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Optimization of banana plantlets acclimatization by hydroponic cultivation

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Received June 09, 2020 Accepted October 10, 2020 ABSTRACT: Acclimatization is the final phase of banana plantlet production by micropropagation. In this phase, the plantlets are grown in a protected environment with high demand for water, fertilizer and labor, until reaching the standard size and quality required for marketing. The aim of this study was to evaluate the possibility of reducing these costs and the duration of the acclimatization phase, using the hydroponic cultivation technique as compared to the conventional system. 'Prata–Anā' banana plantlet performance was measured for both systems under both tropical summer and winter conditions, based upon water and nutrient use efficiency and growing time of the plantlets. In both seasons, the plantlets produced hydroponically presented faster growth of both the shoot and root systems. There was a reduction of 12 days to reach the transplantation point, a significant reduction in plantlet production cost (fertilizers + water) and improved efficiency in space use with an expressive increase in the number of plantlets produced per square meter.

Keywords: Musa spp., soil-less cultivation, water use efficiency, growth

Introduction

The banana crop has high socioeconomic importance throughout the world (Alemu, 2017). According to statistics from the FAO (2020), banana exports contribute approximately US\$ 12 billion per year to the global economy and total banana production reached a volume of 116 billion kg in 2017. Average productivity has increased from 14,000 kg ha⁻¹ in 1993 to 20,000 kg ha⁻¹ in 2017 (FAO, 2018).

In addition to general crop management practices, the quality of the planting material is a crucial input for success in growing commercial bananas. Inputs may represent 70 % of the banana production cost and the plantlets alone account for 36.49 % of these costs (Cardoso, 2006; Tiwari et al., 2019).

Banana plantlets can be obtained by micropropagation whose main advantages, as compared to the traditional method, are the higher multiplication rate and better phytosanitary quality (Ortas et al., 2017; Wong, 1986). The last stage of banana micropropagation is the acclimatization phase, when the plantlets are still growing under protected environmental conditions until reaching adequate size and hardness for their transplantation to the field (Scarinari et al., 2009; Waman and Bohra, 2019).

During acclimatization, the plantlets are growing in pots containing special substrate, and have to be watered and fertilized regularly. During this period there is a significant demand for water and fertilizers, as well as labor [Navarro et al., 1994; Waman and Bohra, 2019]. Because of this, it is important to find alternatives to optimize the use of resources and reduce the duration of this phase.

An alternative may be to adapt the conventional cultivation system to hydroponics, whose use has strongly increased in agriculture. This technique has been pointed out to have significant potential for contributing to food production to meet the needs of a population

on the rise (Sardare and Adname, 2013). This system may optimize the use of space and time use, with earlier transfer of the plantlets to the field, as well as enabling automation, with positive outcomes on resource use efficiency in comparison with the conventional system (Corrêa et al., 2012; Treftz and Omaye, 2016).

Hydroponic cultivation of banana plantlets in the acclimatization phase has not been mentioned in the literature yet and its viability must still be determined. The objective of this work was to compare the performance of hydroponic and conventional systems for the acclimatization of commercial banana plantlets in two seasons.

Materials and Methods

Place and general methodological aspects

The experiments were carried out in a greenhouse of a biofactory of banana plantlets production, located in Cruz das Almas, Bahia, Brazil (12°40′39′′ S, 39°06′23′′ W, altitude of 225 m). The regional climate is classified as dry sub-humid, with average annual rainfall of 1,131 mm, with moderate excess of rain in the winter, and average daily temperature of 23.9 °C (Silva et al., 2016).

The greenhouse was covered by an agricultural film with thickness of 150 microns along with a 50 % shading screen.

Banana plantlets cv. Prata-Anã, obtained by in vitro propagation, were washed to remove the culture medium and then transplanted to trays with 128 cells, each with capacity of 0.0000225 m³ filled with inert material composed of coconut fibers. Two experiments were conducted to compare the performance of banana plantlets cultivated in conventional and hydroponic systems, the first during the winter and the second the summer, as described below.

