Temas Agrarios
ISSN: 0122-7610
ISSN: 2389-9182
revistatemasagrarios@correo.unicordoba.edu.co
Universidad de Córdoba
Colombia

Suarez-Padrón, Isidro E.; López-Díaz, Claudia M.
(*Gynerium sagittatum* Aubl.). A plant of cultural interest.
Temas Agrarios, vol. 26, no. 2, 2021, July-December, pp. 190-202
Universidad de Córdoba
Colombia

DOI: https://doi.org/10.21897/rta.v26i2.2890

REVISTA
TEMAS AGRARIOS

REVIEW ARTICLE

Biotechnology on arrow cane (*Gynerium sagittatum* Aubl.). A plant of cultural interest.

Biotecnología en caña de flecha (*Gynerium sagittatum* Aubl.). Una planta de interés cultural.

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Recibido para publicación: octubre 13 de 2021 - Aceptado para publicación: diciembre 21 de 2021

ABSTRACT

Arrow cane (*Gynerium sagittatum* Aubl.) is a Poaceae associated to human development of the ancient Zenú culture present nowadays in the flat lands of Córdoba and Sucre department in the Colombian north Coast. Despite its importance, arrow cane is not cultivated in large scale and extraction from natural populations is the most common way for plant exploitation with negative environmental and economic consequences for the aboriginal communities. A research program based on biotechnology was implemented to generate information related to plant genetic diversity, plant propagation and genetic resource conservation in order to provide knowledge to contribute to implement technical crop development to minimize the impact of artisans´ activity on the environment and increase sustainability on fiber source for craftsmanship. Accessions from different zones of the Colombian territory were characterized using morphological and molecular markers founding low genetic diversity. Cultivars UC121, "Criolla", "Criolla 1", Costera and "Martinera" were *in vitro* established and micropropagation results indicated that large quantities of high quality, fully adapted, homogeneous plants can be produced from any cultivar. *In vitro* cultured arrow cane plants can be stored by means of slow growth or modified structures such as micro rhizomes developed *in vitro*.

Key words: Wild cane; Zenú; Genetic diversity; *In vitro* culture; Micropropagation.

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RESUMEN

Caña flecha (*Gynerium sagittatum* Aubl.) es una Poaceae que ha estado asociada al desarrollo humano de la antigua cultura Zenú asentada actualmente en las planicies de los departamentos de Córdoba y Sucre en la Costa Norte Colombiana. A pesar de su importancia, la caña flecha no es cultivada a gran escala y la extracción a partir de las poblaciones naturales es la forma más común de explotación de la planta con consecuencias negativas tanto en el ambiente como en la economía de las comunidades indígenas. Un programa de investigación basado en biotecnología fue implementado para generar información relacionada con la diversidad genética de la planta, propagación y conservación de recursos genéticos con el fin de proveer conocimientos que contribuyan a implementar el desarrollo de cultivos tecnificados para reducir el impacto de la actividad artesanal sobre el medio ambiente e incrementar la sostenibilidad en el suministro de fibra para las artesanías. Accesiones de diferentes zonas de Colombia fueron caracterizadas usando caracteres morfológicos y moleculares encontrando una baja diversidad genética. Los cultivares UC121, "Criolla", "Criolla 1" y "Martinera" fueron establecidos *in vitro* y los resultados de la micropropagación indican que grandes cantidades de plantas, completamente adaptadas, homogéneas y de excelente calidad pueden ser producidas de cualquier variedad. Las plantas de caña flecha establecidas en condiciones *in vitro* pueden ser conservadas por largos períodos de tiempo mediante el uso de cultivo lento o la generación de estructuras modificadas como los micro rizomas desarrollados *in vitro*.

Palabras clave: Caña brava; Zenú; Diversidad genética; Cultivo *in vitro*; Micropropagación.

