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IN VITRO SEED GERMINATION, CALLUS INDUCTION AND GERMPLASM CONSERVATION IN PALO DE BALSA [*OCHROMA PYRAMIDALE* (CAV. EX LAM.) URBAN.]

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ABSTRACT: Palo de balsa [*Ochroma pyramidale* (Cav. ex. Lam. Urban.] is a tropical timber tree native of the Americas from Mexico and the West Indies to southern South America. It is an important species in both economic and ecological terms and their wood is precious and is used internationally for model making and locally for rafts, and the kapok for stuffing cushions.The highest percentage of seed germination (75%) was obtained in the treatment 1.0 mg/L GA_3– 2% sucrose. Callus induction and germplasm conservation in explants derived from cultured seedlings were investigated in response to different plant growth regulators. Friable callus induction from hypocotyls and roots was performed on 2.0 mg/L NAA, in the case of auxins, and hypocotyls 2.0 mg/L BAP, and 1.0 and 2.0 mg/L 2iP, in the case of cytokinins. The best treatment on germplasm conservation was MS full-strenght supplemented with 0.5 mg/L ABA. In the re-growth process, the best treatment was MS medium with 0.02 mg/L IAA and 0.02 mg/L GA_3.
Keywords: Mannitol and abscisic acid, Murashige and Skoog medium, Seedlings and plantlets, Shoot elongation, Tissue culture.

INTRODUCTION

The genus *Ochroma* includes only 1 species, *O. pyramidale* (Cav. Ex Lam.) Urb. (syn. *O. lagopus* Sw.). Is a perennial tropical tree belonging to the family Malvaceae-Bombacoideae distributed from Mexico and the West Indies to southern South America; commonly known as topa, palo balsa, pau de balsa or balsa wood. The plant is a tree to 30 m high and 1.8 m d.b.h with smooth grey lenticellate bark; leaves 15x15 – 30x30 cm, ovate often 3–5 lobed; capsule ± woody, narrowly ellipsoid, 15-18 cm long; the lightweight timber (specific gravity 0.13) is used internationally for model making and locally for rafts, and the kapok for stuffing cushions [1, 2].

Other species of the Malvaceae-Bombacoideae family have a great medicinal value. Several parts of the African Baobab (*Adansonia digitata*) have been reported to have interesting antioxidant, antipyretic, analgesic and antiviral properties, and antimicrobial activity [3]. Baobab fruit pulp has a particularly high antioxidant capability mainly because of its high natural vitamin C content; a semi-fluid gum, obtained from baobab bark, is used to treat sores, and it also contains the alkaloid adansonin which has been used for treatment of fever especially which is caused by malaria; antiviral activity was reported against Herpes simplex, Sindbis and Polio, and the extracts from fruits, seeds and leaves are antimicrobial against *Bacillus subtilis*, *Escherichia coli*, *Mycobacterium leprae*, and antifungal against *Penecillium crusto-sum*, *Candida albicans*, and others [3, 4]. Other species as *Bombax ceiba* (silk cotton tree o locally know as semal), has been found to posses strong antiinflammatory, antibacterial, antiviral, analgesic, hepatoprotective, antioxidant, oxytocic, hypotensive, hypoglycaemic, antiangiogenic, antimutagenic, as well as fibrinolysis enhancing activities [5, 6]. An appraisal of this species was recently published by Jain *et al*. (2009) [7]. *In vitro* tissue culture systems on Malvaceae-Bombacoideae species were first established for multiplication from shoot tips of *B. ceiba*; high frequency bud break and multiple shoots were induced from shoot tips on MS medium supplemented with 2.0 mg/L BAP, and the shoots were successfully rooted on half strenght MS medium containing 2.0 mg/L IBA [8]. In *A. digitata*, an efficient protocol for *in vitro* seed germination was developed; thirty to forty five days old seeds showed remarkable sign of growth in 10 days, and 80–90% seed germination occurred in both media (MS half-strength and MS full-strength); in this study, the seedlings were cut into several parts (cotyledonary node, epicotyl, hypocotyl, cotyledonary leaf and root) and these were aseptically transferred in MS medium with different BAP concentrations; however the results were not reported [10]. In case of *Ochroma pyramidale* the literature do not report work in *in vitro* tissue culture.

Despite considerable advance in rooting of cuttings and tissue culture of several tree species as *Eucalyptus* sp. [11], *Acacia mangium* [12], *Cedrela montana* [12], and others, there still is no asexual propagation technique for *Ochroma pyramidale* which could besuitable for production of massive quantities of clones that would be required for comercial scale plantations. The potential for plant propagation, callus induction and plant regeneration from cultured of several explants of *O. pyramidale* has not been studied. As such cultures may provide a useful alternative to other protocols for micropropagation, organogenesis, somatic embryogenesis, cellular suspensions and transformation of *O. pyramidale*, were investigated the effects of several plant growth regulators on *in vitro* seed germination, callus induction and germplasm conservation.