Conventional acclimatization

In the conventional cultivation system, the entire process was monitored daily by a technician in charge of plantlet production, according to the criteria used for large-scale commercial production. Along the acclimatization phase, plants are nourished by foliar fertilization divided into eight applications. For production of 10,000 plantlets by the conventional system, 0.0187 L of fertilizer solution was applied in each spray per plantlet, containing: 14 % nitrogen, 7 % phosphorus, 5 % potassium, 1.5 % magnesium, 0.1 % boron, 1.5 % manganese, 0.05 % molybdenum and 2 % zinc, plus 0.0625 kg of monoammonium phosphate (MAP) and 0.172 kg of ammonium sulfate.

The plants were irrigated manually, with variable frequency of 1.2 to 1.7 irrigations d⁻¹, respectively, in experiment 1 and 2. The number of irrigations per day was determined based upon the visual aspects of the substrate in the morning and afternoon. The substrate changes its color with increasing moisture content from light brown to dark brown.

Hydroponic acclimatization

The hydroponic system used in this study was adapted from the configurations of hydroponic systems applied commercially. The trays containing the explants were placed in recipients measuring 0.48×0.26 m with a capacity of 0.02 m³. The recipients were placed on metal benches at a height of 1.2 m from the floor. The benches were positioned at a slope of 3 % in Experiment I and flat in Experiment II.

The reservoir used had a capacity of 55 L and was attached to an electrical pump with a power rating of 34 W and flow of $0.0002333~\text{m}^3~\text{s}^{-1}$, to move the water from the reservoir to the trays through polyethylene hoses $^{3}\!4$ inch in diameter. The same hoses were also used for the independent drainage system. The nutrient solution was distributed to the trays through microtubes 6 mm in diameter connected to the polyethylene hoses.

The volume of the nutrient solution lost by evapotranspiration was replaced by a system controlled by a float valve installed in the main reservoir and connected to another reservoir containing water from the municipal supply system. A valve was installed between the two reservoirs to support control over the moment of water replacement, providing replenishment every three days in the interval between the recirculation of the nutrient solution. The reservoir with replacement water consisted of a PVC tube with a diameter of 0.15 m and height of 1 m. The structure had a ruler and a transparent microtube to allow monitoring of the volume of water replaced. The pumping system operated with fixed watering intervals of 15 min, each lasting 15 min. The hydroponic system is illustrated in Figure 1.

The nutrient solution was prepared according to the formulation developed by Furlani (1999) for leafy vegetables. The following ingredients were used to prepare 1,000 L of nutrient solution: 750 g of calcium nitrate, 500 g of potassium nitrate, 150 g of monoammonium phosphate, 400 g of magnesium sulfate, 0.15 g of copper sulfate, 0.30 g of zinc sulfate, 1.50 g of manganese sulfate, 1.80 g of boric acid, 0.15 g

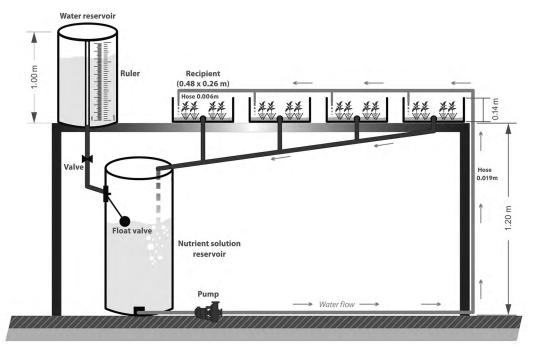


Figure 1 – Diagram of the hydroponic system used in the experiments.

of sodium molybdate, and 16 g of iron Ethylenediamine tetraacetic acid (EDTA) at 13 %.

During plant growth the solution pH was monitored every two days with a pocket pH meter with automatic temperature compensation. Whenever the pH was below 6.0 or above 6.5, it was corrected by applying a solution of NaOH or HCl, respectively. Simultaneously, the electrical conductivity (EC) was measured with a bench meter. The reference adopted for replacement of the nutrient solution was a 25 % decline in EC. However, neither of the two experiments presented any need to replace the solution.

