

Acclimatization and growth of ornamental pineapple seedlings under organic substrates⁽¹⁾

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ABSTRACT

The *in vitro* propagation techniques are commonly used to produce ornamental pineapple seedlings in commercial scale, aiming to attend the growers with genetic and sanitary quality seedlings. However, the choice of the ideal substrate is essential for the acclimatization and growth stage of the seedlings propagated by this technique, since some substrates can increase the seedling mortality and/or limit the seedling growth due to its physical and chemical characteristics. Thus, the aim of this study was to evaluate the acclimatization of ornamental pineapple [*Ananas comosus* (L.) Merr. var. *ananassoides* (Baker) Coppens & Leal] on different substrates. Seedlings with approximately seven centimeters, obtained from *in vitro* culture, were transplanted into styrofoam trays filled with the following substrates: sphagnum; semi-composed pine bark; carbonized rice husk; sphagnum + semi-composed pine bark; sphagnum + carbonized rice husk; and semi-composed pine bark + carbonized rice husk. Each treatment was replicated five times using 10 plants. At 180 days, there were evaluated the following variables: survival percentage, plant height, number of leaves, leaf area, largest root length, and shoot and root dry matter. The substrate semi-composed pine bark + carbonized rice husk presented the lowest mean (62%) for survival percentage. The semi-composed pine bark and semi-composed pine bark + carbonized rice husk treatments presented significant increments in some evaluated biometric characteristics. The semi-composed pine bark is the most favorable substrate for the *A. comosus* var. *ananassoides* acclimatization.

Keywords: *Ananas comosus* var. *ananassoides*, Bromeliaceae, propagation, potting media.

RESUMO

Aclimatização e crescimento de mudas de abacaxi ornamental em substratos orgânicos

Técnicas de propagação *in vitro* são utilizadas visando produzir mudas de abacaxi ornamental em escala comercial, a fim de atender os produtores com mudas de qualidade genética e sanitária. Porém, a escolha do substrato ideal é essencial na etapa de aclimatização e crescimento das mudas propagadas *in vitro*, visto que alguns substratos podem proporcionar a mortalidade das plântulas ou limitar o crescimento das mudas, devido às suas características físicas e químicas. Assim, objetivou-se avaliar a aclimatização do abacaxi ornamental [*Ananas comosus* (L.) Merr. var. *ananassoides* (Baker) Coppens & Leal] em diferentes substratos. Plântulas com aproximadamente 7 cm, oriundas de cultivo *in vitro*, foram transplantadas em bandejas de isopor contendo os seguintes substratos: esfagno, casca de pinus semi-compostada, casca de arroz carbonizada, esfagno + casca de pinus semi-compostada, esfagno + casca de arroz carbonizada e casca de pinus semi-compostada + casca de arroz carbonizada. Cada tratamento foi constituído de cinco repetições contendo dez plantas cada. Aos 180 dias avaliou-se as variáveis: porcentagem de sobrevivência, altura da planta, número de folhas, área foliar, comprimento da maior raiz, e massa seca da parte aérea e raízes. O substrato casca de pinus + casca de arroz carbonizada apresentou média inferior (62%) aos demais tratamentos para a variável porcentagem de sobrevivência. Os tratamentos casca de pinus semi-compostada e casca de pinus semi-compostada + casca de arroz carbonizada apresentaram incremento em algumas características biométricas avaliadas. A casca de pinus semi-compostada é o substrato mais favorável para a aclimatização de *A. comosus* var. *ananassoides*.

Palavras-chave: *Ananas comosus* var. *ananassoides*, Bromeliaceae, propagação, meio de cultivo.

1. INTRODUCTION

In Brazil, Ceara state stands out in the production and exportation of ornamental pineapple stems, which have been widely used in several European and North American countries; ornamental pineapple has been used increasingly in recent years due to its exotic appearance, especially its pleasing colors, as well as long postharvest life (CARVALHO et al., 2009; SOUZA et al., 2012; SOUZA

et al., 2014). Thus, due to the national and international demand for this product, the commercial production can be a promising activity for other states and an alternative for the floriculture products diversification.

The pineapple [*Ananas comosus* (L.) Merr.] presents broad genetic diversity, mainly in Brazil, one of the origin centers and dispersion of these Bromeliaceae (SOUZA et al., 2007; CRESTANI et al., 2010; SOUZA et al., 2012). The most commercialized varieties are *A. comosus* var.

