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In Vitro Propagation of Arrowroot (*Maranta arundinacea* L.)

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ABSTRACT

Arrowroot (*Maranta arundinacea* L.; Marantaceae) is an excellent source of starch (>85%) used in the food industry. The multiplication of this plant is traditionally from rhizomes, which are also a source of starch. *In vitro M. arundinacea* shoot cultures were successfully established from rhizome buds on semi-solid MS medium supplemented with 3 mg.l^{-1} 6-benzylaminopurine in the dark, which was also the best medium for shoot proliferation. Shoots were acclimatized in zeolite and sugarcane filter substrate (1:1) with a 90% survival percentage.

Keywords: buds, darkness, rhizome, tissue culture
Abbreviations: BAP, 6-benzylaminopurine; **ECM**, establishment culture medium; **2-ip**, N^6-(isopentyl) adenine; **TDZ**, thidiazuron

INTRODUCTION

Members of the family Marantaceae are ornamental tropical perennial foliage plants with tuberous rhizomes. This monocotyledonous family member includes 27 genera and about 300 species, whose original areas are the tropical zones of South America. Economically, Marantaceae are an important source of food for the food industry. *Marantha arundinacea* L. from tropical America is also cultivated in tropical regions of the West Indies and Africa. It is an industrial plant from which arrowroot starch (>85% w/w) is produced from the rhizomes of plants grown in the West Indies and Antilles (Scaramuzzi and Apollonio 1997). Kumar and Parrack (2003) showed the feasibility of using arrowroot starch as a low-cost substrate for alkaline protease production from an alkalophilic isolate, *Bacillus lentus* strain MK5-6.

Species belonging to the Marantaceae multiply asexually through plant division or by tip cuttings while multiplication by seeds is rare due to their low germination capacity. The latter, together with the high quality and uniformity of plantlets obtained through tissue culture techniques, and when compared to those originating from rhizome division, provoke a high demand for Marantaceae micropropagation protocols. However, it is difficult to achieve the *in vitro* establishment of *Marantha* cultures from shoot axillary buds excised from adult field-grown plants (Scaramuzzi and Apollinio 1997).

Previously, arrowroot was propagated *in vitro* using emerging buds of rhizome as a source of explants. MS (Murashige and Skoog 1962) solid medium containing BAP (6-benzylaminopurine) at 2 and 3 mg.l^{-1} and NAA (1-naphthaleneacetic acid) at 0.5, 1 and 2 mg.l^{-1} were used for shoot multiplication (Priadi *et al.* 2000). However, for arrowroot clones of Saint Vicent and the Granadines and other Caribbean Islands no available protocols exist.

The aim of this study was to evaluate the culture conditions, type of explants and growth regulators, as a mean of finding a more efficient method for the *in vitro* propagation of *M. arundinacea*.

MATERIALS AND METHODS

Buds arising from cv. 'Criollo' rhizomes (**Fig. 1**) were surface sterilized by immersing for 15 min in 0.25% (w/v) mercuric chloride solution (BDH, UK). Explants were then rinsed three times in autoclaved sterile distilled water.

After disinfection buds were carefully removed under sterile conditions rhizomes were discarded. Buds were sliced into 4 to 5 mm sections with a portion of basal tissue and inoculated onto establishment culture medium (ECM) consisting of MS basal medium (Duchefa, The Netherlands), supplemented with 30.0 g.l^{-1} sucrose (Sigma-Aldrich, USA), 3.0 mg.l^{-1} BAP and 2.5 g.l^{-1} gelrite (Sigma-Aldrich). The influence of two culture conditions (light and darkness) and two explant types (latent and breaking buds) on sprouting were evaluated after 30 days of culture during *in vitro* establishment. In order to evaluate the effects of explant management on shoot and root numbers during *in vitro* proliferation, two treatments were established: 1) decapitation of shoots and 2) combined decapitation and dissection of shoots. Explants in treatment 2 were inoculated onto ECM. On the other hand, the effect of cytokinins (BAP, 2-ip (N^6-(isopentyl) adenine) and TDZ (thidiazuron); Duchefa), all at 13.2 μM (a level that was shown to be effective for other Maranthaceae) on shoot and root numbers were also evaluated during *in vitro* proliferation on MS basal medium supplemented with 30.0 g.l^{-1} sucrose and 2.5 g.l^{-1} gelrite. In both cases, the number of shoots and roots were recorded at 30 days of culture during this phase.

Fig. 1 (**Left**) Shoots obtained from buds in the dark. (**Right**) Different types of explants obtained from rhizome.

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Short Communication

In all experiments, medium pH was adjusted to 5.8 by adding NaOH prior to autoclaving at 121°C and 1.2 kg/cm^2 for 15 min. Explants were incubated in a growth chamber at 25 ± 2°C. Cultures in experiments involving light were grown under cool white fluorescent lamps providing 80 µmol.m^{-2}.s^{-1} photons, with a 16-h photoperiod at 25°C.