Experiment 1

Experiment 1 was conducted in a completely randomized design with eight replications. The source of variation studied was the type of acclimatization, either hydroponic or conventional. The experiment lasted 25 days, at which time more than 50 % of the plants had reached the harvest point corresponding to plant shoot height greater than 5 cm with total root adherence to the substrate.

This experiment was conducted during the winter. The environmental conditions in the greenhouse (average temperature and relative humidity) were monitored by sensors connected to a multiplexer. The variables were logged every 10 min and converted into hourly means. The average temperature during the experiment was 22.8 °C, with a minimum of 17.2 °C and maximum of 31.4 °C, and the relative humidity varied from 40.7 to 100 %, with an average of 83 %.

The EC ranged from 2.08 to 1.91 dS m⁻¹ from the start to the end of the experiment, a decrease of 8.17 %.

Response variables

Twenty-five days after the explants were transplanted to the trays, eight plantlets were sampled to evaluate the following growth variables: plant height (m); leaf area (m²); shoot fresh and dry weight (g); total root length (m) and length in diameter classes (0-2 and greater than 2 mm); average root diameter (mm); and root volume (m³).

The shoots were separated from the roots and weighed on an analytical balance (accuracy of 0.0001 g) to determine fresh weight. The plant height was measured with a ruler and the leaf area determined as described by Potdar and Pawar (1991), using the length and width and the correction according to Eq. 1:

Total Leaf Area =
$$\frac{\Sigma \left[-0.0334 + (L \times W \times 0.8402) \right]}{10.000}$$
 (1)

where: L – length (cm) and W – width (cm).

The shoots were dried in a forced-air oven at 65 °C for 72 h to determine the dry weight on the analytical balance.

The roots were washed until complete removal of substrate and placed in jars with 30 % alcohol, in which

they were kept under refrigeration (5 °C) for evaluation. The root system was analyzed using a scanner and the data were processed by a suitable software program.

Experiment 2

This experiment employed a randomized block design in a 2×7 factorial scheme, with four repetitions, where each experimental unit was composed of 16 banana explants. The first factor denoted the type of acclimatization (conventional or hydroponic) and the second factor the time interval of biometric assessments, in days after transplantation (DAT). Growth was evaluated at 0, 5, 10, 15, 20, 25 and 30 DAT.

The experiment was performed in the summer. The meteorological data were collected by a system developed in the Arduino platform, employing sensors installed 2 m above ground level. Data were recorded every 10 min. The average hourly temperature was 29.7 °C, varying between 23.2 and 43.2 °C and the average relative humidity during the experiment was 69.2 %, with a of 31.7 % and a maximum of 100 %.

The EC of the nutrient solution varied between 2.07 and 1.58 dS m^{-1} from the first to 30 DAT, resulting in a reduction of 23.67 %. However, when the plantlets were grown in the hydroponic system, ready at 18 DAT, the reduction of the electrical conductivity was 9.18 % (1.88 dS m^{-1}).

Growth variables

The same growth parameters were recorded as in Experiment I, namely: plant height; leaf area; fresh and dry shoot weight; total length and volume of the root system. In addition to these metrics, the absolute growth rate was also determined, indicating the increment in dry weight (Eq. 2).

$$AGR = \frac{SDWf - SDWi}{ND} \tag{2}$$

where: AGR is the absolute growth rate (kg d⁻¹); SDWf the final shoot dry weight (kg); SDWi the initial shoot dry weight (kg), and ND the number of days of the experiment (30 days).

Plantlet production efficiency

The efficiency of the use of space in time (number of cycles per year) was derived from the ratio of the time interval (in days) for production of plantlets in each system and the number of days in the year. The plantlet production efficiency (number of plantlets m⁻² yr⁻¹) was obtained from the ratio between the number of plantlets produced per square meter per year by each cultivation system.

