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Influence of silicon and *in vitro* culture systems on the micropropagation and acclimatization of "Dwarf Cavendish" banana

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ABSTRACT. *In vitro* culture systems based on liquid culture media are considered to be more effective than semisolid culture medium systems. Liquid culture media systems provide better nutrient availability for plant tissues, easier culture handling, and the potential for scaling up and automation. However, *in vitro* liquid culture requires more careful handling due to the potential for contamination and the possibility of negative effects, such as hyperhydricity or vitrification, that hinder the growth and development of the plant material. Temporary immersion bioreactors have emerged as a workable alternative for capturing the benefits of liquid media, though semisolid systems are still traditional. Many studies have shown that silicon (Si) is a beneficial plant nutrient. Silicon might have a positive effect in both semisolid and liquid *in vitro* systems. The objective of this study was to evaluate the effect of silicon on the micropropagation and acclimatization of banana plants cultivated *in vitro* by comparing liquid temporary immersion bioreactor technology and semisolid traditional culture systems. Different silicon concentrations (0 and 1 mL L^{-1}) and culture systems (liquid temporary immersion bioreactor and semisolid traditional culture) were evaluated over a 36-day period. The growth characteristics plant size, fresh and dry weight, and number and length of leaves and roots were evaluated. After the 36-day *in vitro* growth period, plants were transferred to a greenhouse for acclimatization and were evaluated after 30 days for the same growth characteristics used in the *in vitro* studies. The temporary immersion bioreactor system resulted in greater growth of banana plants compared to the traditional semisolid system. Temporary immersion bioreactors also showed a positive interaction with Si and resulted in higher values for all growth characteristics in the acclimatization phase.

Keywords: *Musa* spp.; potassium silicate; bioreactor; culture media.

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Introduction

Banana (*Musa* spp.) is considered the fourth most important food commodity worldwide after rice, wheat and maize and is grown on over 10 million hectares in 100 countries (Kishor, Abhijith, & Manjunatha, 2017). The fruit is recognized as a source of carbohydrates, protein, vitamins and minerals (Sinha, Saha, Das, Jena, & Sinha, 2018). This crop has important economic and social impacts throughout the world and is an important source of food (Donato et al., 2006).

Banana is exclusively propagated by vegetative methods. However, the transmission of harmful insects, nematodes, viruses and black Sigatoka disease by field-grown suckers has prompted interest in the use of aseptic culture techniques (Roels et al., 2005). *In vitro* techniques provide a supplement to conventional breeding and have the added benefit of overcoming constraints caused by pests and diseases (Tripathi, 2003). In addition, they allow higher propagation rates for multiplying planting materials, small space requirements regardless of season, and short time requirements (Matsumoto & Silva Neto, 2003).

The use of liquid media for *in vitro* culture and micropropagation systems has many advantages over traditional agar-based semisolid *in vitro* techniques, such as higher multiplication rates, improved *in vitro*

shoot growth, increased nutrient absorption, lower cost, and the potential for automation and up scaling of production (Etiene & Berthouly, 2002; Preil, 2005). Bioreactors are *in vitro* liquid systems that allow the use of large-scale vessels for plant biomass production. Temporary immersion systems (TIS) have been developed to capture the benefits of liquid media culture systems, including low cost, easy culture handling, efficient gaseous exchange, and improved uptake of nutrients and plant growth regulators to ensure maximum growth (Preil, 2005). TIS have been studied for the *in vitro* multiplication of a wide range of tropical crops, such as *Ananas comosus*, *Camellia sinensis*, *Citrus deliciosa*, *Coffea* sp., *Colocasia* sp., *Eucalyptus* sp., *Hevea brasiliensis*, *Manihot esculenta*, *Musa* sp., *Psidium guajava*, *Saccharum* sp., and *Solanum tuberosum* (González, 2005).

