated that 8 mm is the minimum value of the crown size for propagation. The crown diameter is vital in a strawberry seedling since it represents the vigor and the amount of reserves that the individual will have to grow and develop (Carrillo-Mendoza, Rodríguez-Alcázar, & López-Jiménez, 2005). In this sense, the application of IBA did not favor the growth of a larger crown diameter.

Root length

The Pumice substrate was the one that most favored root development (14.50 cm) from 58 DAE (table 3). This response could be favored by the physical and chemical characteristics of this material, among which the high porosity (28.21 %) and moisture retention of 9.44 % stand out, which represented better root oxygenation. However, at 72 DAE, there were no significant differences in the root length of the seedlings grown in perlite (14.50 cm), pumice (14.75 cm), and peat (14.25 cm).

The results of this study are superior to those obtained by Türkben (2008), who employing three rooting means, such as soil: cow dung: sand (1:1:1); peat: perlite + nutrients (1:1) and soil: peat: perlite: sand: cow dung (0.75:1:1:0.75:0.5) recorded root lengths of 10.59 cm, 10.08 cm, and 11.14 cm, respectively. During the three evaluation stages, the shortest root length was obtained with the treatment with water.

The plants subjected to the treatment without IBA had longer root length (table 3). It follows that the endogenous concentration of auxins in stolons is sufficient to develop the root since they are structures with high meristematic activity; therefore, the use of exogenous auxins to ensure rooting is not recommended. Taiz and Zeiger (2006) indicated that the root cells usually contain sufficient or almost sufficient auxin for normal elongation, plus the fact that many cut roots grow *in vitro* for days or weeks without adding auxin, indicating that its likely need for this hormone is satisfied by its ability to synthesize it.

The exogenous application of auxins decreases the response of the stolon to produce roots and increase their length, which may be because separating the stolon from the mother plant may result in changes in response to the exogenous application of auxins that inhibit root elongation, as indicated by Medhi (2002).

Leaf area

The development of the leaf area shown by the seedlings established in pumice in the three evaluation moments were located in the statistical group with the largest leaf area, and those that grew in the treatment with water had the lowest leaf area (figure 1).

The lower development of the leaf area in water could be due to the lack of oxygenation of this substrate and the low absorption of nutrients by the root. The behavior between treatments with and without IBA; the application of IBA affected the leaf area accumulation is shown in figure 2; this may be because when auxins are not used, the formation of roots in the stolon is favored and, hence, leaf development.

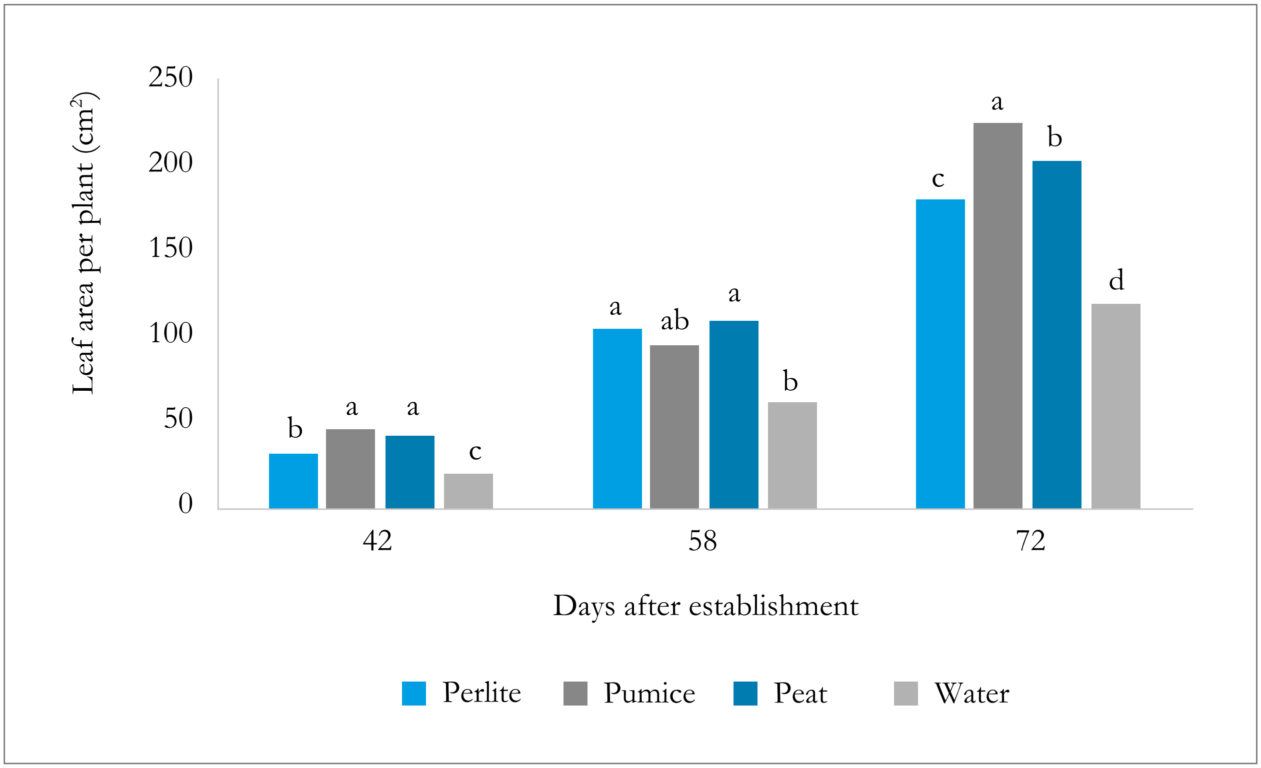

Figure 1. Leaf area development in strawberry seedlings cv. Chandler per substrate.
Source: Elaborated by the authors

