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REPORT

New knowledge for increased efficiency of Araceae micropropagation *in vitro*

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ABSTRACT

In vitro culture is a reliable method for plant multiplication free of microbial contaminants. In this work, a series of research were done to increase the number of explants of Araceae cultivars aimed for propagation in biofactories. Prior treatment of corms with 3.0 % sodium hypochloride for 20-25 min favored the *in vitro* establishment of 85.38 % of apexes. The best choice conditions were MS culture medium with salts and vitamins, further supplemented with 0.5 mg/L 6-BAP, during the establishment phase, and the same medium but with 4.0 mg/L 6-BAP during multiplication. During the establishment phase, undecapitated corms were the main source for explants, obtaining more than 300 meristems per corm. Microbial contamination was reduced from 40 to 2 %, growth of explants increased and also survival index. The best performance was attained during multiplication by applying three subcultures in semisolid medium, combined with temporary or constant immersion semiatutomated systems with culture medium aeration. The multiplication coefficient was further incremented by by applying differen cutting to explants than the traditional one (50 % decapitation of the shoot and bevel cut. A manual was elaborated for the management of plantlets at the acclimatization phase, with the inclusion of several modifications and new contributions to pre-existing methodologies, which further provides novelty and a high scientific impact for taro *in vitro* culture. This work granted the Annual Award of the National Academy of Sciences of Cuba for the year 2019.

Keywords: Alocasia, *Colocasia*, micropropagation, temporal immersion systems, *Xanthosoma*, taro

RESUMEN

Nuevos aportes para el incremento de la eficiencia en la propagación *in vitro* de aráceas. El cultivo *in vitro* es un método confiable para la multiplicación de plantas libre de contaminaciones. En este trabajo se desarrollaron un conjunto de investigaciones para incrementar el número de explantes de aráceas para entregar a las Biofábricas. El hipoclorito de sodio al 3.0 % para la desinfección de los cormos durante 20-25 minutos favoreció el establecimiento *in vitro* del 85.38 % de los ápices. Un medio de cultivo con las sales y vitaminas MS con 0.5 mg/L de 6-BAP en establecimiento y con 4.0 mg/L de 6-BAP en la de multiplicación resultaron las mejores opciones. En establecimiento las yemas de los cormos sin decapitar representaron la mejor fuente de explantes y así: se obtuvieron más de 300 meristemos por cormo, se redujeron las contaminaciones microbianas de 40 % a menos del 2 %, se aumentó el crecimiento de los explantes y el índice de supervivencia. Durante la multiplicación fue mejor realizar tres subcultivos en medio semisólido y emplear los sistemas semiautomatizados de inmersión temporal o de inmersión constante con aireación al medio de cultivo. Se aumentó el coeficiente de multiplicación con el uso de otras formas de corte de los explantes diferentes a las empleadas tradicionalmente (decapitado 50 % del brote y corte a bisel). El diseño de un manual para el manejo de las plántulas en la fase de aclimatización, la inclusión de varias modificaciones y nuevos aportes que mejoran la metodología precedente, le confieren novedad y un alto impacto científico para el cultivo *in vitro* de la malanga. Este trabajo mereció el Premio Anual de la Academia de Ciencias de Cuba para el año 2019.

Palabras clave: Alocasia, *Colocasia*, micropropagación, sistemas de inmersión temporal, *Xanthosoma*, malanga

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Introduction

Quality deterioration of the genetic material in taro plantations due to pathogens' infestations has led to decreased yields and lower commercial availability of this crop [1]. In this scenario, *in vitro* culture provides a useful tool for the fast propagation of healthy plants with full physiologic capacity. However, initial sprouts have to be carefully managed to avoid significant losses due to contamination during the *in vitro* establishment phase and lower multiplication coefficients. This somewhat limits the success of the technique and the scheduled delivery of explants from biofactories [2].

Therefore, in this work, a number of research are summarized, focused on increasing the efficiency of the explants' production process through *in vitro* culture of three taro genuses with both physiologic and sanitary quality. These also established the scientific and practical basis for plant production through somatic embryogenesis.

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Materials and methods

The experiments were carried out at the Vegetal Viotechnology Laboratories of the Institute for Research on Tropical Viands (INIVIT), Cuba, from 2011 to 2019.

Taro clones 'INIVIT MC-2001' and 'Camerún 14' (*Colocasia esculenta* Schott), 'México 1', 'México 8', 'Amarilla Especial' and 'Selección INIVIT' (*Xanthosoma sagittifolium* Schott) [3] were used. Also the cultivar 'Verde Picante' (*Alocasia macrorrhiza* (L) Schott). All were selected from certified seeds at INIVIT, taking care of their clonal morphological properties, sanitation and yield under field conditions. Basal medium was used as culture medium as described by Murashige and Skoog [4] containing inorganic salts and vitamins (MS). Culture medium pH was adjusted to 5.7 with 0.5 mol/L NaOH or 0.5 mol/L HCl, followed by autoclaving sterilization.

