

Effect of different substrates on acclimatization and costs of arrow cane (*Gynerium sagittatum* Aubl.) micropropagated plants

Efecto de diferentes sustratos en la aclimatación y costos de plantas micropropagadas de caña flecha (*Gynerium sagittatum* Aubl.).

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ABSTRACT

To reduce costs associated to *ex vitro* adaptation of arrow cane (*Gynerium sagittatum* Aubl.) plants Cv “Criolla”, the effect of three substrate mixes (Peat, peat + river sand and peat + rice husk) on survival, plant height and substrate associated plant cost were evaluated. Plants were micropropagated in semisolid MS medium supplied with 0.5 mg L⁻¹ BAP. After medium removal, plants were transferred on 72-plug plastic trays filled with the respective substrate treatment. Trays were covered with translucent plastic covers during three days. Thereafter, plants were maintained in a 50% light shade house, fog irrigated twice a day for 1 minute each during 8 weeks. Treatments were distributed in with a complete randomized block design. Data were analyzed with ANOVA and means were separated with Tukey’s mean separation test. Results allowed to evidence that peat + sand resulted in significant increase in survival, plant height and approximately 35% decrease in substrate associated plant cost during acclimatization to *ex vitro* conditions.

Keywords: Arrow cane; Micropropagation; Plantlet; Survival; Transplant *ex vitro*.

RESUMEN

Con el fin de reducir los costos de adaptación *ex vitro* de plantas micropropagadas de caña flecha (*Gynerium sagittatum* Aubl.) cultivar “Criolla”, el efecto de tres sustratos (Turba, turba + arena y turba + cascarilla de arroz) sobre la supervivencia, el crecimiento y el costo de las plantas asociado al sustrato fueron evaluados. Las plantas fueron micropropagadas en medio MS semisólido adicionado con 0.5 mg L⁻¹ de BAP. Después de remover el medio de cultivo, las plantas fueron transferidas en bandejas plásticas de 72 alveolos dispensadas con la mezcla de sustrato respectiva. Las bandejas fueron cubiertas con tapas plásticas transparentes durante 3 días. Las plantas fueron mantenidas en una casa malla con polisombra del 50% de luminosidad y dos riegos diarios de 1 minuto cada uno por nebulización durante 8 semanas. El diseño utilizado fue el de bloques al azar, los datos fueron analizados con ANOVA y los promedios separados con la prueba de separación de medias de Tukey. Los resultados permitieron evidenciar que la mezcla de sustrato formada por turba + arena resultó en incrementos significativos en el porcentaje de supervivencia, altura de planta y redujo en >35% el costo final de las plantas asociado al uso de sustrato durante la adaptación a las condiciones *ex vitro*.

Palabras clave: Caña flecha; Micropropagación; Plantas adaptadas; Supervivencias; Trasplante *ex vitro*.

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INTRODUCTION

Arrow cane (*Gynerium sagittatum* Aubl., $2x = 2n = 72$) is a Poaceae species whose plants in the Americas grow between 0 and 1700 (masl) from Central America, the Antilles to Bolivia and Paraguay. In Colombia, arrow cane plants grow in the West Planes, Central Andean region and the Caribbean Coast where it is cared by aboriginal communities established in the flatlands of Córdoba and Sucre departments since ancient times (GRIN, 2019; Suárez, 2019a). Arrow cane plants are perennial, giant rhizomatous, reed grass with low part of the culms clothed with bladeless sheaths and upper part with unfold leaf blades and open fan-shaped form. Culms range from 5-14 m long and die after flowering. Leaves are bright green 160-230 cm long and 8-14 cm wide; culms can form as much as 200 leaves during life time with 19-28 fresh blades at any time (Kalliola, *et al.*, 1992). Aerial stems are underground interconnected by a net of leafy rhizomes with shoots at the end point that grow at a distance 15-20 cm from the original culm. Both, aerial and underground, structures serve as colonizers of new territories at the same time that prevent soil erosion (Contreras, *et al.*, 1998; Kalliola, *et al.*, 1992; Suárez, *et al.*, 2013). The leaf central nerve is the raw material used by natives to make a colorful variety of worldwide known handicraft, included "Sombrero Vuelto" raised as Colombian Cultural Symbol by Congress: Arrow cane crafts are considered an antique mechanism for religious, artistic and political expression and a legacy of the Zenú tribe of Indians (Artesanías de Colombia, 2019).