At 30 DAT, the mortality (%) of the plantlets was determined in the two acclimatization systems by a count of both the dead and the living as follows (Eq. 3):

$$MR(\%) = \frac{DS}{DS + LS} \times 100 \tag{3}$$

where: MR – mortality rate, DS – dead plantlets, LS – live plantlets.

Volume of water and fertilizers applied

The volume of water applied during the experiment in the conventional system was determined by recording the flow through the hose (1.55 \times 10⁻⁴ m³ s⁻¹). This allowed for determining the number of plantlets irrigated in a 1 min period, obtaining an average of 3.2 \times 10⁻⁶ m³ per plant irrigation, with a frequency of 1.7 irrigations d⁻¹.

The volume of water used for replacement in the hydroponic system was obtained by applying the volumes of water recorded between the replacement events, and taking into consideration the time interval in days, as expressed in Eq. 4.

$$V = \frac{(Lf - Li)\pi D^2}{4n\Delta t} 10^3 \tag{4}$$

where: V – the volume applied (m³ per plant d⁻¹); Lf – the final water level in the automatic supply reservoir (m); Li – the initial water level in the automatic supply reservoir (m); D – the internal diameter of the automatic supply reservoir (m); Δt – the time interval between readings (days); and n – the number of plants cultivated in the tray in time interval Δt .

The water use efficiency (m³ per plantlet) was calculated as the ratio between the volume of water applied and the shoot's dry weight of the plantlets.

The efficiency of fertilizer use efficiency was calculated as the accrued cost of fertilizer application to 10,000 plants during the plantlet cultivation cycle.

Statistical analysis

In Experiment 1, the means of the treatments were compared by the paired T-test (p < 0.05), after transforming the data to log (×) when necessary to satisfy the prerequisites for the statistical test used.

In Experiment 2, the data were submitted to analysis of variance using the Exp.Des.pt in the R Core Team software package (version 4.0.2). The means of the variables between the acclimatization methods were analyzed by the F-test at a probability of p < 0.05. For a comparison between the evaluation periods, linear and polynomial regression models were fitted, taking into account the coefficient of determination and presenting if significant at least p < 0.05 level (F-test). Where interaction of the factors was detected, the variables in each factor were separated. The mortality percentages were compared by the unpaired T-test at 5 % significance.

Results

The plantlets grew faster in the hydroponic acclimatization process than in the conventional one under the same microclimatic conditions.

Experiment I

Effects of the acclimatization method on shoot fresh and dry weight, plant height, total leaf area (p < 0.01) and total root system length (p < 0.05) (Figure 2) were evident, but no effect on the root system volume (p = 0.0854) was detected. The use of the hydroponic acclimatization of 'Prata–Anã' banana plantlets enabled increases of 126, 123, 127, 69 and 61 % in the variables' shoot fresh and dry weight, total leaf area, plant height and total root system length, respectively.

There was a significant effect (p < 0.05) on the root length in the diameter interval class 0 to 2 mm (Figure 3). Plants acclimatized in the hydroponic system presented a smaller proportion of roots with a small diameter, with a tendency for higher production of roots in all diameter classes evaluated (Figure 3).

Experiment II

Growth variables

In this experiment, in addition to the isolated effect of the acclimatization method on plantlet growth, as observed in Experiment I, there was interaction between the acclimatization method and date of evaluation (days after transplantation) for plant height, leaf area, shoot fresh and dry weight (p < 0.01) and total root system length (p < 0.05). The root system volume was influenced only by the time factor (p < 0.01).

Plant height, shoot fresh and dry weight, leaf area and total root system length of the plantlets increased progressively during the experimental period, with linear and polynomial fits as a function of time after transplantation (Figure 4). However, the growth rate was higher for the plantlets grown in the hydroponic system compared to the conventional system. At 30 DAT, the hydroponic system produced increases in these variables of 31, 76, 37, 120 and 38 %, respectively, as compared to conventional acclimatization.