Cómo citar

Suarez-Padrón, I.E. and López-Díaz, C.M. 2021. Biotechnology on arrow cane (*Gynerium sagittatum* Aubl.). A plant of cultural interest. Temas Agrarios 26(2): 190-202. https://doi.org/10.21897/rta.v26i2.2890

INTRODUCCIÓN

Arrow cane (*Gyneriun sagitattum* Aubl.) (Poaceae) is a plant species that grows from Central America, the Antilles to Bolivia and Paraguay. In Colombia, arrow cane is the most important plant species for Zenú aboriginal people, a millenarian indigenous community established since pre-Columbus times in the flatlands of Córdoba and Sucre Departments. Zenues use the plant for home construction, tools for hunt and fish, musical instruments, feed animals, natural medicine, and the leaf central nerve to manufacture the most famous worldwide Colombian handicrafts (Artesanías de Colombia, 2019; SIC, 2019; Lopez y Suarez, 2018). Arrow cane crafts sales are the most important source of income for a legally organized Indian reservation called RISAS (Resguardo Indígena de San Andrés de Sotavento), a community with around 20000 aboriginal people (DANE, 2019).

Arrow cane large commercial crops are inexistent and most fiber is extracted from natural populations; it is estimated that craft sales increase in the past 20 years have contributed to decrease >50% plant natural populations, negatively impacting on wetland preservation, ecosystems stability and increasing fiber costs for artisan activities (Araméndiz *et al*., 2005). Limitations to plant large crop plantations are based on the inability to provide large quantities of good quality plant material for crop initiation. Since seeds are not viable for germination and plant production, initial studies to find a workable propagation system focused on plant growth from cuttings and new shoot growth; however, propagule size needed for rooting and time for new shoot growth resulted highly inefficient (Hernández *et al*., 2005). A research program to study arrow cane based on biotechnology techniques was set to minimize plant limitations for crop production by the Plant Biotechnology Research Group, Institute of Applied Biotechnology for the Colombian Caribbean of the Universidad de Córdoba.

To assess genetic variability accessions from different origins of the Colombian territory were morphological and molecular characterized using agronomic characters and amplified fragments length polymorphism (AFLPs), respectively. Results from both methodologies revealed a low genetic diversity among accessions because of asexual propagation and anthropic movement of plant material from place to place (Araméndiz *et al*., 2005; Rivera *et al*., 2009). Micropropagation was used as an attempt to develop an efficient method for plant material production. Cultivar UC121 was successfully introduced *in vitro* and micropropagation resulted in a high multiplication rate (12 new shoots from a single explants); plants fully adapted to ex vitro conditions. Cultivar “Criolla”, the most preferred for artisans, was then micropropagated and multiplication rates doubled compared to UC121. Double phase medium was tested as a way to reduce cost by gelling agent elimination; results showed a 20% cost reduction per micropropagated shoot.

Micropropagation protocols were extended to cultivars “Criolla 1” and “Martinera” with multiplication rates above 10 new shoots per explant for both cultivars. Besides micropropagation, *in vitro* storage as a way for genetic resource preservation has been also implemented. Arrow cane plants were cultured during a year on the same medium conditions evidencing that reduced nutrient and low carbon source induced a better response to prolonged culture conditions. Alternatively, modified stems (micro rhizomes) were observed to provide a suitable structure for plant propagation and conservation (Suárez *et al*., 2009; Pastrana and Suárez, 2009; Suárez *et al*., 2013; Suárez *et al*., 2017, Pérez *et al*., 2019).

The present review will show how the arrow cane biotechnology program has allowed knowing that genetic diversity of the species in Colombia is low and strategies must be implemented to increase genetic variability. Arrow cane conventional plant propagation methods are inefficient; however, micropropagation protocols can supply large quantities of high-quality plants for commercial crop establishment, and *in vitro* culture can be used as a tool for a safe long term genetic resource conservation strategy.

Arrow cane (*Gynerium sagittatum* Aubl.): Origin, distribution, botany and importance.