Large scale cultivation of arrow cane plants to produce fiber for craft activities has been hindered by the lack of an efficient plant propagation system. Seeds are highly unviable and cuttings need large stem pieces to root (González, 1997). This situation has increased pressure on natural populations as the major source to obtain fiber for crafting. Reports

show micropropagation throughout culture of explants with pre-existing meristems as an efficient alternative method for mass clonal propagation due to high multiplication rates, genetic stability and complete plantlet recovery (Suárez, *et al.*, 2009; Pastrana and Suárez, 2009; Suárez, 2019b). Despite benefits of micropropagation for large production of clonal plant material, costs of micropropagated plants are still a disadvantage for low cost agricultural systems such as arrow cane cultivation. To lower costs in arrow cane micropropagation, conventional semisolid culture media compared to double phase medium system were evaluated; shoot costs were reduced by 40% when plants were proliferated in double phase medium (López and Suárez, 2018).

Likewise, *ex vitro* transfer and hardening of micropropagated arrow cane plants without transparent plastic covers not only reduced labor costs, but also increased plant growth without affecting plant survival (Pico, 2019). Transplant to *ex vitro* conditions is the most critical stage for plant survival in micropropagation (Suárez, *et al.*, 2020). *Ex vitro* acclimatization of arrow cane micropropagated plants has traditionally been done by transferring *in vitro* grown plants into high cost peat substrate because of high water retention that contributes to full plant recovery (Suárez, *et al.*, 2013; Suárez, *et al.*, 2020). Substrate mixes are intended to provide support to the plants, retain moisture, allow drainage and provide nutrients, especially for root growth and development; however, using locally available materials such as sand, crops residues, compost, rice husk and soil contribute to lower substrate associated costs (Dias, *et al.*, 2018; Pascual, *et al.*, 2018; Waman, *et al.*, 2019). In the present research, the effect of peat and two substrate mixes on plant recovery and growth were evaluated and cost associated with the *ex vitro* acclimatization stage calculated.

MATERIALS AND METHODS

Plant material

Plant material was obtained from *in vitro* maintained Cv “Criolla” plants during a year with monthly transfers to fresh medium. Explants consisted of three-stem clusters established in semisolid MS (Murashige and Skoog, 1962) medium with (in mg L⁻¹) BAP (Benzilaminopurine) (0.5), myo-inositol (100), sucrose (30000), thiamine HCl (0.4) and solidified with TC Agar (7000). Medium pH was adjusted to 5.7-5.8 with HCl or KOH previous to agar addition. The medium was sterilized by autoclave at 120 °C and 1,2 psi. A single explant was established per 125 cm³ borosilicate flask with 30 mL medium aliquots. Flasks were covered with two layers of heavy-duty aluminum foil and sealed with Parafilm®. Cultures were stored at 25 °C with 12 hours photoperiod (40 μmol m⁻¹ s⁻¹) provided by white cool fluorescent lamps with transfers to fresh medium every four weeks.

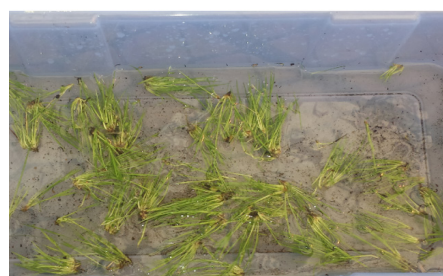
Substrate preparation and evaluation

Three substrate mixes (volume:volume): peat, peat + rice husks (1:1) and peat + sand (1:1) were prepared and evaluated. Sand was obtained from river shores and washed with potable water followed by sterile deionized water. Peat was obtained from commercially available product (Pindstrup®) while rice husk and sand were locally obtained and disinfected by heat (180 °C) in an oven for 48 hours. Parts were mixed, moistened and covered with polyethylene during 48 hours before use. Substrate mixes were individually dispensed in 72-plug plastic trays 48 hours before plant transfer to *ex vitro* conditions.