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erectifolius, *A. comosus* var. *bracteatus* and *A. comosus* var. *ananassoides* (CARVALHO et al., 2009). Similarly, to other Bromeliaceae species, pineapples have extraordinary features for ornamental uses, such as potted plants, cut flowers and landscaping. According to Souza et al. (2007) and Souza et al. (2012), these genotypes present shapes and color diversity that stand out for their beauty, originality, and durability of their fruits and leaves.

In order to maintain the specific characteristics for each genotype and to obtain pathogen-free plants, the *in vitro* propagation is the most adequate technique; and it is widely used for pineapples' propagation. However, a suitable substrate must be chosen to avoid losses and other disorders, such as attack by root pathogens and/or slow growth, during the seedlings acclimatization and growth stages. Several materials have been indicated as substrate for ornamental pineapple acclimatization, such as those based on coconut powder or fiber (BOMFIM et al., 2007; AZEVEDO et al., 2008; CUNHA FILHO et al., 2008; BOMFIM et al., 2011) and the mixture of vermiculite and earthworm humus (1:1, v/v) (SILVA et al., 2008).

However, the availability of these materials varies according to the region and it may add cost to the seedlings production, due to the shipping. As an option to reduce costs, mixtures of different materials can be made. Gonçalves et al. (2000) report that suitable substrates for seedlings propagation, by seed and cutting, can be obtained through the mixture of 70 to 80% of an organic component (cattle manure, eucalyptus or pine bark, sugarcane bagasse, earthworm humus and others residues) and 20 to 30% of an element used to increase macro porosity (carbonized rice husk, biomass boiler ash, carbonized sugarcane bagasse, among others).

Furthermore, it is important to be aware about the physical and chemical characteristics of the substrates,

such as apparent density, in order to handle them properly. Adequate measurements of apparent density are essential to calculate and understand other important substrate attributes for plant growth as total porosity, aeration space and available water (FERNANDES and CORÁ, 2004).

Thus, the aim of this study was to evaluate the acclimatization and growth of ornamental pineapple seedlings on different substrates.

2. MATERIAL AND METHODS

Ornamental pineapple seedlings (*Ananas comosus* var. *ananassoides*) with approximately seven centimeters length, propagated *in vitro* on ½ MS culture medium, were transplanted into styrofoam trays (25.0 × 25.0 × 5.0 cm) filled with the following substrates: sphagnum, semi-composed pine bark, carbonized rice husk, sphagnum + semi-composed pine bark (1:1, v/v), sphagnum + carbonized rice husk (1:1, v/v) and semi-composed pine bark + carbonized rice husk (1:1, v/v). Each treatment was replicated five times using 10 plants and distributed in a completely randomized design.

The styrofoam trays were drilled for easy irrigation drainage and held in greenhouse covered by polyethylene film and black plastic screen (Sombrite®) with 70% of solar radiation retention. The seedlings irrigation was conducted daily, according to each substrate necessity, in order to maintain the water content close to the water retention capacity of the substrates.

The physical and chemical characterization of the substrates was carried out at the beginning of the experiment, according to the methodology proposed by Kämpf et al. (2006) to assess the apparent density (g L⁻¹), water retention capacity (mL L⁻¹), pH and electrical conductivity (µS cm⁻¹) (Table 1).

Table 1. Physical and chemical characterization of the substrates based on apparent density (AD), water holding capacity (WHC), pH and electrical conductivity (EC).

Substrates*	AD (g L ⁻¹)	WHC (mL L ⁻¹)	pH	EC (µS cm ⁻¹)
SPH	15.9	459.1	4.2	130.0
PB	274.4	378.7	4.7	52.5
CRH	152.4	579.1	5.5	57.0
SPH + PB	148.6	452.8	4.4	82.5
SPH + CRH	85.7	470.7	4.8	119.5
PB + CRH	205.2	470.5	5.1	79.5

*Sphagnum (SPH), pine bark (PB), carbonized rice husk (CRH).

After 180 days of growth, were evaluated the survival percentage and seedlings growth based on the biometric characteristics: largest root length (cm); plant height (cm); number of leaves; leaf area (mm²), using the Image J software; and the shoot and root dry matter (g). The data were submitted to analysis of variance and the means compared by Tukey's test at 5% probability using the statistical software SISVAR (FERREIRA, 2011).