Arrow cane (*Gynerium sagittatum* Aubl.) is a plant species native from West India and widely distributed along the neotropics. In the Americas, arrow cane is found from South Mexico, Central America and the Antilles to Bolivia and Paraguay, always associated with floodplains, swamps and river shores, and grows between 0 and 1700 m.a.s.l.; several studies associate arrow cane as a colonizer for forest succession in rivers with high channel migration. In Colombia, arrow cane is found in the Cauca and Magdalena valley rivers, shores of Samaná River, South of Cauca and Valle departments, Sonsón and Medellín (Antioquia), San Martín Plains and Nechí river shores. Arrow cane is well adapted to the Colombian Atlantic Coast environmental conditions where grows in the planes of Córdoba and Sucre departments. The plant is endemic to Sinú and San Jorge river valleys and is cultivated at low scale by aboriginal communities in the municipalities of Montelibano, San Carlos, Tuchín, Chimá, Canalete, Valencia, Los Córdobas, San Andrés de Sotavento and Montería (Hitchcock, 1936; Conert, 1961; Pérez, 1978; Croat, 1978; Howard, 1979; Uribe, 1982; Salo *et al*., 1986; Kalliola *et al.*, 1992).

The arrow cane plant is a perennial, giant, rhizomatous, reed grass with erectus stems that grow from a single point forming culms. Low part of the culms are clothed with bladeless sheaths while the upper part has the unfold leaf blades with an open fan-shaped form. Culms can be 5 to 14 m long and die after flowering. Leaves are bright green 160 to 230 cm long and 8 to 14 cm wide; a culm can form as much as 200 leaves during lifetime with 19 to 28 fresh blades at any time. Culms are interconnected with an underground net of strong leafy rhizomes that originate from old culms and serve as source for new shoots working to prevent soil erosion. Floral biology of *Gynerium sagitatum* has been scarcely studied. Most culms flower and set seeds some do not flower at all. Flowers grow in staminate no feathery spikelet, 3 mm long, hyaline glumes, purple colored and pubescent at the base. Fruits are narrow and oblong about 1 mm long covered with silky hairs that favor wind and water dispersion. The plant is dioecious, wind pollinated and seed index is about 1,67 million kg^{-1} (Howard, 1979; Pohl, 1983; Francis, 1983; Schnee, 1984; Kalliola *et al*., 1988; Kalliola *et al*., 1992; Araméndiz *et al*., 2009; Suárez *et al*., 2013).

Arrow cane is the keystone of the ancient Zenú culture, an aboriginal group that populated Colombian Atlantic coast planes since pre-Columbus times. The plant has been utilized to construct houses, make arrows to hunt and fish, manufacture ornamentals, use as medicine and feed animals. Old culms are used to make musical instruments and the central nerve is the source to manufacture around 50 types of handicrafts, and among them *Sombrero Vueltiao* is the most worldwide known and declared as Colombian Cultural Symbol by Congress; figures found in the crafts are associated with music, religious and political expression of the *Zenú* people. Crafts sales are the main source of income for *Zenú* communities established in the *Resguardo*

Indígena de San Andrés de Sotavento, an Indian Reservation with around 20.000 people established in seven municipalities in the Sucre and Córdoba Departments; however, increased sales have impacted negatively in arrow cane natural populations since few crops are established and most fiber needed for craftsmanship is extracted from the wild. Because of the crafts industry activity, it is believed that arrow cane natural population has decreased more than 50%, jeopardizing not only the economic sustainability of the *Zenú* people for the future but also impacting the ecosystem by disturbing wetlands ecosystems, increasing erosion and displacing associated species (Valencia, 1987; Serpa, 2000; Araméndiz *et al*., 2009).

Molecular characterization

Arrow cane (2n = 72 or 2n = 76), also known as wild cane, brave cane, can cane, carrizo or chusque, is systematically classified as class Magnoliopside, order Poales, family Poaceae, genus *Gynerium*, species *Gynerium sagittatum* (Aubl.) P. Beauv. (GRIN, 2018; IT IS, 2018). Two distinct types of Gynerium sagittatum has been reported: a “large” typical of small and medium size meandering rivers, stems are 8-14 m long, soft, round and unbranched. The “small” type is characteristic of large rivers with meandering or anastomotic channels, stems 5-8 m long, oval or flattened culms and commonly branched. Unbranched culms, long leaves and culms, rhizome distribution and predominant clonal propagation may indicate that “large” type plants predominate in the Colombian North Coast (Ferreyra, 2018; Kalliola *et al*., 1988; Watson y Dallawitz, 2018; Araméndiz *et al*., 2009).

In a study to assess genetic resources of arrow cane in Colombia, 38 accessions were collected from Antioquia, Córdoba, Caldas, Norte de Santander, Santander and Valle departments. Out of 38.28 were successfully rooted and established as a germplasm collection in the Universidad de Córdoba Experimental Field.