It is important to note that according to the regression equations (Figure 4), at 18 DAT the plantlets grown in the hydroponic system had reached the same average height as that reached under conventional acclimatization at 30 DAT, accelerating development of the upper system by 12 days. For shoot fresh weight, this result was also attained at 18 DAT, while for shoot dry weight and total root system length these results were achieved at 24 DAT and for leaf area at 16 DAT. The absolute average growth rates by dry weight were 0.008422 and 0.005465 kg d⁻¹, respectively, for the hydroponic and conventional acclimatization.

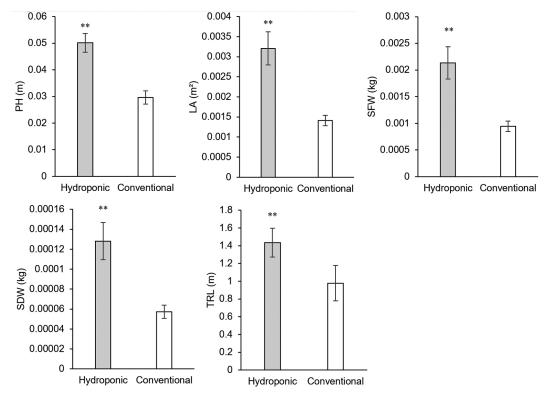


Figure 2 – Plant height (PH), leaf area (LA), shoot fresh weight (SFW) and shoot dry weight (SDW), and total root system length (TRL) of 'Prata–Anā' banana plantlets 25 DAT grown conventionally and hydroponically. Means of eight repetitions and respective standard errors. **significant at 1 % by the T–test.

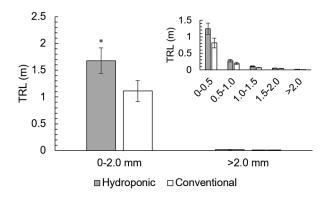


Figure 3 – TRL = Total root system length in diameter classes and in classes with smaller intervals. *significant at 5 % by the T-test.

Plantlet production efficiency

The main criterion usually applied for determination of plantlets at adequate selling point is their height, in addition to the substrate totally filled with the roots as a more qualitative variable. Thus, we chose plant height as the criterion to determine the readiness for sale. Applying this criterion, the estimated efficiency of space utilization over time was 20.3 cycles yr⁻¹ for hydroponic acclimatization and 12.2 yearly cycles for

the conventional system. This means an increase of approximately 66 % in the number of plantlets produced in a year by using hydroponic acclimatization. The plantlet harvest points were determined to be 18 and 30 DAT for the hydroponic and conventional system, respectively.

As regards plantlet production efficiency, taking into consideration 897 plantlets m^{-2} , we estimated potential outputs of 18,119 and 10,943 plantlets m^{-2} yr⁻¹ for the hydroponic and conventional systems, respectively. The respective mortality rates at 30 days after transplantation were 4.3 % and 18.4 % (Figure 5) for the hydroponic and conventional systems.

Volume of water and fertilizers applied

Water consumption to produce 10 thousand plantlets was $1.634 \times 10^{-4} \, \text{m}^3$ per plant for the conventional system and $6.440 \times 10^{-5} \, \text{m}^3$ per plant in the hydroponic system, providing a reduction of $60.58 \, \%$ during the cycle of 18 and 30, respectively. Water utilization efficiency was much higher for the hydroponic system as, on average, for each liter of water $0.002216 \, \text{kg}$ of shoot dry weight was produced, whereas in the conventional system only $0.001273 \, \text{kg}$ of shoot dry weight was obtained for one liter of water used, a difference of 74.08 % between the systems studied.