3. RESULTS AND DISCUSSION

Ornamental pineapple seedlings acclimatized on different substrates presented losses around 10%, except to the seedlings acclimatized on semi-composed pine bark + carbonized rice husk (PB + CRH) mixture, which was observed 38% of seedlings mortality (Table 2).

Table 2. Survival percentage of *Ananas comosus* var. *Ananassoide* plants at 180 days of acclimatization under organic substrates.

Substrates*	Survival (%)	
SPH	92.0	a1
PB	87.0	a
CRH	90.0	a
SPH + PB	93.0	a
SPH + CRH	89.0	a
PB + CRH	62.0	b
C V (%)	19.0	

*Sphagnum (SPH), pine bark (PB), carbonized rice husk (CRH). 'mean followed by the same letter in the column do not differ by Tukey's test at 5% of probability.

The acclimatization stage is considered one of the elements that guarantees success during the seedlings production when propagated *in vitro*, since the seedlings are exposed to new growth conditions causing biotic and abiotic stresses, such as water loss, tissue dehydration, synthesis process reduction, among others (BOMFIM et al., 2011; CUNHA FILHO et al., 2008; OLIVEIRA et al., 2010).

Once the substrate used during the acclimatization directly affects the survival and growth of seedlings propagated by *in vitro* method (BOMFIM et al., 2007; LONE et al., 2008; STEFANELLO et al., 2009), it can be considered one of the factors responsible for the seedlings production success. However, it is observed in Table 1 that the physical and chemical characteristics of semi-composed pine bark + carbonized rice husk substrate were similar to other substrates, so those properties did not influence directly on the seedlings survival percentage.

Silva et al. (2008), working with *Ananas comosus* var. *erectifolius* acclimatization on substrate of vermiculite +

humus (1:1, v/v) obtained values for seedlings survival ranging from 73 to 100%. Similarly results were described by Braga et al. (2011) for 'Gomo de Mel' pineapple acclimatized on different substrates, obtaining survival percentage between 60 and 100%. On the other hand, Moreira et al. (2006) studying 11 substrates for 'Pérola' pineapple acclimatization verified seedling survival percentage close to 95%, regardless the substrate. Thus, it can be inferred that the seedlings survival percentage is related to the characteristics of the materials utilized as substrate.

Regarding the global plants growth on the substrates, there was recorded significant differences among them (Table 3). These results corroborate those described by Moreira et al. (2006) in 'Pérola' pineapple and Oliveira et al. (2014), in three banana cultivars; both cultures acclimatized on different substrates. Was observed that substrate semi-composed pine bark provided the highest means for leaf area and shoot dry mass.

Table 3. Biometric characteristics evaluated in *Ananas comosus* var. *ananassoides* plants at 180 days of acclimatization under organic substrates. Largest root length (LRL), plant height (PH), number of leaves (NL), leaf area (LA), shoot and root dry matter (SDM, RDM).

Substrates*	LRL (cm)		PH (cm)		NL	
SPH	23.55	ns	20.36	b ¹	14.41	bc
PB	30.31		29.56	a	23.25	a
CRH	23.01		19.24	b	13.02	c
SPH + PB	28.83		21.21	b	14.49	bc
SPH + CRH	24.59		24.83	ab	16.00	bc
PB + CRH	26.11		23.54	ab	19.23	ab
C V (%)	15.00		16.50		15.29	
Substrates*	LA (mm ²)		SDM (g)		RDM (g)	
SPH	7,198.05	d	1.05	b	0.17	bc
PB	20,001.15	a	4.18	a	0.49	a
CRH	7,325.33	d	1.03	b	0.13	c
SPH + PB	9,584.32	cd	1.20	b	0.21	bc
SPH + CRH	11,408.61	bc	1.63	b	0.22	bc
PB + CRH	13,312.22	b	2.41	b	0.34	ab
C V (%)	16.40		41.07		36.22	

*Sphagnum (SPH), pine bark (PB), carbonized rice husk (CRH). ^{ns}non-significant. ¹mean followed by the same letter in the column do not differ by Tukey's test at 5% of probability.

