Article

Effect of water quality and substrates in the production of stevia seedlings (*Stevia rebaudiana* Bertoni)

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Abstract

The production of seedlings by cuttings for the sowing of stevia, is one of the most used methods in Mexico and in the world, for the economic and its relative ease to make them; however, the production of *Stevia rebaudiana* Bertoni seedlings with cuttings is limited by the use of very hard waters with high electrical conductivity, so that sometimes the roots do not develop and the cuttings dry up. The objective of the work was to evaluate three types of substrates and three water qualities, in the development of *S. rebaudiana* seedlings in the state of Yucatan. The study was carried out in Muna Yucatan, and three types of substrates were tested (commercial foam, moss-based mixture and coconut fiber) and three qualities of irrigation water (2 130 ppm, 1 230 ppm and 330 ppm of dissolved solids total) in the production of seedlings of the Morita II variety. The 100 cuttings were established per treatment and the length (cm) of the root and the height of the seedling were measured every 10 days. An effect of the type of substrate ($p< 0.05$) on seedling height and root size was found. With regard to water quality, an effect was found on the seedling height ($p< 0.05$), but not on the length of the root ($p> 0.05$). It is concluded that the substrate based on mixture of moss and coconut fiber formed roots in less time and produced higher seedlings. This is an advantage since the production time is reduced and there is a greater probability of survival of seedlings to the transplant.

Keywords: root development, cuttings, salinity.

Reception date: march 2018
Acceptance date: may 2018

Introduction

The S. *rebaudiana* is a perennial herbaceous plant that belongs to the Asteraceae family. It is a native shrub native to the mountain slopes of Paraguay, grows between 65 and 80 cm in height and propagates sexually in its natural habitat, but also asexually for commercial plantations (Jarma *et al*., 2010; Goyal *et al*., 2010; González, 2011).

Stevia produces non-caloric sweeteners such as stevioside and rebaudiosides. For this reason, the crop has spread from its center of origin Paraguay and Brazil, to almost the entire world; however, the studies on the agronomic management of this species are limited, particularly related to their water needs and their sensitivity to both water deficits and excess salts in the soil (González *et al.*, 2016).

The sexual reproduction of *S. rebaudiana* is by cross-fertilization (alogama), a condition that causes great phenotypic variability and in the composition of the active principles in the leaves, which generates an opportunity to select outstanding plants for breeding purposes. Seed reproduction for commercial plantations is not recommended due to the existing variability and vegetative or asexual reproduction is suggested (Ramírez *et al*., 2011). In Mexico there is a great interest in the cultivation of stevia and one of the main limitations for its expansion and development in the country is the lack of vegetative material. At present there is a demand for seedlings not satisfied, so that it is necessary to have technologies for their production that allows the expansion of their cultivation in the country.

Propagation is an important point within the production system of *S. rebaudiana*, the asexual one being through cuttings the most used method at present, since it allows to conserve the phenotypic and genotypic characteristics of the progenitor plant (Landazuri and Tigrero, 2009; Ramírez *et al.*, 2011). On the other hand, they are more economical than those obtained through tissue culture.

To successfully achieve the production of stevia seedlings, two main factors must be considered: the substrate that supports the seedling and the quality of the irrigation water. A substrate is a support medium, which stores and supplies nutrients, water and air to the root system. The purpose of a substrate is to promote good growth, within the space of a container, and to prepare the seedlings for a successful transplant (Alvarado and Solano, 2002).

One of the main factors to consider in the quality of the water for irrigation is the presence of salts, since all the water that is used in the irrigation contains a certain quantity of dissolved salts. The suitability of water for irrigation depends in general on the types and quantities of salts it contains. All salts from irrigation waters have an effect on water-soil-plant relationships and soil properties, and indirectly on plant production (Anonymous, 1995).

The substrates and water quality used for the production of seedlings are parameters that must be selected for the proper development of the seedling cutting. In the state of Yucatan, the production of stevia has faced problems such as the high salinity of water, which can diminish the capacity of the production potential of seedlings and to date it is unknown which substrate is the most indicated for its production in protected structures. In Mexico, research into the culture of *S. rebaudiana* is emerging, and there is very little information on the effects of the interaction between the type of substrate and water quality in the production of stevia seedlings. Due to this, the objective of the work was to evaluate different types of substrates and three qualities of water, in the development of *S. rebaudiana* seedlings in the southern zone of the state of Yucatán.

