Original Article

In vitro micropropagation of Alternanthera Ficoidea L. by callus formation on MS medium

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Abstract

In this research work, the in vitro propagation of Alternanthera ficoidea, an ornamental plant belonging to the Amaranthaceae family native to South America, was carried out. This plant is considered important due to the presence of important pharmaceutical products such as tannins, saponins, phytols, carotenoids, xanthophylls and polyphenols, which is why it can be included as a medicinal herb as well as being used colloquially as a tranquilizer and as a treatment for gastritis. This method guarantees to obtain a large number of homogeneous explants from the mother plant tissue, taking advantage of the division and regeneration capacity that plant cells possess when they are in adequate conditions for their growth. To achieve this in vitro establishment, three disinfection methods were evaluated, obtaining a greater number of viable explants with the use of 0.1% mercuric chloride for 5 min and greater shoot growth with the combination of phytohormones (1.5 + 1.5 mg.L-1 of AG3 and BAP respectively) reaching an average shoot length of 3.7 cm. These seedlings were acclimatized to nursery conditions.

Keywords: in vitro propagation, disinfection, phytohormones and acclimatization

INTRODUCTION

It is a genus of herbaceous plants belonging to the *Amaranthaceae* family, comprising up to 170 genera and a relatively high number of invasive weeds (1). Many of these species are aquatic plants native to the South American continent. The Amaranthaceae family is recognized in Peru with 13 genera and 72 species (2) (3), of which up to 2006, eight species and two varieties in six genera were recognized as endemic, found in the desert scrub and meso-Andean regions (4).These plants have mostly medicinal properties as is the case of A. sessilis, which is used to treat fever, snake bites, dysentery, diarrhea, and skin. Internal and external inflammation problems include acne and pimples (5). A.brasiliana, known as lancetilla, used by the indigenous community of Colombia (Tikuna of the upper Amazon) as an analgesic and to relieve gastrointestinal problems (6). Some species, such as A. caracasana HBK, present antimicrobial activity (7), besides being used to treat different diseases already mentioned (Aguilar, 1994).

The species Aternanthera ficoidea (Figure 1), commonly known as sanguinarea due to the brownish red color of its leaves and stem, is a perennial plant found mostly in gardens as an ornamental. It is used in an infusion to treat gastritis and as a tranquilizer (8). This work aims to elaborate a protocol for the micropropagation of *Alternanthera ficoidea L.* for its subsequent use in the extraction of antioxidants or phenolic compounds as well as natural dyes used in the food and textile area.

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Figure 1 Alternathera ficoidea L

Materials and methods

Reagents and plant samples

For the elaboration of the culture medium of Murashige & Skoog (1962), stock solutions of Macronutrients, Micronutrients, Vitamins and Phytohormones (Merck, Darmstadt, Germany) were prepared and kept at 5 °C for its conservation, complemented with 30 g of Sucrose and as gelling material 7 g of Agar - Agar. The pH of the culture medium was adjusted to 5.7 ± 1 before being autoclaved at a pressure of 1.2 atm at 120 °C for 15 min as Dogan *et al*. (58) weighed on an OHAUS SCOUT PRO 200g Analytical Balance, model SP202.

The plant species were obtained from the Chaclacayo Nursery located in Chaclacayo (altitude: 647 m.a.s.l., Latitude: 11° 58'37.7" South, Longitude: 76° 46'27.6" West), in the city of Lima, Peru. And an analysis of taxonomic determination was carried out in the facilities of the Herbarium of the Michael Onew Dillon Scientific Institute "Herbario Sur Peruano" (HSP).

Mercury chloride (Merck, Darmstadt, Germany), Ethanol 96° (Driproquim, Arequipa, Peru), Tween-20 (Merck, Darmstadt, Germany) were used for the evaluation of the appropriate disinfectant.

These methods were performed inside the Laminar Flow Chamber Company C4, using glass instruments and forceps previously sterilized in the HW KESSEL Autoclave, model 290104.

Evaluation of the disinfection method

Three disinfection treatments were performed: **T1:** 96° ethanol for 30 seconds with 1% sodium hypochlorite, then washed with sterile water for 4 minutes, **T2:** 96° ethanol for 30 seconds, 2.5% (v/v) sodium hypochlorite and 0.1% tween-20 solution for 7 minutes. They were then washed with sterile water. Mantovani *et al*. (59) and **T3:** Mercuric chloride 0.1% for 5 minutes. Then they were washed with sterile water. According to Gnanaraj *et al.* (9). The factors evaluated in this study were the number of non-contaminated (viable and necrotic) and contaminated (bacteria and fungi) explants.

Stimulation of indirect organogenesis

The explants of Alternanthera ficoidea L. were seeding was carried out by placing five nodal segments per flask. Experimental conditions were photoperiod for 16 hours of light/ 8 hours of darkness, a light intensity of 2000 to 2500 Lux provided by fluorescent tubes at a temperature of 20 ± 3°C. The prepared culture medium was supplemented with a concentration of 0.01 mg/L ANA and 0.2 mg/L BAP for callus induction. There were 12 experimental units and the evaluation was performed every 15 days where the explant length (cm) was evaluated after callus formation.