In addition, it is important to highlight that this technique is already used for efficient banana multiplication (Alvard, Côte, & Teisson, 1993; Lemos, Ferreira, Alencar, Oliveira, & Magalhães, 2001) and that it provides the advantage of increasing the absorption of nutrients. In this system, the plant is in direct contact with the culture medium, so silicon can be efficiently absorbed. The benefits of silicon have been associated with several indirect effects, such as increased photosynthetic capacity, increased total chlorophyll content, reduced transpiration, increased plant growth and increased cell mechanical resistance (Zhuo, 1995). The presence of Si in the cell wall may increase cellulose, hemicellulose and lignin contents, increasing cell stiffness (Barbosa Filho, Snyder, Fageria, Datnoff, & Silva, 2001). As a result, *in vitro*-derived plantlets show higher survival rates during acclimatization when supplemented with Si.

The objective of this study was to compare semisolid and liquid *in vitro* culture systems (i.e., TIS) for the growth and development of banana plants.

Material and methods

The study was performed at the Laboratory of Ornamental Horticulture and Biotechnology at the Tropical Research and Education Center (TREC) of the University of Florida (UF), in Homestead, Florida, United States.

Explants of the "Dwarf Cavendish" banana established *in vitro* were inoculated in bioreactors containing MS liquid medium (Murashige & Skoog, 1962) supplemented with 30 g L^{-1} sucrose and 4 mL L^{-1} 6-benzyladenine. Potassium silicate (K_2SiO_5) was added to the MS medium at a concentration of 1 mL L^{-1}. The MS medium without the addition of Si was used as the control. The pH was adjusted to 5.7 before autoclaving at 121°C for 20 min. The same procedure was followed for the semisolid medium, which was solidified with 3.0 g L^{-1} Phytagel (Sigma-Aldrich, St. Louis, MO).

Subsequently, in a laminar flow hood, the shoots were separated, and 2-3 cm explants were transferred either into bioreactors containing 1,000 mL of liquid MS culture medium or glass containers (baby food jars) containing 50 mL of semisolid MS culture medium. The treatments were the culture system (liquid TIS and semisolid traditional culture) in the presence (1 mL L^{-1}) or absence (0) of silicon. Cultures were maintained in a growth room at 27 ± 2°C under LED light (50 µmol m^{-2} s^{-1}) with a 16h photoperiod for 36 days.

Growth characteristics, including plant height (PH), pseudostem diameter (PD), plant fresh (FW) and dry weight (DW), number of leaves (LN) and roots (RN), and their lengths (LL and RL) were evaluated after 36 days of *in vitro* culture.

Subsequently, plants were transferred to the greenhouse for acclimatization and evaluated for the same growth characteristics after 30 days.

The experiment was established in a completely randomized 2 x 2 factorial design: Silicon (with and without) x Culture System (liquid and semisolid). Two bioreactors per treatment containing 15 explants per bioreactor were used for the liquid culture system, while six containers with 3 explants per container were used for the semisolid culture system. Data were subjected to analysis of variance (ANOVA), and the means were compared by Tukey's test at a 5% level of significance using the SISVAR statistical program (Ferreira, 2011).

Results and discussion

After 36 days of *in vitro* culture, the growth characteristics did not differ significantly for the interaction between the bioreactor system and Si. Therefore, these factors were evaluated separately, showing that Si contributed to an increase in growth characteristics, including LN, RN, PD, and FW (Table 1). Similar to the results obtained in our study, Sivanesan and Park (2014) and Rodrigues et al. (2017) demonstrated that Si

enhances the growth and development of several species, including an increase in the number of leaves and fresh weight of plants grown *in vitro*. Particularly for bananas, Si has been shown to increase the height, fresh and dry weight (Asmar et al., 2011), and increased pseudostem diameter (Asmar et al., 2013) of *in vitro* shoots.

Table 1. Leaf number (LN), root number (RN), pseudostem diameter (PD), and plant fresh weight (FW) of *Musa* spp. grown *in vitro* in liquid culture (TIS bioreactors) with or without silicon (Si) for 36 days. Si was applied as potassium silicate (K_2SiO_3, 1 g L^{-1}).

Silicon (Si)	LN	RN	PD (mm)	FW (g)
With Si	6.250 a	9.250 a	7.933 a	3.362 a
Without Si	5.000 b	8.375 b	6.618 b	3.003 b
CV (%)	10.68	6.62	9.38	8.47

*Means followed by the same letter within columns are not significantly different by Tukey's test ($p \leq 0.05$).