A morphological characterization was carried out using 12 qualitative characters such as internodal color, culm wall, wall texture, leaf orientation, leaf arch, blade pubescence, leaf base pubescence, sheath color, branching, flowering and fiber texture, and 10 quantitative characters such as plant high, tillering index, low and upper internodal distance, stem diameter, leaf number, leaf length, leaf wide, midrib size and shaft length. The collected data was analyzed using a multiple correspondence analysis and classification according with Ward Aggregation Hierarchical Ascendant Method (Ward, 1963). The results allowed to conclude that there was low genetic variability among collected accessions; specially, the ones collected inside Córdoba department. Different denomination of accessions is the result of personal and cultural origin rather than based on evolutionary changes in genotype or phenotype; however, the wide distribution of arrow cane all over the Colombian territory reduces risks of genetic erosion (Araméndiz *et al*., 2009).

In an attempt to increase sensitivity of the genetic analysis, molecular approaches to characterize arrow cane collections were reported. To develop an AFLPs analysis protocol, genomic DNA was isolated from 25 arrow cane accessions obtaining pure, low degraded DNA that was used as a template for digestion with EcoRI and MseI. The digested DNA fragments were adapted with ACA, ACT and ACC for EcoRI terminals and CAG, CTA, CAT and CAA for MseI terminals. The adapted fragments were amplified using PCR (Polymerase Chain Reaction) with eighth primer combination and AFLP fragments were separated in a 6% polyacrylamide electrophoresis vertical gel. The presence (1) or absence (0) was plotted in a binary matrix for grouping several variables.

Initial analyses allowed to detect that only four pair of primers were polymorphic, and therefore were selected and used for a definitive analysis. The collected data showed that fragments ranged between 20 and 330 bp length, primer combination polymorphism ranged between 25.8 and 39.53%, E-ACC/M-CAG was the most polymorphic primer combination (38%), a total of 223 loci were generated. Primer combination with the lowest loci number resulted in the highest number of monomorphic loci (Nei and Li, 1979; Rivera *et al*., 2009).

To assess genetic variability and association with geographical origin, AFLPs were used to analyze 25 *Gynerium sagittatum* Aubl. accessions. The results showed low genetic difference and low geographical origin correlation; however, the Dice similarity index (Dice, 1945) discriminated three groups based on fiber characteristics, such as softness, resistance, flexibility, important for crafts work. A first group comprised 13 accessions collected from inside Córdoba department distinguished by soft fiber texture, low pubescence and thick stem wall, consequent with accessions named as "Criolla" by local farmers and desirables for handicraft production. Groups two and three clustered accessions from Antioquia, Córdoba and Caldas departments with rough texture, abundant pubescence in leaves and shafts, thin stem wall, acute leaf angle and thick stem. Low correlation between accession clusters and geographical origin was attributed to clonal propagation of the plants and flow of plant material for planting *vía* anthropic methods (Rivera *et al.*, 2008; 2009).

Propagation

Arrow cane can propagate naturally by seeds or rhizome growth, or directed by cuttings. When vegetative propagation occurs, culms produce new rhizomes with shoots at the end point that grow in free spaces at a distance 15 - 20 cm from the original culm. Long distance dispersal also occurs when stems self-cut and water transports them elsewhere; these fragments are observed to root profusely. Viable seeds germinate 3-7 days after imbibition at 20-30 °C (Kalliola *et al*., 1988; 1992).

"Large" type plants flower abundantly but seed viability is low; therefore, clonal propagation by rhizome growth and shoot development is the standard method for plant multiplication. Besides clonal propagation, seed germination and seedling development is frequent for "small" type plants. An experiment using sexual propagules to evaluate germination and seedling growth from both "small" and "large" type of plants, resulted in very low germination and seedling development (1%) for "large" type plants while "small" type fully germinated and seedling growth reached 50 cm 4 months after germination. In Colombia, under humid Caribbean conditions, seed propagation has not been reported (Pohl, 1983; Francis, 1983; Kalliola, 1992; Aramendiz *et al.*, 2009).