Materials and methods

Location of the experimental site. The work was carried out in a protected structure located in the Experimental Uxmal Site, in the municipality of Muna, Yucatán, which has a subhumid warm climate with regular rains in summer, average annual temperature of 25.2 °C, average maximum temperature of 36 °C in the month of May and 14.3 °C as the minimum average in January.

Seedling production It was carried out in a shadow house of 8 x 24 m with a waterproof roof and anti-aphids mesh on the sides. The Morita II variety was used for the production of seedlings, the cuttings were selected from a mother plantation of approximately six months, with a size of 12 to 15 cm long and 2 to 3 mm in diameter, preferably from the apical ends of branches not flowered, since it has been found that they have a better development (Ramírez *et al*., 2011).

Unicel trays of 33 cm wide x 67 cm long and 6.5 cm high with 200 cavities and drilling in the base for drainage were used to establish the seedlings (Lozano and Ramírez, 2017). In the case of phenolic foam, pieces of 2.5 x 2.5 x 3.5 cm were cut for use in the cavities of the trays. The trays were irrigated once a day until saturation, so that the cuttings were kept moist with the water that corresponded to them according to each treatment. Purified water produced using a softener, reverse osmosis and ultraviolet light and well water from the experiment site in Muna was used.

Variables evaluated. For the production of seedlings, two factors were studied, type of substrate and irrigation water quality.

The commercial substrates evaluated were three: substrate 1. Light and inert phenolic foam with a pH of 6 to 6.5 and a cation exchange capacity (CIC) of 1.9, substrate; 2. Formulated with a uniform combination of *Sphagnum canadiense* moss and vermiculite with pH between 5.9 to 6.2 and a CIC of 27 and substrate; and 3. Formulated with coconut fiber and has a pH of 5.7 to 6.3 and a CIC of 39.

The water types were three and the following chemical characteristics were considered: total dissolved solids (SDT), pH, electrical conductivity, sodium absorption ratio and interchangeable sodium percentage among others. The SDTs were determined in the field with a digital HM model EZ equipment with the following readings; High SDT (2 130 ppm SDT from well water from Muna Yucatán), medium SDT (1 230 ppm SDT, 50:50 well water and purified water) and low SDT (330 ppm SDT from purified water) and it was complemented with the analyzes of the water from the deep well (60 m) considered as treatment 1, water from the treatment of purified water treatment 3 and a mixture of both as treatment 2 (Table 1).

Table 1. Chemical characteristics of the water used to irrigate S. *rebaudiana* seedlings.

Parameters	T1	T2	T3	Level
Total dissolved solids (ppm)	2 130	1 230	330	250 a 1 500
pH	7.11	7.00	6.92	6.5-8.0
Electrical conductivity (mS cm^{-1})	3.04	2.00	1.27	0.2. a 2.0
Sodium absorption ratio	10.82	5.24	4.23	<10
Exchangeable sodium percentage (%)	0.88	1.55	2.22	

The response variables were: root length, for which five samples of each treatment were measured 12 and 22 days after the cuttings were established in the trays and height of seedlings which were measured ten random samples on days 3, 12 and 27 after planting.

Statistical analysis. A factorial analysis 3 x 3 (9 treatments) was carried out with 100 seedlings per treatment, the seedling being the unit of repetition. An analysis of variance and a comparison of means were made by the Duncan method, to establish the differences between the treatments, using the Statgraphics Centurion XV program.

Results and discussion

Seedling height

For the development and growth of seedlings, the substrate used is a fundamental factor, since it contributes to the quality of the seedling (Ortega, 2010). Currently there are a lot of materials that can be used for the production of substrates and their choice will depend on the plant species to propagate, propagating type, sowing season, propagation system, cost, availability and characteristics of the substrate (Hartman and Kester, 2002).

According to its elaboration, there are several types of substrates used for the production of seedlings, since in few occasions the soil is used as a substrate due to the difficulty in its handling, for which reason it is necessary to select a material that replaces it so much in its physical as well as chemical properties, such as cation exchange capacity, porous space, water retention capacity, pH and electrical conductivity (Valdéz and Benavides, 2013).