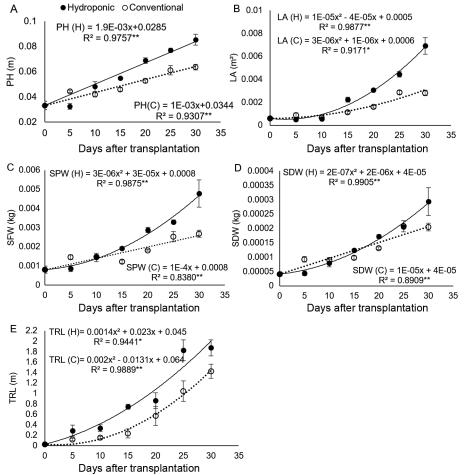


Figure 4 – Plant height (PH, A), leaf area (LA, B), shoot fresh weight (SFW, C), shoot dry weight (SDW, D) and total root system length (TRL, E) of banana plantlets under conventional (C) and hydroponic acclimatization (H) in function of days after transplantation. **significant at 0.05 level and *significant at 0.01 level according to the F test.

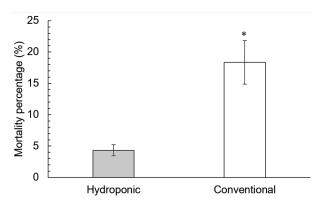


Figure 5 – Mortality percentage of banana plantlets by the two acclimatization systems. *significant at 5 % by the unpaired T-test.

The total cost of water and fertilizers used to prepare the nutrient solution for the production of 10,000 banana plantlets was 46.3 % higher in the hydroponic system. On the other hand, the overall cost

of the hydroponic acclimatization system was 57.6 % lower than that of the conventional system, when taking into account the plants' nutrient uptake as assessed by electrical conductivity variation of the nutrient solution at 18 DAT (Table 1, Figure 6).

Discussion

In both experiments, we observed faster increases in the variables shoot fresh and dry weight, leaf area, plant height and total root system length of banana plantlets cultivated under the hydroponic system in comparison to the conventional system (Figures 2, 3 and 4). We suggest that this plantlet growth acceleration is due to the greater availability of water and nutrients in the hydroponic system than in the conventional one, as has also been reported by Jensen (1997), Rouphael et al. (2018), Sardare e Admane (2013) and Savvas (2003) for many agricultural species.

The nutrient solution used in the hydroponic system contains high concentrations of nutrients, which promote faster growth. In this respect, Taiz et al. (2014)

Inputs	CONVENTIONAL		HYDROPONIC			
	Quantity	Cost (US\$)	Preparation of the solution		Water and fertilizers consumed (9.18 %)	
			Quantity	Cost (US\$)	Quantity	Cost (US\$)
1. Water applied (L)	1.634	2.1273	644	0.8376	644	0.8376
Subtotal (US\$)		2.1273				0.8376
2. Fertilizers						
Fertilizer solution (mL)	18.75	0.1734	_	_	_	_
MAP (g)	62.5	0.1200	157.2	0.3007	-	-
Ammonium sulfate (g)	172	0.2048	_	_	-	_
Calcium nitrate (g)	-	-	786	1.1310	-	-
Potassium nitrate (g)	-	-	524	0.9631	-	_
Magnesium sulfate (g)	-	-	419	0.3672	-	-
Copper sulfate (g)	-	-	0.157	0.0005	-	_
Zinc sulfate (g)	-	-	0.314	0.0013	-	-
Manganese sulfate (g)	-	-	1.572	0.0295	-	_
Boric acid (g)	-	-	1.886	0.0092	-	_
Sodium molybdate (g)	-	-	0.157	0.0240	_	-
Iron EDTA 13 % (g)	-	-	16.8	0.1716	-	-
Subtotal (US\$)	-	0.4963	_	3.0018	-	0.2749
TOTAL (US\$)		2.6236		3.8394		1.1125

MAP = monoammonium phosphate; EDTA = iron Ethylenediamine tetraacetic acid.

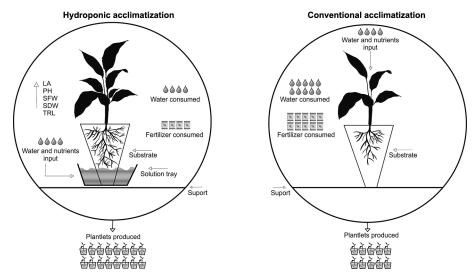


Figure 6 – Banana Plantlets production under hydroponic and conventional acclimatization.

reported that modern nutrient solutions contain the highest concentrations of nutrients possible without causing toxic and saline effects, which leads to high growth.