For the leaves number per plant, only the semi-composed pine bark substrate contributed to increase this variable (23 leaves per plant). Concerning to the leaf area, the higher mean was also verified for semi-composed pine bark substrate. However, when using only the carbonized rice husk as substrate or sphagnum and its mixtures (SPH, SPH+PB and SPH+CRH) it was observed the lowest number of leaves (13 leaves per plant) and consequent reduction of leaf area. The plants acclimatized in semi-composed pine bark present leaf area two times greater than those acclimatized in sphagnum or in sphagnum combined with other material.

As the number of leaves per plant and leaf area are important characteristics and may be related to the high seedlings survival and growth in the field (SANTOS et al., 2004, MOREIRA et al., 2006), preference should be given to the substrates that promote these characteristics. The larger plants leaf area may be more efficient in solar energy absorption, as well as dry matter production. This effect could be verified for the plants that grew on semi-composed pine bark substrate, which accumulated three times more dry matter on shoot compare to the other substrates (Table 3). In reference to the pattern of these variables, the substrates sphagnum (SPH) and carbonized rice husk, pure or in mixture, have possibly reduced the index because of the high water retention capacity of these materials.

The elevate water content in the substrate can reduce the oxygen availability and damage the root respiration (AZEVEDO et al., 2008). Thus, it is important to emphasize that when there is a mixture of materials, the

substrates features, mainly physical, can be improved and reflects positively on the plants growth. This effect was also verified for other biometric characteristics analyzed, which did not present adequate results in carbonized rice husk substrate, but presented satisfactory results for the PB + CRH mixture (Table 3). It is possible to infer that the addition of semi-composed pine bark to the carbonized rice husk increased the apparent density (205.21 g L⁻¹) and decreased the water retention capacity (470.45 mL L⁻¹) of the mixture when compared to the pure carbonized rice husk (Table 1). It is important because in Bromeliaceae species, higher water content available in the substrate could damage pineapple plants growth, since these plants present a lower transpiration rate (SILVA and SILVA, 2006).

The semi-composed pine bark acts as a conditioner and improves the physical structure of the substrates, such as porosity, allowing efficiently gases exchange between substrate and environment. Formulations that decrease the substrate macroporosity create unfavorable conditions for the seedlings establishment and growth, because increasing the micropores amount in the substrate decreases aeration (BLOUIN et al., 2008; SUGUINO et al., 2011). In addition, the substrates semi-composed pine bark and PB + CRH used in this study presented physical properties which are in agreement with those reported in the literature to describe a good substrate, e.g. apparent density between 200 and 400 g L⁻¹ and water holding capacity ranging from 40 to 50% of the substrate volume (VERDONCK et al., 1981; KÄMPF, 2000). Regarding the substrates chemical characteristics, pH and electrical conductivity (EC), all

substrates also presented adequate values to ornamental plants cultivation (VERDONCK et al., 1981; KÄMPF, 2000; COLOMBO et al., 2016).

For the roots dry matter, the semi-composed pine bark was superior when compared to the other materials, whereas the substrate carbonized rice husk, sphagnum, and sphagnum + semi-composed pine bark presented the lowest value for this characteristic. Thus, it is important to observe the root growth in the acclimatization stage, since the roots generated *in vitro* are not functional when transplanted; therefore, new roots must be grown *ex vitro* (MOREIRA et al., 2006), in order to ensure the seedlings survival and performance at the field. Despite the favorable effect of the PB + CRH substrate for some biometric characteristics, this mixture presented the lowest percentage of seedling survival (62%), which could be an unfavorable point to choose this substrate.

4. CONCLUSION

The substrate semi-composed pine bark, presents the best conditions for acclimatization and growth of the pineapple ornamental seedlings.

AUTHORS CONTRIBUTION

R.C.C.: Creation of the idea, experiment installation and conduction at the greenhouse, laboratory analysis and data collection, statistical analysis of data, manuscript preparation. **V.F.:** Experiment installation and conduction at the greenhouse, laboratory analysis and data collection. **M.A.C.:** Laboratory analysis and data collection, manuscript preparation. **D.U.C.:** Laboratory analysis and data collection, manuscript preparation. **S.R.R.:** Orientation of the work, important suggestions incorporated to the work. **R.T.F.:** Orientation of the work, important suggestions incorporated to the work.

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