Evaluation of the effect of phytohormones

The effect of growth regulators such as 6-benzylaminapurine (BAP) and gibberellic acid (AG3) was evaluated at different concentrations (Table 1) and each one of them had three replicates per treatment; the length reached by the seedlings in each treatment was evaluated during 45 days.

Table 1. Phytohomonas AG3 and BAP treatments.

Treatment	AG 3 (mg/L)	BAP (mg/L)
1	1	0
2	1.5	0
3	2	0
4	0	1
5	0	1.5
6	0	2
7	1.5	1
8	0.5	1.5
9	1.5	1.5

Rooting and acclimatization

For rooting stimulation, the MS medium was supplemented with 1 mg/L of ANA; the seedlings with a root length of not less than 5 cm were selected from the rooting stage; these seedlings were removed from the flasks and washed several times with sterile water to eliminate as much agar as possible and avoid future contamination, they were placed in a container containing topsoil and worm humus 2:1 respectively. The containers were covered with plastic bags to maintain humidity and were brought to nursery conditions.

Results

Disinfectant selection

In order to select the appropriate method for obtaining viable explants free of contamination, the three treatments mentioned above were compared. Table 2 shows the percentage of viable explants compared to contaminated explants.

Table 2. Explants obtained during the evaluation of disinfection treatment.

TREATMENT	N° Uncontaminated Explants			N° Contaminated Explants		
	% Viable	%Necrops	TOTAL	%Bacteria	% Fungi	TOTAL
T1	13.33	60.00	73.33	26.67	0.00	26.67
T2	42.31	19.23	61.54	26.92	11.54	38.46

T3	61.40	26.32	87.72	10.53	1.75	12.28

Evaluation of the effect of phytohormones

This stage shows the effect on the length caused by growth regulators such as BAP and AG3 in different concentrations on the explants, expressed in centimeters, the evaluation was carried out over 45 days.

Table 3. Length achieved with phytohormone stimulation.

Treatment	AG 3 (mg/L)	BAP (mg/L)	Length (cm)
1	1	0	1.9
2	1.5	0	2.4
3	2	0	2.9
4	0	1	1.7
5	0	1.5	1.8
6	0	2	1.8
7	1.5	1	1.9
8	0.5	1.5	2.8
9	1.5	1.5	3.7

Figure 1. *In vitro* propagation process of *Alternanthera ficoidea L.* A) nodal segments of plant material, B) stimulation of callus formation with 0.1 mg.L-1 of ANA, D) shoot with Treatment 9 (1.5 + 1.5 mg.L-1 of AG3+ BAP), E) multiplication stage, F) shoots in MS medium supplemented with ANA and BAP, G) rooting and H) acclimatization.

Discussion

Disinfectant selection

According to the results obtained, the adequate treatment, was T3, which used mercuric chloride at a concentration of 0.1% for 5 minutes, as shown in Table 1. According to research work carried out in India, this method of disinfestation is the most effective in terms of in vitro propagation of Alternanthera (9), (10), (11) and (12).

It should be noted that the different authors mention using various rinsing times to eliminate the remains of HgCl2 due to its high toxicity to human health, especially the respiratory tract, and being a potent environmental pollutant.

Evaluation of the effect of phytohormones

Table 3 shows that T9, consisting of a mixture of 1.5 mg/L of AG3 and 1.5 mg/L of BAP, had a growth of 3.7 cm. This indicates that this concentration is the most adequate for

stimulating the growth of the aerial part of Alternanthera *ficoidea* L.

A comparison between both regulators during the 45 days showed a greater growth of explants using AG3 at different concentrations (1, 1.5 and 2 mg/L) than with 2 mg/L of BAP. Furthermore, in previous studies carried out by Muñoz *et al*. (13), the effect of BAP on growth stimulation in the in vitro multiplication stage was evaluated, reporting that good results were obtained at a concentration not exceeding 2 mg/L. In contrast, these concentrations negatively affect the rooting stage due to the remains of BAP in the plant.

Taking as reference the works done in India with A. versicolor where the adequate concentration for the maximum formation of leaves was evaluated with 1 mg/L IAA and 1mg/L of BAP, they obtained 98% of multiplied viable explants (10) and with the use of A. sessilis the adequate concentration of cytosines (BAP and KIN, TDZ and Adenine sulfate Ads) for the explant growth was compared, having a better result at a concentration of 1mg/L of BAP + 1mg/L (11). These data corroborate the results obtained in this work, so it could also be affirmed that the adequate concentrations of growth regulators in combination for Alternanthera species should be homogeneous.

Conclusion

The in vitro micropropagation is a quite simple and effective method if it is required to obtain homogeneous species and in a great quantity of a species conditioned by the climatic seasons, as in the case of Alternanthera ficoidea L. This method allows them to take advantage of this disadvantage, acclimatizing them to have them in any season of the year. And to be able to carry out future research about its medicinal properties and reddish pigment.

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