Additionally, under the conventional system used in this study, representing a typical commercial system for acclimatization of banana plantlets, the irrigation and fertilization are performed manually, often lacking uniformity, and are less frequently applied when compared to the hydroponic system, in which the nutrient solution was offered automatically every 15 min in carefully controlled doses (AlShrouf, 2017).

The absence of a significant difference between the treatments studied regarding root system volume may be related to a smaller allocation of photoassimilates to the roots, giving priority to the shoots, due to the low demand for root production in media containing high nutrient concentrations (Taiz et al., 2014), as in the case of hydroponic production.

On the other hand, we suggest that the greater root system length in the hydroponic treatment, without a difference in volume, was a result of the greater production of roots with smaller diameters. Higher production of roots with smaller diameters is desirable

in the production of plantlets since it increases the chances of survival in the field due to the greater vigor of the plants (Wu et al., 2016).

The regression equations generated (Figure 4) indicated that the plantlet harvest point for sale was reached earlier, at 18 days after transplantation, under the hydroponic system, a reduction of 12 days in the acclimatization period. This result was expected which represents a greater number of plantlets to be produced per time unit in the same space, an increase of about 65 % as compared to the conventional system. This also reduces the need for labor due to both the faster plantlet production and the automation feasibility (Wootton-Beard, 2019).

Similar results were reported by Castellane and Araújo (1994), who observed for lettuce, bell pepper and tomato annual productivities of 52, 16 and 18 t ha⁻¹ yr⁻¹, when grown in a conventional field system, and yields of 313, 96 and 375 t ha⁻¹ yr⁻¹ when cultivated in hydroponics, respectively, due to the faster growth cycle.

The higher water use efficiency in the hydroponic system corroborates Putra and Yuliando (2015) and Sardare and Admane (2013), all reporting that the closed-cycle hydroponic system is more efficient in water use due to the recapture of the nutrient solution not used by the plants to the reservoirs at the end of watering for subsequent reuse.

When counting the quantity of fertilizers used in the nutrient solution, the hydroponic system resulted in a higher production cost based on the water and fertilizers. However, only part of the fertilizers was used providing a cost reduction opportunity (Table 1). This reduced use of fertilizers in the hydroponic system was the result of recycling the nutrient solution, since the nutrients were readily available to the roots and none were lost to lixiviation because of the closed system (Savyas, 2003).

To the best of our knowledge, this is the first article reporting the acclimatization of banana plantlets in a hydroponic system for commercial purposes. For banana, hydroponics has only been used to understand aspects of uptake of nutrients, toxic elements and pollutants (Cornelis et al., 2012).

The results found in this study, carried out in partnership with a biofactory for the production of banana plantlets to supply the Brazilian market, are noticeably positive considering the growth acceleration of the plants promoted by the hydroponic acclimatization, resulting in faster production of banana planting material. In addition, the system proposed reduces cost due to lower volumes of water and fertilizers consumed and less labor needed. In spite of the higher initial costs for the implementation of the hydroponic system, over the long term the return is higher due to the greater automation and more efficient use of inputs and space in time, as has been reported for other crops (Sardare and Admane, 2013).

Conclusions

The use of the hydroponic system yielded a significant reduction in the time needed for acclimatization of banana plantlets, more efficient use of space in time and lower demand of water compared to the conventional system.

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Authors' Contributions

Conceptualization: Coelho Filho, M.A.; Toyosumi, I.S. Data acquisition: Toyosumi, I.S. Data analysis: Coelho Filho, M.A.; Toyosumi, I.S. Design of methodology: Coelho Filho, M.A.; Toyosumi, I.S.; Silva, T.S.M. Software development: Silva, T.S.M. Writing and editing: Coelho Filho, M.A.; Toyosumi, I.S.; Soares, T.M.; Azevedo Neto, A.D.; Melo, D.M.

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