A varied number of research were done to proposed an adjusted process for the micropropagation of *Xanthosoma* and *Colocasia* cultivars. Notably, for the first time it was included an *in vitro* methodology for cultivars of the *Alocasia* genus.

New treatments are reported, increasing the efficiency at the stage of establishment, consisting on the disinfection with 2.5 and 3.0 % sodium hypochloride and alcohol 70 % for 20, 25 and 30 min; different 6-bencylaminopurine (6-BAP) concentrations (0.1, 0.3, 0.5 and 0.7 mg/L); the use of explants from shoots of decapitated and sprout corms, axillary shoots yet protected by leaves. Also for the multiplication stage by studying the influence of the type and concentration of the plant growth regulators, the physical status of the culture medium, and the use of semi-automated culture systems. Particularly, new ways are described to manage buds with cuts stimulating the growth of explants, following seven treatments that combine 50 and 75 % decapitation of buds and bevel cuts from the base, longitudinal area and the apical bud, respectively.

For the first time, conditions were assayed and standardized for the *Alocasia* genus, establishing the parameters required for plants' establishment, multiplication, rooting and acclimatization stages.

Results and discussion

A methodology was adjusted for the establishment *in vitro* and multiplication stages of cultures of explants of *Xanthosoma* and *Colocasia* taro cultivars. This supported the increase in the number of explants bearing physiological and sanitary quality as to be provided to Cuban biofactories. The use of 3.0 % sodium hypochloride to disinfect taro corms for 25 min supported the decrease down to 2 % of microbial contamination and a rise on efficiency up to 85.38 % during the in vitro establishment of axillary shoots' apexes. The use of the MS culture medium was implemented, further supplemented with 0.5 mg/L 6-BAP during the establishment stage [5]. Moreover, the efficiency of the *in vitro* establishment phase was increased by using shots from unbehaded corms for cultivars of the three genera. It was possible to produce more than 300 meristems each as alternative procedure. Simultaneously, the growth speed of explants and survival indexes were increased [6].

The MS medium supplemented with 4.0 mg/L 6-BAP was implemented, the three subcultures done in semisolid medium [7]. Besides, it was demonstrated the usefulness of the temporal immersion system at this stage, further introducing the constant immersion with aeration of the culture medium. This significantly increased the multiplication coefficients (8.76 times) [8].

Another novel contribution comprised the demonstration of the advantages of in vitro management of buds with different types of cuts, thereby increasing the multiplication coefficients in *Xanthosoma sagittifolium* Schott and *Colocasia esculenta* Schott through a simple micropropagation method [2].

All these supported the rise in the production of taro explants for commercial cultivars with physiological and sanitary quality to provide enough explants to biofactories, as well as for research purposes and export.

A novel methodology was implemented for the *in vitro* propagation of plants of the *Alocasia* genus from phase 0 until acclimatization. This included disinfection with 3.0 % sodium hypochloride for 20 min at the establishment phase, the lack of any growth regulator added at that phase and on rooting, and 6-BAP concentrations were reduced to 3.0 mg/L at the multiplication phase [9].

The best morphophysiological growth of explants was attained on semisolid medium, together with a 3.07 multiplication coefficient in average after three subcultures. The multiplication coefficient rose to 12.6 with the addition of the temporary immersion systems for *Alocasia* spp. micropropagation. In fact, this was the first report on the implementation of an efficient and reproducible protocol for the micropropagation during acclimatization for this genus [9].

These results are highly relevant to promote the development of this genus in Cuba, guaranteeing the multiplication of cultivars of interest, aimed for human and animal food, and for commercial export. This is an Araceae genus barely used in Cuba, but having a high usage potentiality since its nutritional content ranges 21-26 % per leaf.

The implementation of the methodology proposed for managing in vitro plants supported the elaboration of a manual for plant management at the acclimatization phase (Figure 1). The procedure was primarily conceived for *Xanthosoma* spp., but it was validated later on for cultivars of *Colocasia* and *Alocasia* genuses [9].

There were previous reports on the micropropagation of some cultivars of *Xanthosoma* spp and *Colocasia* [10-12]. But our results summarize the best outcomes and add new improvements for a higher efficiency during the *in vitro* processing of other cultivars. In fact, we obtained 85 % survival during establishment, high multiplication coefficients, low bacterial contamination indexes, a better use of plant growth regulators and high plantelet survival upon acclimatization.