MATERIALS AND METHODS

Plant materials and seed desinfestation

Elite 50-year-old *O. pyramidale* tree was selected from Refugio de Vida Silvestre Laquipampa (Lambayeque, Peru). The selection was based on the trees anatomical features: straight trunks at least 12 m long and 80 cm diameter. Mature fruits were collected from this tree between october 2011 and january 2012. This material was immediatelly transferred to the laboratory until that spontaneous opening occurred, and madure seeds were obtained. The resulting seeds were kept at room temperature in a black paper bag for a maximum period of 3 months. The external cover of seeds (kapok) was removed manually and washed in running tap water and a commercial detergent; posteriorly, seeds were surface sterilized by immersing them in a 70% (v/v) ethylic alcohol for one min and in 5.25% (v/v) sodium hypochlorite solution (Clorox®) containing a few drops of polyoxyethylene sorbitan monolaurate (Tween 20®) for 5 to 10 min, followed by five rinses of 1 min each with sterile distilled water.

Culture media and culture conditions

All media consisted of full-strenght MS [13] salt formulation containing the following ingredients: thiamine HCl (1.0 mg/L), myo-inositol (100 mg/L), 2 to 3% sucrose and 0.6% agar-agar. The disinfected seeds were germinated aseptically in the MS formulation supplemented with two concentrations of GA_3 (0.5 and 1.0 mg/L). The seeds were scored daily for germination and the breakthrough of the radicle from the seed coat were used as the criterion for germination [14]. In the callus induction, three types of auxins (2,4-D, NAA and IAA) and three types of cytokinins (BAP, KIN and 2iP) were applied in two concentrations (1.0 y 2.0 mg/L, respectively), and for germplasm conservation, two concentrations of MS salts (full-strength and half-strenght MS of inorganic salts) were assessed in factorial combinations with two concentrations of mannitol (4 and 6%) and IBA (0.1 and 0.5 mg/L). The pH of all the culture media was adjusted to 5.7 ± 0.1, with KOH and HCl, before autoclaving. For all experiments, 25 mL of the medium was aliquoted into 150x25 mm test tubes, covered with polypropylene tops, and autoclaved for 20 min at 121 ^oC and 1.05 kg cm^{-2}. One explant was cultured per tube. Cultures were incubated at 26 ± 2^oC under a 16-h photoperiod with the light intensity of 70 µmol m^{-2} s^{-1} photosynthetic active radiation provided by cool white fluorescent tubes; only the callus cultures were incubated in the dark.

Callus induction and germplasm conservation

Cotyledons, hypocotyls and roots of 15-d-old seedlings were used as explants for callus induction, and apical shoots (1.5–2 cm long) obtained from *in vitro* germinated seedlings of 30-d-old were incubated in the culture medium of the germplasm conservation. Each treatment comprised 15 explants and was performed twice. The experiments were evaluated every 60 (for callus induction) and 180 days (for germplasm conservation).

Statistical analysis

Results were processed and analyzed by analyses of variance (ANOVA) and the Duncan multiple range test ($p \leq 0.05$) in order to compare treatment means. All the statistical analysis were carried out the IBM SPSS Statistics 20 software [15].

RESULTS AND DISCUSSION

Seed germination

The results of the *O. pyramidale* seed treatments are presented in the table 1. On seed germination, radicle emerges in 7–9 days and complete expansion of cotyledons between 25 to 30 days. After 60 days, the germination of seeds without GA_3 and 2 and 2.5% sucrose was 15 and 60%, respectively. The highest percentage of seed germination (75%) was obtained in the treatment 1.0 mg/LGA_3 – 2% sucrose, when seeds were disinfected by immersing in 5.25% sodium hypochlorite for 8 min. When being increased the disinfection treatment to10 or more minutes reduced germination less than 10%, showing deformed embryos and cotyledons damaged (data not showed in table); likewise, the increase in the concentration to 3% sucrose reduced the germination rate to 55%. After 30 days of culture, the contamination rate was relatively low with 1.5 and 8.7% for bacteria and fungi, respectively. In both treatments, 2.5% sucrose with 0.5 and 1.0 mg/L GA_3, apical shoots elongation were significantly longer than in other treatments; alike, the leaves and roots number were also higher in these treatments. No shoot hyperhydricity was observed in the *in vitro* palo de balsa cultures.