Cutting propagation was attempted to develop a system for rapid sustained production of plant material for commercial crop planting. The effect of type of cutting (rhizome or culm fragments), cutting longitude and position in the substrate were evaluated. Results showed that aerial 3-4 nut cuttings, vertically positioned in the substrate resulted in higher rooting percentage, a greater number of shoots and longer shoots compared to rhizome portions or shorter culm sections, horizontally positioned in the substrate. However, a different experiment under similar conditions reported a higher rooting percentage for horizontally positioned 3-4 nut culm sections. External auxin supply was used as a mechanism to improve rooting levels in cuttings. Culms sections of cultivar "Criolla" consisting of 2, 3 or 4 nuts (30, 40 and 50 cm, respectively) were treated with 0, 400, 800, 1200, 1600 or 2000 mg L^{-1} naphthaleneacetic acid (NAA)

or indolebutyric acid, and their combinations. Auxins were dissolved in sterile-distilled water and cuttings were immersed 5 cm from the base during 1 hour in the dilution.

Treated cuttings were, thereafter, established in plastic bags (1.100 cm^3) containing a silky:sand (1:1) substrate. After 60 days, rooting was common for all treatments, including those non-auxins treated; there were no statistical differences among treatments for root number and root length, root number range was 2-3 per cutting, root length was 3-4 cm, and total rooting was between 75% (control) and 100% (1200 mg L^{-1} ANA + IBA) (Hernández *et al*., 2005; Araméndiz *et al.,* 2009).

Micropropagation

Cutting propagation studies showed that arrow cane is an easy to root plant species, even when no exogenous auxin is supplied. However, cuttings should be long enough to provide at least one underground node for root formation, and at least 2-3 nodes above soil level for shoot growth. A 3-4 node section cutting is equivalent to 30-40 cm long; therefore, a maximum 2-3 cutting per adult culm may be obtained for rooting and shoot development. This fact makes arrow cane propagation by cuttings inefficient for production of large number of plants despite easiness for root formation. Micropropagation is a technique that allows massive clonal propagation of plants under controlled environmental conditions, in absence of microorganisms and supplied with culture media with organic and inorganic compounds that provide all nutrients for growth and development. Micropropagated plants grow in closed recipients (*in vitro*), under heterotrophic nutrition, usually is more space and time efficient than external propagation methods and has been attempted as an alternative for high quality, massive production of arrow cane plant material (Ball, 1946; Murashige, 1974; Kane, 1996; Gielis, 1999; Suarez *et al*., 2013).

Micropropagation from pre-existing meristems consists of in vitro establishment of sterile explants containing at least one bud followed by its repetitive elongation in a cytokine-supplied medium (Debergh and Maene, 1981; Read, 1988; Davis, 1995; Kane, 1996; Chawla, 2003; Leifert and Cassells, 2001). The first study on arrow cane micropropagation of was performed using a cultivar named UC121 (Stands for Universidad de Córdoba 121) obtained from the arrow cane collection established at the Universidad de Córdoba Experimental Field (Montería - Colombia). Explant consisting of surface-sterilized nodal segments (2.0 cm long) with a single axillary bud were cultured in three different semisolid MS (Murashige and Skoog, 1962) (Full strength, half strength and full strength plus 0.3 mg L^{-1} BAP with 0.1 mg L^{-1} NAA) to evaluate establishment conditions. Furthermore, to evaluate multiplication rate, *in vitro* established explants were transferred onto semisolid MS medium supplied with different BAP concentrations (0; 0.5; 1; 2 or 4 mg L^{-1} BAP) or BAP (0.3 mg L^{-1}) combined with NAA (0.1 mg L^{-1}).

Once multiplied, shoots were transferred onto rooting media consisting of semisolid MS with different NAA (0, 1, 2, 3 or 4 mg L^{-1}) to evaluate rooting percentage, roots per explant and root length. Finally, rooted and rootless micropropagated shoots were transferred *ex vitro* onto peat substrate with fog irrigation for four weeks to evaluate plant survival. Data showed that culture of explants in MS medium supplied with 0.3 mg L^{-1} BAP and 0.1 mg L^{-1} NAA resulted in higher survival percentages (80%) during establishment and higher multiplication rates (12 new shoots per explant) after 12 weeks in culture. Shoots rooted in all treatments; however, NAA supply statistically increased (20X) root number per explant and decreased >3X the root length

after four weeks. *Ex vitro* transferred rooted and rootless plants fully adapted to external conditions. The overall results showed that arrow cane could be *in vitro* established and micropropagated, rooting was not necessary for *ex vitro* recovery and large amounts of plants can be produced from a single explant, becoming an efficient alternative for planting material production (Suarez *et al*., 2009).