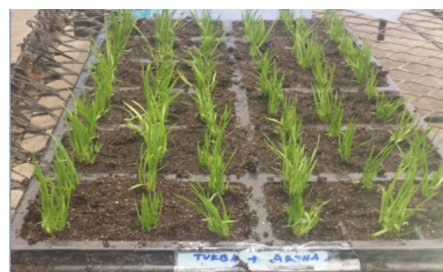
Ex vitro transfer

In vitro cultivated Cv “Criolla” plant clusters maintained in multiplication medium during

four weeks were removed from the flasks, the medium residue was washed out with sterile distilled water (Figure 1a) and clusters (3 cm long) were established in plugs previously filled with the respective substrate mix. In each plug, a cluster was placed and the substrate around it compacted (Figure 1b). Once transplanted, plants were sprayed with sterile distilled water and trays were covered with translucent plastic covers (Figure 1c). Trays were maintained in a shade house (50% Saram®) with three sprays a day using sterile distilled water. After three days, plastic covers were side moved to allow air exchange; and after five days, trays were uncovered and maintained with fog irrigation twice a day (9:00 AM and 4:00 PM) for 1 minute each.



(1a)



(1b)



(1c)

Figure 1. Shoot plant clusters of *Cynerium sagittatum* Aubl. Cv “Criolla” plants removed from culture medium (1a), transplanted in substrate mix (1b) and maintained during *ex vitro* acclimatization (1c).

Research consisted of a one-way factorial experiment where the effect of substrate mix (Three treatments) on plant survival and growth was evaluated. Treatments were distributed with a complete randomized block design where each tray was the block with three replicates per treatment for a total of 648 experimental units. Eight weeks after the transplant, the number of survived plants was registered and the survival percentage calculated. For each treatment, 10 plants were randomly selected, plant height data were registered, analyzed with ANOVA ($\alpha = 0.05$) and means separated with Tukey Test ($\alpha = 0.05$). Estimation of substrate effect on plant cost was calculated based on substrate value and amount used, and number of plants recovered 8 weeks after the transplant.

RESULTS AND DISCUSSION

Transfer to *ex vitro* and plantlet recovery

Survival percentage under *ex vitro* conditions was 90% for plants transplanted in peat + sand mix, 85% for plants transplanted in peat and 60% for plants transplanted in peat + rice husk mix (Figure 2). ANOVA allowed detecting statistical differences ($Pr < 0.0001$) as a result of the treatments. The Tukey test on collected data showed that survival percentage of plant transplanted in trays filled with peat + sand mix was statistically higher than those transplanted in peat alone and peat + rice husk mix.

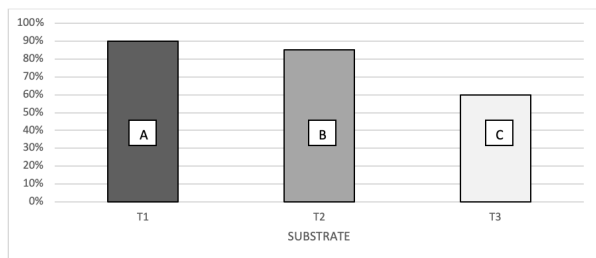


Figure 2. Percentage of *Gynerium sagittatum* Aubl. Cv “Criolla” micropropagated plants that survived after eight weeks of being transplanted in peat + sand mix (T1), peat (T2) and peat + rice husk mix (T3) [Values with the same letter are not different according to Tukey test ($\alpha = 0.05$)].

Plant height

The ANOVA allowed detecting statistical differences ($Pr = 0.0042$) in plant height data as a result of the effect of substrate mixes. Tukey mean separation test showed that plants transplanted in trays filled with peat + sand mix (9.18 cm) were significantly higher than the plants transplanted in peat + rice husk (8.43 cm) and those transplanted in peat alone (7.43 cm) (Figure 3).

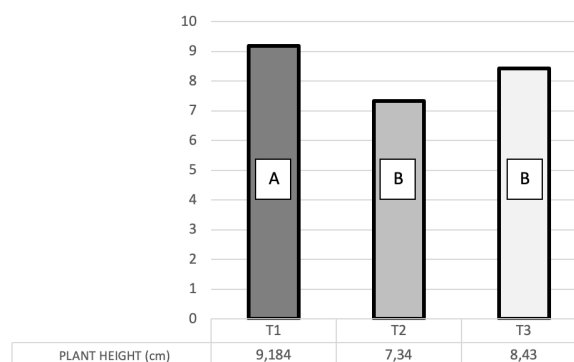


Figure 3. Plant height of *Gynerium sagittatum* Aubl. Cv “Criolla” micropropagated plants after eight weeks of being transplanted in peat + sand (T1), peat (T2) and peat + rice husk (T3) [Values with the same letter are not different according to Tukey test ($\alpha = 0.05$)].