Seeds of *O. pyramidale* are dispersed by wind, numerous, small, about 5 mm long, pyriform, oily, and imbedded in the pale brown trichomes of the capsule; the behavior is orthodox, and in natural conditions germination is 70 and 80 percent and root protrusion occurs in 15 to 22 days [16], similar results to those observed in the present study. In others Malvaceae-Bombacoideae species as *Adansonia digitata*, an endangered medicinal tree, the seeds have very hard coats and germination is usually less than 20%, and the dormancy can be attributed partly to the testa and partly to the pulp [17]; most probably, natural regeneration of African Baobab is poor because of browsing animals and uncontrolled bush fires, therefore, vegetative propagation is reported to be advantageous [18]. To mitigate this problem a efficient protocol was developed for *in vitro* micropropagation via seed germination of *A. digitata*. In effect, seeds were harvested from the mother tree after every 15, 30, 45, 60 and 75 days from the beginning of the fruiting season, but only seeds of 30 and 45 day old, without pre-treatment of scarification, shown germination rates between 80–90%; mature seeds 60 day old, without pre-treatment of scarification, and 75 day old soaked in concentrate nitric acid (HNO_3) for 24 h and soaked in tap water for 1 – 10 days, the seeds failed to germinate on both MS half-strength and MS full-strength medium [9]. In the present study, the pre-treatment with NaOCl solution for 10 min or more was as detrimental as the pre-treatment with HNO_3 in *A. digitata* seeds reported by Singh *et al*. (2010) [9].

In other studies, seeds of *A. digitata* were chemical scarificated with concentrate sulfuric acid (H_2SO_4 at 95%) for 12 h and then rinsed thoroughtly with sterile distilled water; posteriorly, the seeds were surface disinfected with bleach (NaOCl, chlorometric 8º) for 5 min [17, 19]. After chemical scarification and disinfection, previously pulped baobad seeds offer a germination rate of 90% after 20 days of culture [19], slightly higher than observed in the present work.

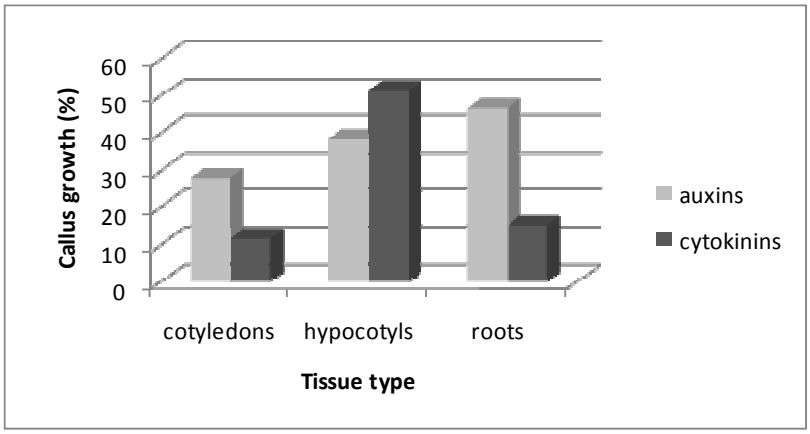

Fig-1. Effect of the auxins (2,4-D, NAA and IAA) and cytokinins (BAP, KIN and 2iP) in the callus growth (+++) in three explants types (cotyledons, hypocotyls and roots) of *O. pyramidale* after 60 days of culture.

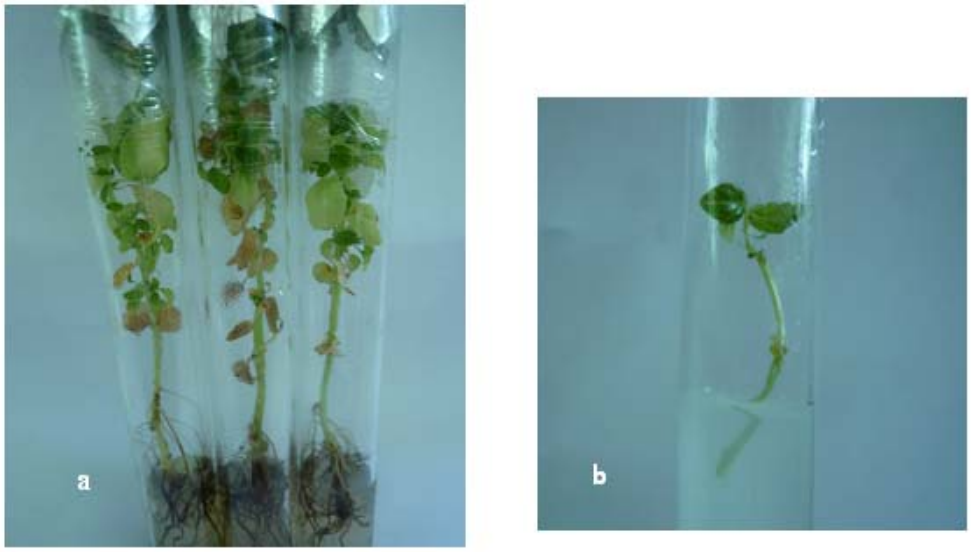

Fig-2. *In vitro* conservation of *Ochroma pyramidale*. a. Plantlets on *in vitro* conservation in MS full-strenght supplemented with 0.5 mg/L ABA, after 21 months of culture. b. Re-growth process, after 21 months of conservation in MS medium supplemented with 0.02 mg/L IAA and 0.02 mg/L GA_3.