Fiber flexibility and resistance are the main characteristics artisans use to select arrow cane plants for craftsman work, and cultivar "Criolla" is one of the preferred (Aramendiz *et al.,* 2009). To develop a micropropagation protocol for "Criolla" plants, surface-sterile 2 cm long nodal segments containing an axillary meristem were *in vitro* established onto semisolid MS medium supplied with four different BAP (0, 0.5; 1, 2 or 4 mg L^{-1}) concentrations, and a treatment with 0.3 mg L^{-1} BAP combined with 0.1 mg L^{-1} NAA. Multiplied shoots were *in vitro* rooted with several NAA concentrations (0, 0.5; 1, 2 or 4 mg L^{-1}) and, thereafter, rooted and rootless shoots were transplanted *ex vitro* for plantlet recovery.

The results showed that "Criolla" explants proliferated statistically better (25 new shoots per explant) when cultured with 0.5 mg L^{-1} BAP than those cultured with 0.3 mg L^{-1} BAP combined with 0,1 mg L^{-1} NAA as observed for cultivar UC121 (12 new shoots per explant). In addition, despite the excellent levels of rooting *in vitro* (100% rooting and >12 roots per explant when cultured with NAA supply), rooted and rootless micropropagated shoots fully adapted (100%) to ex vitro conditions; reinforcing the capacity of arrow cane micropropagated plants to adapt to ex vitro conditions without *in vitro* rooting. The results of this study, confirmed that arrow cane plants can be micropropagated for supplying massive number of high-quality plant material for commercial crops (Pastrana and Suarez, 2009).

Besides "Criolla", other local cultivars are used by arrow cane artisans. Cultivars "Martinera" and "Costera" are also known because softness and flexibility of their fiber; however, fiber resistance seems to be lower than "Criolla", and therefore are used for different types of crafts such as necklaces, wallets, shoes and other appliances (Aramendiz *et al.,* 2009). To provide a micropropagation protocol for these cultivars, shoots were isolated from field grown crops and planted in a soil:sand (1:1) substrate contained in plastic pots (20 x 35 cm) and maintained in a shade house (80% shade) with daily irrigation. Actively grown axillary buds were removed using a surgical blade, surface-sterilized with a 1.25% sodium hypochlorite solution during 15 min, washed three times with sterile deionized water inside a laminar flow hood and established onto MS semisolid medium supplied with (in mg L^{-1}) myo inositol (100), thiamine HCl (0.4), sucrose (30.000) and TC agar (7.000).

Once adapted to *in vitro* conditions, shoots were transferred onto semi solid MS medium supplied with different BAP (0; 0.5; 1 or 1.5 mg L^{-1}). Maximum multiplication rate resulted in 130 new shoots per explant for "Martinera" and 30 new shoots per explant for "Costera" when shoots were cultured in medium supplied with 1 mg L^{-1} BAP. Shoot length for both cultivars were 3.8 cm and 2.7 for "Martinera" and "Costera", respectively, at the same BAP concentration.

Multiplied shoots were cultured in rooting media to evaluate the effect of NAA different concentrations (0; 0.5; 1 or 1.5 mg L^{-1}) on rooting percentage, root number per explant and average root length. After 6 weeks in culture, shoots cultured with 0.5 mg L^{-1} NAA produced a significant higher number of roots (4X for "Martinera" y 2X for "Costera") than those cultured in simple media with no NAA. Number of roots per explant was as higher as 70 for "Martinera" and 50 for "Costera" when

NAA supply was 1.0 mg L^{-1}. Average root length was always higher in roots produced by shoots cultured in non-NAA supplied media; however, for this variable, short and thick roots are preferred for easiness to transfer from in vitro to *ex vitro* conditions. *In vitro* multiplied shoots from both cultivars were fully adapted to ex vitro conditions. The results of the study provided the bases for mass production of arrowcane plants cultivars "Martinera" and "Costera" through micropropagation (Suárez *et al*., 2020).