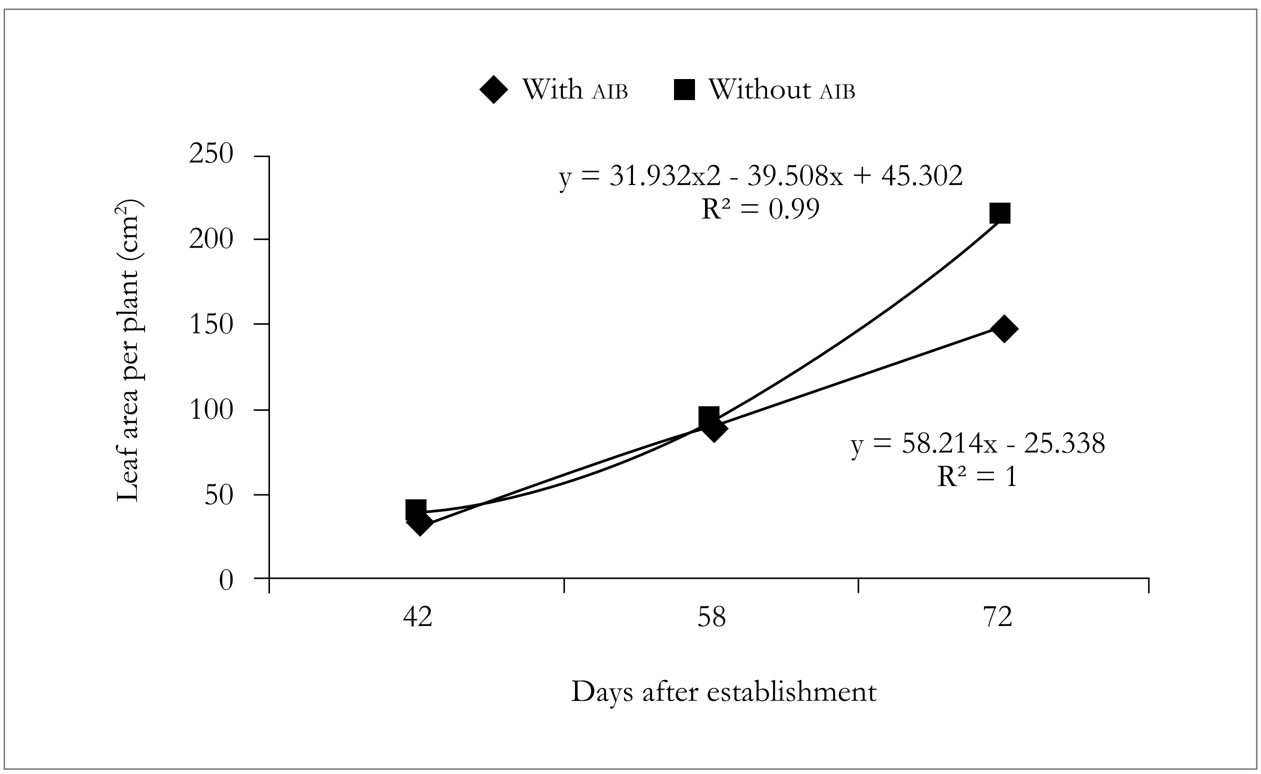

Figure 2. Effect of the application of IBA in leaf area production in strawberry cv. Chandler seedlings.
Source: Elaborated by the authors

Fresh and dry stem weight

The fresh weight of the stolon stems showed no differences between treatments during 42 and 58 DAE; a fresh weight of 10.32 g was obtained in the third sampling with the peat treatment.

These results are similar to those found in other strawberry cultivars such as Festival (14.23 g) and CP-Zamorana (11.52 g), which were propagated in cultivated beds with soil (Rodríguez-Bautista, Calderón-Zavala, Jaen-Contreras, & Curiel-Rodríguez, 2012) and there is a notable difference compared to stolons that were placed in water (5.35 g). The exogenous application of IBA did not increase the fresh weight. Without the application of IBA, the accumulation of fresh matter from seedlings increased. The dry weight of the stem increased with the use of solid substrates (perlite, pumice, and peat) compared to the use of water as a substrate (table 3); this result was 50 % lower than what Rodríguez-Bautista et al. (2012) reported in CP-Zamorana strawberry (4.74 g of stem weight).

Dry root weight

At 72 DAE, the treatments with peat and pumice stood out by obtaining a higher dry root weight with 1.15 and 0.82 g, respectively. These values are, however, lower than those reported by Carrillo-Mendoza et al. (2005) of 1.54 g per plant. In the treatment without exogenous IBA, the accumulation of total dry matter, both from the root and from the vegetative organs, increased.

Root ball quality

The pumice substrate obtained the value of 1 because it achieved 100 % integrity of the root ball, showing good posture and detachment ease from the container used. The substrate that showed compaction was peat and its rating was 2 with 90 % integrity in the root ball, since it releases parts of the substrate and shows some cracks when it is separated from the container. The root ball formed with the perlite substrate was assigned the category of 4 according to the scale proposed by Quesada and Méndez (2005), since at the end of the container used, it left at least 50 % of the root ball and, therefore, it is not suitable for the production of strawberry seedlings with root ball.

Conclusions

The use of pumice as a substrate allows obtaining higher quality seedlings compared to perlite, peat, and water substrates. The seedlings that grew in pumice exceeded the other substrates in leaf area values. Regarding seedling height, crown diameter, number of leaves, fresh and dry stem weight, and root length, it was statistically among the substrates that had the highest values. The exogenous application of IBA in strawberry stolons affected the procurement of quality seedlings.

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Disclaimers

All authors made significant contributions to the document and agree with its publication; furthermore, the authors state that there are no conflicts of interest in this study.

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