For the interaction between the culture system (semisolid and liquid TIS bioreactors) and Si, no significant differences were observed after 36 days of *in vitro* culture for growth characteristics, including PH, LN, RN, RL, PD, and FW. Therefore, these variables were evaluated separately. The TIS liquid culture system in the bioreactors showed increased growth characteristics for PH, LN, RN, PD, and FW. In contrast, the semisolid cultures showed increased RL (Table 2). In our study, the use of liquid culture media in bioreactors promoted increased growth characteristics, likely due to the improved nutrient uptake. Lemos et al. (2001) showed that liquid media in both permanent and temporary immersion bioreactor systems increase the contact area of the explant with the media, which favors greater absorption of nutrients and water by *in vitro* tissues and organs. In similar studies, liquid media that were either stationary (Costa, Faria, Londe, Ribeiro, & Damascena, 2016; Siqueira, Santos, Salomão, Silva, & Barros, 2013) or agitated (Costa et al., 2016) promoted increased pseudostem diameter, leaf number, and fresh mass of *in vitro* banana shoots compared to those in semisolid media.

Table 2. Leaf number (LN), root number (RN), plant height (PH), root length (RL), pseudostem diameter (PD), and plant fresh weight (FW) of *Musa* spp. grown *in vitro* under liquid (TIS bioreactors) and semisolid (baby food jars) culture systems for36 days.

Culture System	LN	RN	PH (cm)	RL (cm)	PD (mm)	FW (g)
Liquid	6.875 a	10.375 a	9.500 a	2.563 b	8.410 a	3.681 a
Semisolid	4.375 b	7.250 b	6.052 b	3.650 a	6.140 b	2.681 b
CV (%)	10.68	6.62	6.74	18.04	9.38	8.47

*Means followed by the same letter within columns are not significantly different by Tukey's test ($p \leq 0.05$).

There was a significant interaction between the addition of Si and the type of culture system, whereby Si contributed to increased plant DW in the liquid culture system (TIS bioreactor). Increased plant DW was also observed in the liquid culture system when Si was not present (Table 3). Increased shoot and root dry matter as a result of Si fertilization has been well reported (Epstein, 1994), including an increase in the fresh and dry weight of *in vitro* banana shoots (Asmar et al., 2011). The efficacy of liquid culture systems, specifically temporary immersion systems, has also been demonstrated. The liquid medium had a strong influence on the development and multiplication rate of micropropagated banana plants compared to that in conventional growth on a semisolid medium (Alvard et al., 1993; Etienne et al., 1999; Costa et al., 2016). This was demonstrated in this study, as most growth characteristics of the banana plants were higher in the liquid medium than in the solid medium (Tables 2 and 3).

Table 3. Plant dry weight (DW) of *Musa* spp. grown in liquid (TIS bioreactors) and semisolid (baby food jars) *in vitro* culture systems with or without silicon (Si) for36 days. Si was applied as potassium silicate (K_2SiO_3, 1 g L^{-1}).

	Culture System	
Silicon (Si)	Liquid	Semisolid
	DW (g)	
With Si	0.4165 aA	0.1983 aB
Without Si	0.3320 bA	0.2243 aB
CV (%)	13.03	

*Means followed by the same lowercase letter in the columns (Si) and uppercase letter in the rows (culture system) are not significantly different by Tukey's test ($p \leq 0.05$).

During the acclimatization of the *in vitro*-derived banana shoots, no significant differences were observed for leaf length (LL) or root dry weight (DW). However, Si application resulted in increased root number (RN) and increased plant fresh weight (FW) of *in vitro*-derived banana plantlets under

acclimatization (Table 4). These results are similar to those reported by Asmar et al. (2013), where Si application promoted increased shoot fresh weight of acclimatized *in vitro*-derived banana plants when compared to those of the control (no Si). However, no difference was observed in root number (Asmar et al., 2013).

These results are extremely important because after the acclimatization process, the plants are planted in the field, and these improved characteristics (increased root number and plant fresh weight) can lead to the greater growth and development of these plants.

Table 4. Root number (RN) and root fresh weight (FW) of *Musa* spp. plantlets after 30 days of acclimatization. Plantlets were cultivated *in vitro* under a liquid culture (TIS bioreactors) system with or without silicon (Si) for36 days. Si was applied as potassium silicate (K_2SiO_3, 1 g L^{-1}).