Micropropagated plants are usually costly as a result of reagents used, labor employed and labs facilities needed for production. Reduction of costs of micropropagation protocols is always a challenge, and gelling agents used for semisolid media are always a target (Serrano-Martínez *et al*., 2011; Scherwinski *et al*., 2012; Senapati, 2015). In an attempt to reduce costs for arrow cane micropropagated plants, clusters of three stems of *in vitro* established plants of "Criolla" cultivar were established into 750 cc polycarbonate flasks containing double-phase medium consisting of 100 ml MS semisolid medium dispensed in the bottom of the recipient, and added with 30 ml liquid MS medium on top of the semisolid phase. Four explants were established in each container and the liquid phase was replenished every two weeks.

This system was compared to conventional semi solid system where similar explants were cultivated in 30 ml MS semisolid medium per flasks with transfers to fresh medium of the same formulation every four weeks. Media formulation for both treatments was MS (Murashige and Skoog, 1962) supplied with (in mg L^{-1}) myo inositol (100), sucrose (30.000), thiamine HCl (0.4) and BAP (0.5); the semisolid phase was gelled with Phytagel® (3.000) (Sigma Co.). The results showed that double-phase medium statistically increased the number of shoots per explant, the total number of shoots and induced a significant increase in shoot length than semisolid medium. Additionally, cost analysis revealed that using double-phase medium for arrow cane micropropagation can lower plant cost by 20% compared to conventional semisolid medium with periodical transfer to fresh new media (López and Suárez, 2018). A second study to evaluate the double-phase medium strategy was performed including cultivars "Criolla", "Martinera" and "Criolla 1" plants. The results not only reinforced multiplication rates (>20 new shoots per explant) for "Criolla" plants, but also showed that "Martinera" and "Criolla 1" plants proliferate efficiently at rates of 20 and 10 new shoots per explant, respectively. The results also made possible to provide high quality plant material for two more cultivar for crop establishment (López *et al*., 2021).

Despite plant propagation per se, *in vitro* culture of arrow cane has been used to evaluate alternatives as a phytoremediator plant. *In vitro* established "Criolla" plants in semisolid MS medium with (in mg L^{-1}) thiamine-HCl (1), myo-inositol (100), pyridoxine-HCl (0,5), nicotinamide (0,5), glycine (2), sucrose (2500), agar (4500), and BAP (1) were supplied with different mercury (Hg) (0, 5, 10, 15, 25 or 75 µg) concentrations. Plants were stored at 25 C with 12 h photoperiod (50 µmol m^{-2} s^{-2}). After 20, 40 or 60 days, tissue samples from roots and stem-leaves were processed and total mercury (THg) measured; likewise, plant toxicity symptoms were evaluated. The results showed that plant toxicity and THg content in all tissues increased with time and Hg accumulation was higher in roots than in stem-leaves.

The fact that plants grew in high Hg concentrations in the culture medium, THg levels found in plant tissues were high (>14 µg THg g^{-1}) and toxicity symptoms, such as chlorosis and necrosis, were relatively low, indicated that arrowcane is a high tolerant

Hg plant with great potential to be used as soil phytoremediator in mining mercury contaminated areas (Ortega *et al*., 2011).

Genetic resource conservation

Germplasm collections are necessary for plant breeding programs to readily access to genetic resources for different studies or to provide plant material to growers. For species like *Gynerium sagittatum* germplasm conservation is especially costly because no viable seed is produced, plant collections must be stored in vivo in the field, spreading growth habit requires high cost for maintenance and usually they have to be relocated to avoid plant mix in the field. *In vitro* conservation of germplasm is an alternative to in vivo field collections since costs are reduced because of lower space, plant material is free of pests and diseases, there is no risk for hazardous events, such as flooding or winds, and plants remain ready for growth and development once needed. There are several techniques to store plants material in vitro such as cell tissues, shoots, cryopreserved tissues or energy reserve organs (Kane, 1996).