Of the three substrates evaluated in this work for the production of stevia seedlings, the results indicated that there were significant statistical differences ($p< 0.05$) in the height of the seedlings between substrates. It can be seen in Figure 1 and 2, that substrates 2 and 3 based on mixture of moss and coconut fiber (11.4 and 10.8 cm in height respectively), showed a greater growth with respect to substrate 1 (6.8 cm) of commercial foam. This may be due to the fact that chemically inert substrates, such as synthetic foam (substrate 1) act only as a support for the plant, while the rest are also involved in adsorption processes and are chemically active as the substrates, 2 and 3 (a base of moss and coconut fiber) since it has been reported that they intervene in the fixation of nutrients (Pastor, 2000).

Figure 1. Development of stevia seedlings 22 days after sowing in three different substrates.

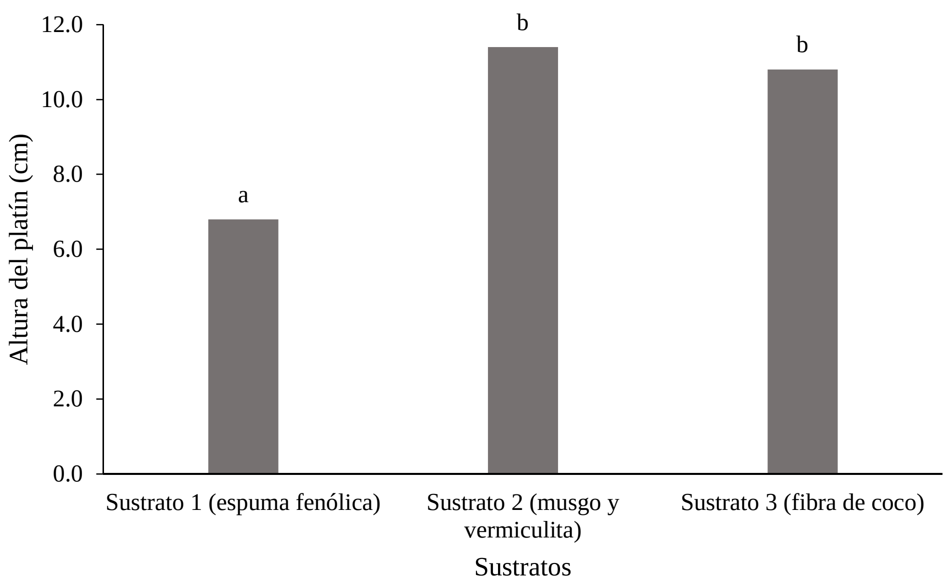

Figure 2. Height of the stevia seedling in three types of substrates.

The substrate 1, because it is a solid, inert, single-component substrate with a low cation exchange capacity (CIC), only acted as support for the seedlings, which resulted in the lowest growth in this type of substrate.

The substrate 2 based on moss and vermiculite, has a great water absorption capacity and a better CIC. The moss absorbs up to 20 times its dry weight and the vermiculite which is a mineral composed of aluminum-iron-magnesium silicate, has a structure in plates, which generates a high proportion of surface area/volume and therefore a high capacity of moisture retention (Bunt, 1988). These characteristics were adequate for the production of stevia seedlings under the conditions used in the experiment, which agrees with that reported by Gaudig *et al*. (2014), who indicated that moss possesses adequate physical and chemical properties for the production of diverse seedlings.

The substrate of coconut residues (substrate 3) presented an adequate growth of stevia seedlings, probably because it is a substrate of homogeneous granular structure, with high total porosity, high aeration capacity and water retention, low bulk density, pH between 5 and 6 and highly stable physical structure (Jasmin *et al*., 2003; Arévalo *et al*., 2016).

The concept of water quality for irrigation refers to water characteristics that may affect soil and crop resources in their long-term use Bosch *et al*. (2012). To evaluate the water quality for irrigation, three main criteria must be defined: salinity, sodicity and toxicity (Gholami and Shahinzadeh, 2014; Asamoah *et al*., 2015).

To measure the quality of irrigation water, direct and indirect variables are considered. The direct variables to measure water quality for irrigation are (1) salinity; (2) sodicity; and (3) alkalinity and specific ionic toxicity. Indirect variables, also called dependent environment are (1) tolerance of crops to salinity; (2) tolerance of soils to salinity, sodicity and alkalinity; (3) irrigation management; and (4) climate (Aragües, 2011).