Some authors consider *in vitro* culture techniques as expensive procedures. Nevertheless, that is a relative

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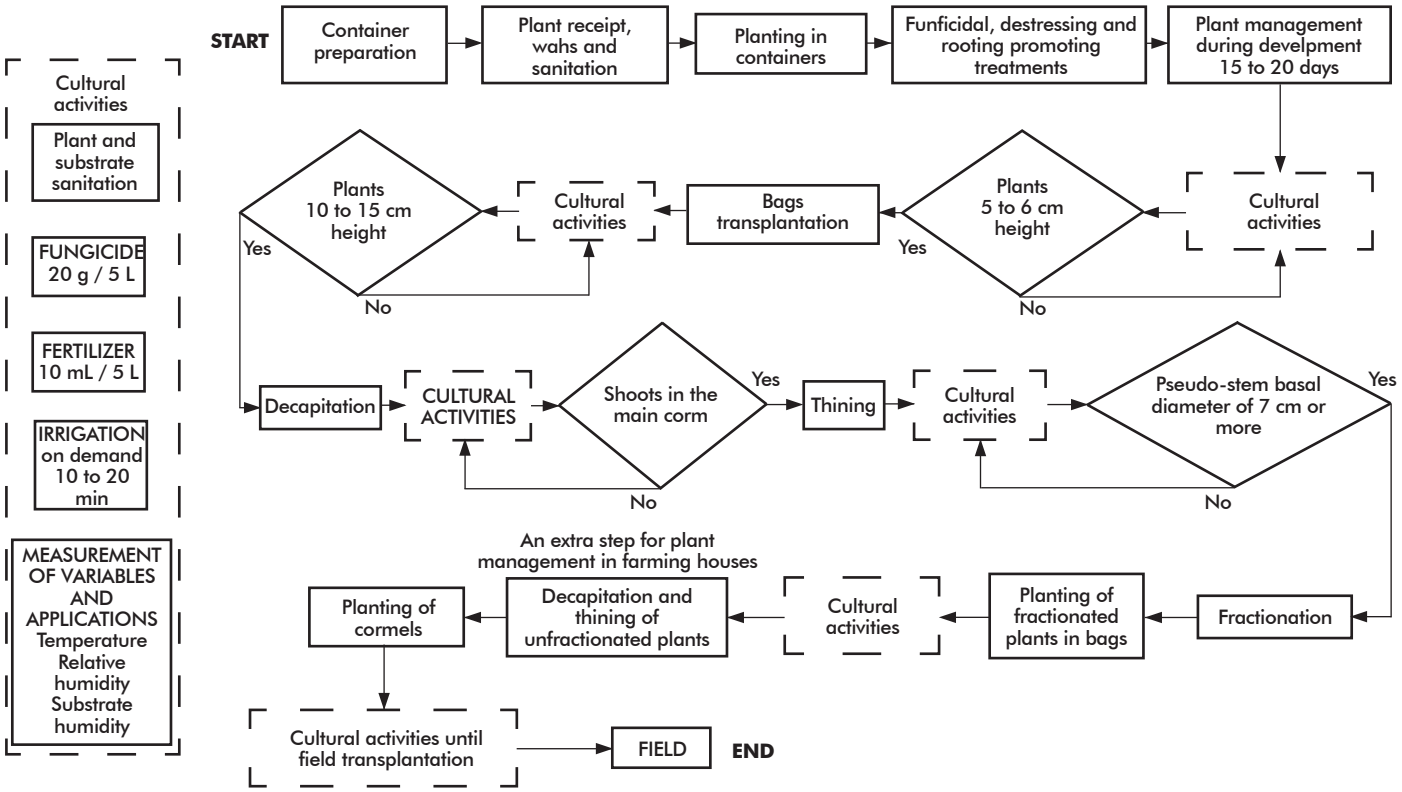

Figure 1. Flow diagram for culture activities during the management at the acclimatization phase of *in vitro* produced plants.

consideration since through such techniques, planting material of high genetic and phytosanitary quality is produced. This becomes an investment for a higher vegetative development of plantations, leading to higher agricultural yields as demonstrated in a series of crops. Particularly In tato cultivation, micropropagation leads to a rejuvenation of commercial cultivars, the generation of new clones and their fast introduction into available agroproduction systems. For instance, it is possible to reduce in up to two years the introduction of a new cultivar [13].

In vitro culture can be useful also for sanitation of vegetal materials, further increasing or recovering their productive potential. In Cuba, for instance, it has proven useful to significantly reduce the incidence of the dry rot in taro [1].

The efficient application of these methods, as proposed herein, provides an essential alternative to increase the quality of planting material for higher yields. It also accelerates the introduction of new cultivars of taro through biofactories, with a substantial practical contribution to suffice for agricultural needs.

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Relevance of the study

It was generated for the first time a complete methodology, reproducible and efficient for the micropropagation of *Alocasia* cultivars, up to the acclimatization phase of *in vitro* plants, which supports the development of this crop in Cuba. It integrates novel improvements for the efficiency of the in vitro culture of cultivars from the *Xanthosoma*, *Colocasia* and *Alocasia* genuses.

Adjusted and improved protocols were also generated, from the selection and desinefction of explants until the acclimatization phase. It was proven that a survival higher that 85 % is achievable, with high multiplication coefficients due to the combination with cutting procedures of explants and the use of temporary immersion systems, a better use of plant growth regulators, the reduction of microbial contamination indexes down to 2 %, reaching an overall 98.3 % survival of plantelets in acclimatization.

Conflicts of interest statement

The authors declare that there are no conflicts of interest.

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