In vitro long-term conservation for arrow cane plants has been achieved. *In vitro* established shoots of arrow cane "Criolla" cultivars were cultured in different media composition (MS strength and sucrose concentration) to evaluate their effect on long term culture conservation and *in vitro* plant survival. Seven semisolid media composition (MS + 15 g sucrose, MS + 30 g sucrose, MS + 60 g sucrose, ½ MS + 15 g sucrose, ½ MS + 60 g sucrose, ¼ MS + 15 g sucrose or ¼ MS + 60 g sucrose) solidified with 7 g TC agar (Sigma Co.) were evaluated. A single shoot cluster (3 stem per cluster) was established in 250 cc glass flasks containing 30 ml medium. Cultures were covered with two layers of heavy-duty aluminum foil, sealed with Nescofilm® and stored for 12 months at 25 °C with 12 h photoperiod (40 µmol m^{-2} s^{-2}). The results showed that after a year, *in vitro* maintained arrow cane plants survived from 70%, when cultured in MS + 30 g sucrose, up to 96.7% in ¼ MS + 60 g sucrose. Shoots cultured in ¼ MS + 60 g sucrose proliferated more than those cultured in any other media formulation and shoot length of shoots was not statistically different for all treatments (Suarez *et al*., 2013).

In a different study, *in vitro* rhizome growth has been induced as a way to develop modified vegetative organs for plant propagation and/or conservation. *In vitro* established arrow cane "Criolla" shoots were transferred onto semi solid MS medium supplied with a combination of different BAP (0; 2.2; 4.4 or 8.8 µM) with abscisic acid (ABA) (0; 1.39; 3.78 or 7.57 µM) concentrations at several sucrose (87000, 165000 or 263000 µM) levels. Flasks (250 cc with 30 ml medium) were covered with two-layers of heavy-duty aluminum foil, sealed with Nescofilm® and maintained at 25 °C with 12-hour photoperiod (40 µmol photons m^{-2} s^{-1}) provided by white cold fluorescent lamps. After six weeks in culture, the results showed that media supplied with 263000 µM sucrose, 2.22 µM or 4.44 µM BAP and 1.89 µM ABA concentrations induced significant higher number of rhizomes compared to lower sucrose levels with no BAP/ABA or 8.88 µM BAP with 3.78 µM or 7.57 µM ABA.

However, when transferred to *ex vitro* conditions, only rhizomes developed from shoots cultured in media supplied with 4.44 µM or 8.88 µM BAP with ABA at 263000 µM sucrose were able to survive >30%, and develop new shoots that emerge as new plants. Rhizomes are specialized plant stems that serve as propagation units and storage organs that remain dormant under adverse environmental conditions. As an underground structure, *in vitro* grown rhizomes do not require acclimatization when transferred *ex vitro*; therefore, saving time and resources

for plant recovery. On the other hand, given a specific culture medium formulation, rhizomes could be maintained *in vitro* for long periods of time reducing growth and development. Induction of *in vitro* rhizome formation is a strategy that can be used for both, plant propagation and germplasm conservation of arrow cane (Suarez *et al*., 2017).

CONCLUSION

Despite centuries of use by ancient communities, arrow cane is a non-domesticated plant species and knowledge about its biology and crop production techniques are scarce. During the past 15 years, the Institute of Applied Biotechnology for the Colombian Caribbean Research Program on Arrow Cane has been able to generate information about genetic diversity, propagation and conservation. Today is clear that non-conventional strategies must be implemented to increase genetic diversity for plant evolution and to sustain a plant breeding program, germplasm can be stored under *in vitro* conditions for long term and growers can count on micropropagation protocols to obtain any amount of high-quality plant material for commercial crops.

Arrow cane has a wide range of use; despite crafts and indigenous uses, home construction, biofuel and phytoremediation are some of the future uses for this plant species and some research is needed to provide more information. So far, genetic and agronomic evaluations, as well as *in vitro* culture studies, have solved questions regarding desirable cultivars and source of planting material required for large scale crop plantation.

Conflicts of Interest

The manuscript was prepared and reviewed with participation of all authors, who declare that there is no conflict of interest that jeopardizes the validity of the results presented.

The present research program was supported by Universidad de Córdoba, Vicerrectoria de Investigacion y Extension

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