For the present study, information was available on the indicators that measure salinity, such as electrical conductivity (CE) and total dissolved solids (SDT), on sodicity, such as sodium absorption ratio (RAS), alkalinity a through pH and toxicity such as the contents of chlorides, boron and sodium. Regarding the independent variables and because the sowing was carried out in a protected structure, in containers with substrates and the same water management, only the tolerance of S. *rebaudiana* to salinity was contemplated.

Among the indicators evaluated on water quality, it was found with respect to the pH of the irrigation water that in the three treatments it tends mainly to a water of neutral pH (7.11 the highest and 6.9 the lowest). Waters with very high or very alkaline pH, hinder the absorption of nutrients in plants as they are precipitated and very acid pH can solubilize very toxic elements such as aluminum (Arzola *et al.*, 2013). In the present study none of the waters presented high alkalinity problems (> 8).

With regard to the RAS Lingaswamy and Saxena (2015) suggest using the criterion proposed by Richards that classifies it as follows: < 10 excellent, 10 to 18 good, 18 to 26 doubtful, > 26 not recommended. The RAS in T1 is good and T2 and T3 is excellent. Therefore, in the case of the RAS, there was no problem, especially due to the type of irrigation with a garden shower and the use of substrates.

The CE is an indicator of the total dissolved salts in the water and its classification is a subject that has been much discussed and questioned (Tartabull and Betancur, 2016) since, to consider its effects, not only should this isolated indicator be taken, but also the texture of the medium, precipitation and tolerance to the salinity of the crop. For the case of the waters of T2 and T3 they were within the range of 0.2 to 2 considered as suitable for agricultural irrigation and the water of T1 with restrictions.

Another indicator closely related to the CE is the SDT, and according to the classification by the degree of restriction of use as irrigation water, it is considered that a water with SDT of <450 mg L^{-1} has no restriction of use, waters with 450-2,000 mg L^{-1} of SDT have a slight or moderate restriction and waters with SDT of > 2 000 mg L^{-1} present a severe restriction Cortes *et al*. (2009).

Due to this, it was expected that the seedlings irrigated with water with low CE and low content of SDT were the best, but did not show such behavior. This could be due to various factors such as the content and type of salts in the irrigation water, since the purified water due to its purification process had a sodium absorption ratio of 10.82 (high according to what is recommended for water from irrigation of <10) and interchangeable sodium percentage of 2.2, higher than well water with 3.91 and 0.81 of sodium absorption ratio and interchangeable percentage of sodium respectively. Another aspect that influences is that *S. rebaudiana*, is considered a plant moderately sensitive to salinity (González *et al*., 2016), which allows it to support saline waters like those of T1, without an apparent damage in the development of the seedlings.

The height of the seedlings presents significant statistical differences ($p< 0.05$) with respect to the quality of irrigation water, agreeing with that reported by various authors, who have observed changes in the growth of the stem of different plants by varying the salinity of the plant. Water, mainly a decrease in growth due to an increase in salinity (Pares and Basso, 2013; Ruiz *et al.*, 2014).

It was observed that the height of the seedlings that received water from T1 (9.12^{a} cm) and T2 (10.24^{b} cm) were statistically different ($p< 0.05$); however, they were equal to seedlings irrigated with water with T3 (9.71^{ab} cm) (Figure 3).