Rooted shoots were transplanted into a substrate consisting of zeolite mixed in a 1: 1 ratio with sugarcane filter substrate. Trays were placed in a greenhouse (600 µmol.m^{-2}.s^{-1}, 28 ± 2°C) under humidity tents (90% RH) to prevent desiccation during the first two weeks.

Experiments were completely randomized. Variations among treatments means were analyzed using ANOVA and Tukey's test at the 5% level.

RESULTS AND DISCUSSION

Effect of different culture conditions (light and darkness) on *in vitro* establishment of arrowroot buds

Darkness significantly affected the sprouting of arrowroot buds (**Table 1**): higher bud sprouting could be achieved within 1 week; this response could be maintained throughout the entire evaluation period of 30 days (data not show). However, statistically fewer buds sprouted in light compared to darkness. Even though the percentage of response increased in the second week in light, this percentage was still below the level in the dark.

Etiolating of branches before shoot tip culture cut favors the *in vitro* establishment and proliferation of mature chestnut (*Castanea sativa*) and oak (*Quercus*) trees (Ballester *et al.* 1989). Prolonged etiolation in that study facilitated *in vivo* and *in vitro* rooting.

Choi *et al.* (2001) showed that incubation for 4-5 weeks in the dark was the most important condition to regenerate the shoots of two cultivars of *Diospyros kaki*, and was is essential for adventitious shoot formation in cv. 'Fuyu'. Kirdmanee *et al.* (2004) could develop *Zingiber* buds on MS medium in the dark for 1 month.

Evaluation of explant type in the establishment of buds

There is no difference whether latent buds or sprouting buds from rhizome are used on the percentage sprouting although more buds form from latent buds than from sprouting buds within 60 days (**Table 2**).

Apical and axillary buds have been successfully used in many species. In species that are vegetatively propagated, young shoots have been the best source of explants (Villalobos *et al*. 1982). In general, younger and less differentiated tissues result in the best response *in vitro.* Chiari and Bridgen (2002) achieved highest plantlet regeneration (74.1%) in *Alstroemeria* from meristem axillary buds from rhizomes.

Evaluation of explants treatment in the multiplication of arrowroot buds

When shoots are decapitated and sectioned, there is an increase in the number of buds formed although an opposite trend is observed with respect to the number of roots formed (**Table 3**). Although banana micropropagation was conventionally achieved through the decapitation of shoot pseudostems, recently Madhulatha *et al.* (2004) used decapitated shoot apices of cv. 'Nendran' to evaluate cytokinin pulses. In fact, micropropagation of bananas and plantain at the commercial level requires that decapitated shoots be cut longitudinally into two or four sections, depending on the size of corms.

Parthanturarug *et al.* (2003), when studying explants type, achieved the best results in terminal buds of *Curcuma longa* that had been decapitated and cut longitudinally into four sections. Teixeira da Silva and Tanaka (2006) showed that decapitation of protocorm-like bodies (PLBs) in hybrid *Cymbidium* was essential for successful PLB proliferation, without which only shoots would develop, and undesired morphogenic response in PLB proliferation.

Table 1 Sprout of arrowroot buds under light and darkness conditions.

Different condition	Sprouting (%)
Light	54.4 b
Darkness	86.6 a

Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's test.

Table 2 Effect of bud type on sprouting during *in vitro* establishment of arrowroot.

Explant type	Sprouting (%)	№ Buds/rhizome
Breaking buds	88.86 a	1 b
Latent buds	83.30 a	5.5 a

Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's test.

Table 3 Effects of explant manipulation on shoot and root numbers during *in vitro* proliferation of arrowroot.

Treatments	№ shoots	№ roots
Decapitated shoots	1 b	3.13 a
Decapitated and sectioned shoots	2.27 a	0.13 b

Different letters indicate significant differences at $P \leq 0.05$ according to Tukey's test.

Table 4 Behavior of arrowroot shoot (within 30 days) proliferation with different cytokinins.

Treatment	Shoot №
BAP	2.4 a
2iP	1.7 b
TDZ	0.1 c

Means with the same letters are not significantly different at the 5% level. (Kruskal-Wallis, Student-Newman- Keul's test)

Evaluations of different cytokinins on the multiplication of arrowroot shoots

BAP was the most effective cytokinin for shoot proliferation (**Table 4**) and is generally a good cytokinin for shoot proliferation in many plants species, e.g. chrysanthemum (Teixeira da Silva 2005).

Salvi *et al.* (2002) achieved the best proliferation in *Cucurma longa* with 10 µM 2iP, but in liquid culture medium and within 8 weeks. Arrowroot buds did not respond to TDZ, and only some explants swelled, but no shoots differentiated. Huetteman and Preece (1993) recommended 1-10 µM TDZ for woody plant shoot proliferation. TDZ can, however, cause abnormalities or be toxic as shown by Kim *et al.* (1997) in *Liquidambar styraciflua*.

Although the findings of this study indicate some promising results for arrowroot proliferation and micropropagation, and even though rooting percentage and acclimatization was successful (90% based on 40 plantlets), many more plant growth regulator combinations and *in vitro* culture conditions need to be tested.

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