Cost analysis

The costs of substrate mixes used during plant transfer to *ex vitro* conditions varied from peat (US\$5.52), peat + rice husk (US\$4.14) and peat + sand (US\$3.72), according with the amounts of materials used. The estimated cost of substrate mix associated with the total number of 216 transplanted plants in each substrate mix and the final number of plants recovered in each treatment, show that the highest economic efficiency in plant cost unit occurs when plants are transplanted in peat + sand mix, followed by peat alone and peat + rice husk with the highest proportional cost (Table 1).

Micropropagated plants are costly because of use of specialized infrastructure, reagent costs, qualified personnel and labor (Ahloowalia and Savangikar, 2002; Aziz and Al-Taweel, 2019). Implementing strategies to lower micropropa-

Table 1. Cost analysis of *ex vitro* acclimatization of *Gynerium sagittatum* Aubl. Cv “Criolla” micropropagated plants transplanted in different substrate mixes.

Substrate	Substrate cost (US\$)	Survival (%)	Final plants	Plant cost (US\$)
Peat + sand	3.72	90	195	0.019
Peat	5.52	85	184	0.030
Peat + rice husk	4.14	65	140	0.032

gated plant costs is always a challenge, and studies to increase cost efficiency in micropropagation of several plant species has been reported (Raghu, *et al.*, 2007; Sahu and Kumar, 2013; George and Manuel, 2013; Kadam, *et al.*, 2018). In arrow cane, mechanisms used to decrease plant costs during micropropagation incorporate several aspects. López and Suárez (2018) evidenced that using double phase (semisolid-liquid) culture media reduced by 40% costs of Cv “Criolla” micropropagated shoots and significantly increased *in vitro* multiplication rate of Cvs “Criolla”, “Criolla 1”, “Martinera” and “Costera” (López, 2018; Suárez, *et al.*, 2020). In a different strategy, Pico (2019) demonstrated that *ex vitro* transplanting and hardening of micropropagated Cvs “Criolla”, “Martinera” and “Costera” plants without plastic covers did not affect survival rate and plant growth, but instead decreased costs because of lower labor demand.

Several substrate mixes have been used to increase survival during *ex vitro* adaptation of various micropropagated plant species. Bonilla, *et al.*, (2015) increased 3x the survival rate and improved adaptation of micropropagated *Manihot sculenta* plants when used a solid humus + rice husk (1:1) substrate mix. Palacios-Arriaga, *et al.* (2019) increased survival percentage of genetically modified rose plants regenerated through somatic embryogenesis when the transplanting was done in a peat + perlite (1:1) substrate mix. Espinosa-Reyes *et al.* (2019) reported >85% survival and

increased plant height during *ex vitro* adaptation of *Morus alba* micropropagated plants transplanted in substrate mixes with different amounts of soil, cow manure and zeolite.

Sand is an inert material that provides density, increase drainage and favors air exchange in substrate mixes; in contrast, peat and rice husks favor water retention and moisture (Verhagen, 2009; Walczak, *et al.*, 2002; Londra, *et al.*, 2018). Pérez-Alonso, *et al.* (2016) evaluated the effect of cachaza compost and zeolite, mixed or alone, on *ex vitro* adaptation of *Aloe vera* micropropagated plants, observing that compost alone contributed to 100% plant survival and better plant growth and development because of substrate aeration. Gil, *et al.* (2017) working on *ex vitro* transfer of micropropagated *Morus alba* plants reported an increased percentage of *ex vitro* adapted plants, higher number of rooted plants, increased mean number of leaves per plant and larger leaves when plants were transferred in a sand + moss + humus substrate mix, correlating the results with the sand characteristics that provided to the mix. The present work evidenced that arrow cane micropropagated plants showed a higher survival rate, a better growth and development, and resulted in a lower costs when the transplanting was carried out in a peat + sand substrate mix. These results allow increasing accessibility of the arrow cane micropropagation technology to plant growers and artisans.

CONCLUSIONS

- The peat + sand (1:1) substrate mix significantly increased plant survival in arrow cane Cv “Criolla” micropropagated plant transferred to *ex vitro* conditions.
- Arrow cane Cv “Criolla” micropropagated plants transplanted to *ex vitro* conditions in a peat + sand (1:1) mix substrate grew significantly higher compared to plants transplanted in peat alone or peat + rice husk mix.
- *Ex vitro* transfer of arrow cane Cv “Criolla” micropropagated plants using a peat + sand (1:1) substrate mix reduces plant costs associated to transplant by 35% with respect to plants transplanted in peat alone and by 37.6% when the transplant is done using peat + rice husk (1:1) substrate mix.

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