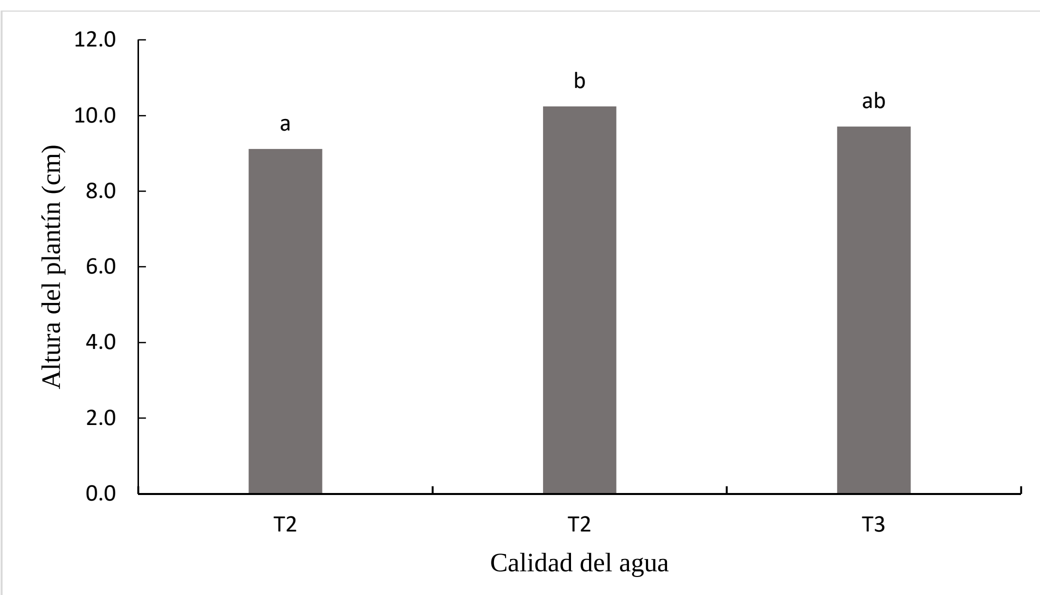

Figure 3. Height of stevia seedlings in three water qualities.

Due to these results, sodium played an important role in the growth of the seedlings since, although SDT and CE were low in the water of T3, the percentages of sodium absorption were adequate in the intermediate water quality, in addition It is possible that the cuttings require a certain amount of minerals to develop properly, making the irrigation water of T2 has been better.

Analyzing the response of the height of the seedlings with water from T1, it was observed that from day 3 of sowing the cutting, significant statistical differences were found ($p < 0.05$) between the type of substrates, being less the development of seedlings in substrate 1 (Table 2). This same behavior was observed on day 12 and 22, as well as with the seedlings obtained with irrigation water from T2 and T3. The best development of the seedling with substrate 2 and substrate 3 was very marked on the different sampling days and under any type of water quality. However, the best performance was of substrate 2 under the quality of water with medium SDT at 22 days after planting the cuttings.

Table 2. Average height (cm) of stevia seedlings in three water qualities and three substrates.

	Water quality Treatment 1		
	Substratum 1	Substratum 2	Substratum 3
3 days	4.9 ± 0.33^{a}	5.7 ± 0.52^{b}	5.8 ± 0.45^{b}
12 days	6.6 ± 0.75^{a}	8.8 ± 0.89^{b}	8.8 ± 0.95^{b}
22 days	8.7 ± 1.75^{a}	15.8 ± 3.25^{b}	15.7 ± 2.58^{b}
	Water quality Treatment 2		
	Substratum 1	Substratum 2	Substratum 3
3 days	5.1 ± 0.21^{a}	6.1 ± 0.67^{b}	5.5 ± 0.51^{a}
12 days	6.4 ± 0.67^{a}	10.1 ± 1.7^{b}	10.3 ± 1.15^{b}
22 days	10.0 ± 1.16^{a}	20.8 ± 4.78^{b}	19.0 ± 2.19^{b}
	Water quality Treatment 3		
	Substratum 1	Substratum 2	Substratum3
3 days	4.9 ± 0.54^{a}	6.0 ± 0.55^{b}	5.6 ± 0.65^{b}
12 days	6.6 ± 0.72^{a}	9.7 ± 1.21^{b}	8.8 ± 1.08^{b}
22 days	8.3 ± 1.51^{a}	20.0 ± 2.76^{b}	18.0 ± 2.61b

[a,b] Different literals in the same row indicate significant statistical differences ($p < 0.05$)

Analyzing the results of heights for water quality in the growth time in the different substrates, the cuttings were observed on the 22nd day of the cuttings, a significant effect ($p < 0.05$) of the type of water, finding a better development in the seedlings with water of T2 and T3.

Root length

The development of the root of the seedlings was influenced by the type of substrate ($p < 0.05$) with substrate 2 being the best, followed by substrate 3 and finally substrate 1 (Figure 4). On day 12 of development, the length of the roots, presented an average of 4.4 cm and 5.8 cm 22 days after planting the cuttings. Due to its characteristics the moss-based substrate allows a better rooting and an optimal development of the seedlings. It has been, reported that it has good physical and chemical characteristics and besides being good for stevia, it proves to be efficient for the production of forest and vegetable plants (García *et al*., 2001; Rangel *et al*., 2002).