Silicon (Si)	RN	FW (g)
With Si	9.00 a	5.87 a
Without Si	6.88 b	3.94 b
CV (%)	15.47	20.25

*Means followed by the same letter within columns are not significantly different by Tukey's test ($p \leq 0.05$).

There was a significant interaction between Si and the culture system, and Si addition resulted in a higher number of leaves and an increase in pseudostem diameter in liquid culture (bioreactors). As mentioned earlier, this is likely due to the improved nutrient uptake from liquid media (Lemos et al., 2001). However, higher values for plant height (PH), root length (RL), pseudostem diameter (PD), leaf fresh weight (FW), and leaf dry weight (DW) were observed in the semisolid medium without Si. In contrast, the addition of Si resulted in increased PH, RL, PD, FW, and DW in the liquid medium (Table 5).

Table 5. Growth characteristics of *Musa* spp. plantlets after 30 days of acclimatization, including leaf number (LN), plant height (PH), pseudostem diameter (PD), root length (RL), leaf fresh weight (FW), and leaf dry weight (DW). Plantlets were cultivated *in vitro* under liquid culture (TIS bioreactors) and semisolid culture (baby food jars) systems with or without silicon (Si) for36 days. Si was applied as potassium silicate (K_2SiO_3, 1 g L^{-1}).

	Culture System	
Silicon (Si)	Liquid	Semi-solid
	LN	
With Si	12.00 aA	9.00 aB
Without Si	9.00 bA	8.25 aA
CV (%)	9.59	
Silicon (Si)	Liquid	Semi-solid
	PH (cm)	
With Si	8.00 aA	7.33 aA
Without Si	6.17 bB	8.50 aA
CV (%)	13.44	
Silicon (Si)	Liquid	Semi-solid
	PD (cm)	
With Si	11.77 aA	10.52 bB
Without Si	9.03 bB	12.13 aA
CV (%)	6.85	
Silicon (Si)	Liquid	Semi-solid
	RL (cm)	
With Si	18.00 aA	18.88 aA
Without Si	12.00 bB	19.25 aA
CV (%)	7.29	
Silicon (Si)	Liquid	Semi-solid
	FW (g)	
With Si	8.24 aA	7.96 aA
Without Si	4.31 bB	8.62 aA
CV (%)	20.77	
Silicon (Si)	Liquid	Semi-solid
	DW (g)	
With Si	0.4980 aA	0.6160 aA
Without Si	0.2867 bB	0.7373 aA
CV (%)	24.85	

*Means followed by the same letter lower case in the columns (Si) and upper case in the rows (culture system) are not significantly different by Tukey's test ($p \leq 0.05$).

Asmar et al. (2013) reported similar results in banana, with higher values for dry and fresh weight and PD when Si was added to the culture medium.

Ziv (2010) reported similar results in sun star (*Ornithogalum dubium*), and the addition of silicon to the bioreactor liquid medium resulted in higher values for dry weight and the dry weight/fresh weight ratio.

The increase in dry weight may be due to the deposition of amorphous solid silicon but may also be due to the effects of silicon on the other nutrients in the medium, allowing balanced availability and absorption (Epstein, 1994).

This study demonstrated that liquid culture systems such as temporary immersion bioreactors provide an increase in most growth characteristics of banana shoots in the multiplication phase compared to those of bananas grown in a semisolid medium. In addition, the *in vitro* cultivation of banana shoots with Si in liquid culture systems such as temporary immersion bioreactors increased plantlet growth compared to that in the semisolid medium. However, in the absence of Si, the semisolid medium provided better plantlet growth than the liquid medium in the acclimatization phase.

Additional studies are warranted to assess different immersion parameters, such as frequency and duration, as they affect the multiplication rates of large-scale clonal *in vitro* propagation of banana. In addition, different concentrations of Si should be evaluated in combination with bioreactor systems for fine-tuning *in vitro* plant growth and development.

Conclusion

The liquid medium was more efficient than the solid medium for the *in vitro* multiplication of banana shoots.

In the acclimatization phase and with added silicon, the liquid medium was more efficient than the solid medium for increasing plantlet growth. However, in the absence of Si, the solid medium was more efficient than the liquid medium.

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