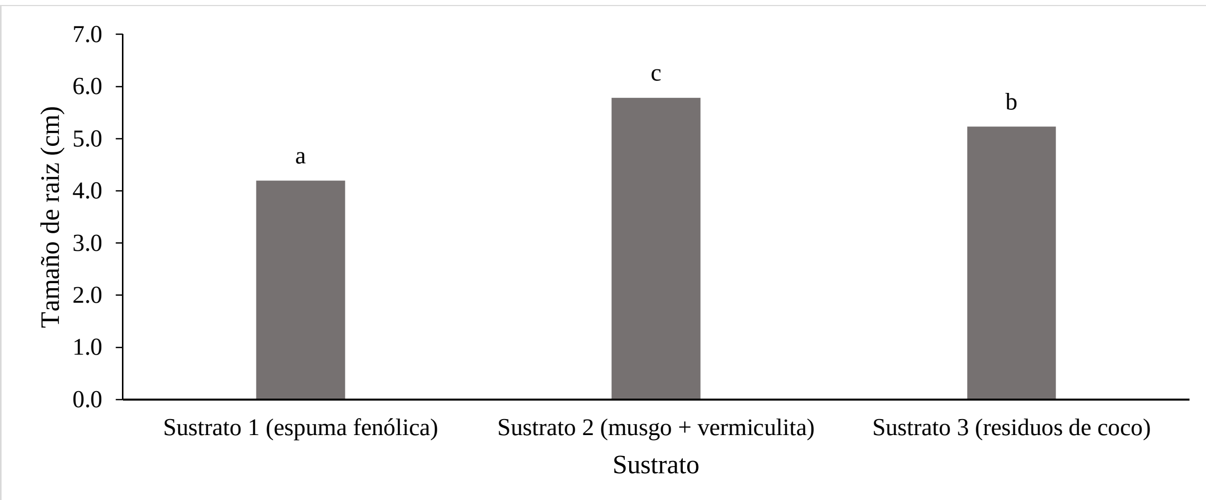

Figure 4. Root length of stevia seedlings in three types of substrate.

Although the quality of the water had a significant effect on the height of the seedlings, the same effect on the length of the roots was not found being 5.0, 5.1 and 5.1 cm for T1, T2 and T3 respectively.

When analyzing the size of roots individually, it was found that with water from T1 and T3, substrate 2 and 3 were the best (Table 3); however, water from T2 was better on substrate 2. On day 22 regardless of water quality, substrate 3 (5.9^{b} cm) and substrate 2 (6.1^{b} cm) were better than substrate 1 (5.1^{a} cm).

Table 3. Average root length (cm) of stevia in three water qualities and three substrates.

	Water quality Treatment 1		
	Substratum 1	Substratum 2	Substratum 3
12 days	3.8 ± 0.89[a]	4.8 ± 0.80[b]	4.7 ± 0.66[b]
22 days	4.9 ± 0.65[a]	5.5 ± 0.71[b]	5.4 ± 0.96[b]
	Water quality Treatment 2		
	Substratum 1	Substratum 2	Substratum 3
12 days	2.8 ± 0.78[a]	5.8 ± 1.13[c]	4.5 ± 1.1[b]
22 days	5.1 ± 0.42[a]	6.5 ± 0.61[b]	5.6 ± 0.22[a]
	Water quality Treatment 3		
	Substratum 1	Substratum 2	Substratum 3
12 days	3.4 ± 1.39[a]	4.7 ± 0.73[b]	4.5 ± 0.89[b]
22 days	5.2 ± 0.91[a]	6.3 ± 0.45[ab]	6.6 ± 0.42[b]

[a,b] Different literals in the same row indicate significant statistical differences ($p < 0.05$).

Based on these results, it was observed that the quality of the water did not affect the size of the roots; however, although it was not measured, it was perceived that the secondary roots were more abundant in the seedlings irrigated with water of T2 and T3.

Conclusions

The substrates used were an important factor in the development of the *S. rebaudiana* seedlings and the growth of their roots.

The best results in the development of seedlings and root length of *S. rebaudiana* were obtained with substrates 2 (based on coconut fiber) and substrate 3 (mixture based on moss and vermiculite).

With the substrates 2 (coconut residues) and substrate 3 (moss- based mixture), seedlings with an adequate development for the transplant were obtained after 22 days.

The water quality had an effect on the height of the *S. rebaudiana* seedlings, but not on the length of the roots.

The water purification process lowers the electrical conductivity, but decreasing the amount of calcium and magnesium increases the RAS, which lowers the quality of the irrigation water.

With substrates 2 and 3 it is feasible to produce the *S. rebaudiana* seedlings with waters whose electrical conductivity is up to 2.0 mS cm^{-1}.

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