Table –1: Effect of the gibberelic acid (GA_3) and sucrose on *in vitro* germination seeds of *O. pyramidale* after 60 days of culture.

Treatments		Germinated seeds (%)		Morphogenic responses		
GA_3 (mg/L)	Sucrose (%)	+	-	Shoot elongation (mm)	Number of leaves per shoot	Number of roots per shoot
0.0	2.0	15.0	85.0	0.75 a	0.20 a	0.61 a
0.5	2.0	20.0	80.0	1.13 ab	0.43 a	1.09 ab
1.0	2.0	75.0	25.0	3.83 ab	1.43 ab	3.39 ab
0.0	2.5	60.0	40.0	2.17 ab	0.70 ab	2.49 ab
0.5	2.5	15.0	85.0	6.72 b	2.13 b	5.47 ab
1.0	2.5	50.0	50.0	6.27 b	2.2 b	5.94 b
0.0	3.0	30.0	70.0	1.55 ab	0.60 a	1.25 ab
0.5	3.0	40.0	60.0	3.04 ab	0.70 ab	2.57 ab
1.0	3.0	55.0	45.0	4.54 ab	1.26 ab	4.16 ab

Values with different letters in the same column are significantly different ($P \leq 0.05$)

Table –2: Effect of auxin type on callus induction in cotyledons, hypocotyls and roots of *O. pyramidale* after 60 days of culture.

Treatments (mg/L)			Callus induction (%)											
2,4-D	NAA	IAA	Cotyledons				Hypocotyls				Roots			
			-	+	++	+++	-	+	++	+++	-	+	++	+++
0.0	0.0	0.0	70	30	-	-	90	10	-	-	80	20	-	-
1.0			-	-	60	40	10	20	50	20	-	-	30	70
2.0			20	10	20	50	30	50	20	-	-	30	20	50
	1.0		20	40*	20*	20*	30	-	-	70	30	-	30	40
	2.0		10	-	30	60	-	-	10	90	10	-	-	90
		1.0	-	80*	20*	-	-	40	30	30	-	10	60	30
		2.0	20	70*	10*	-	-	40	40	20	20	50	30	-

*Compact callus

-, without callus formation; +, callus covers 1/3 of the explant; ++, callus covers ½ to 2/3 of the explant; +++, callus covers the whole explant.

Table –3: Effect of cytokinin type on callus induction in cotyledons, hypocotyls and roots of *O. pyramidale* after 60 days of culture.

Treatments (mg/L)			Callus induction (%)											
BAP	KIN	2iP	Cotyledons				Hypocotyls				Roots			
			-	+	++	+++	-	+	++	+++	-	+	++	+++
0.0	0.0	0.0	100	-	-	-	100	-	-	-	90	10	-	-
1.0			50	50	-	-	90	10	-	-	20	60	20	-
2.0			-	100	-	-	-	-	-	100	50	30	-	20
	1.0		20	80	-	-	20	80	-	-	40	10	10	40
	2.0		40	60	-	-	-	50	30	20	70	20	-	10
		1.0	20	20	40	20	-	-	-	100	60	20	-	20
		2.0	-	10	40	50	-	-	10	90	40	60	-	-

-, without callus formation; +, callus covers 1/3 of the explant; ++, callus covers ½ to 2/3 of the explant; +++, callus covers the whole explant.

Callus induction

For establishment of callus cultures, explants including cotyledons, hypocotyls and roots, were inoculated and incubated in MS media with different concentrations of auxins (2,4-D, NAA and IAA) and cytokinins (BAP, KIN and 2iP). In the case of auxins, from the three types of explants tested, hypocotyls were the most responsive in terms of friable callus induction followed by root explants, reaching in both explants until 90% (+++) in the treatment 2.0 mg/L NAA; however, compact callus and roots proliferation were observed in cotyledons in 1.0 NAA mg/L (+ to +++) and 1.0 and 2.0 mg/L IAA (+ and ++) treatments (Table 2).

In the case of cytokinins, hypocotyls were the most responsive in terms of friable callus (90 to 100%) in the treatments 2.0 mg/L BAP (+++) and 1.0 and 2.0 mg/L 2iP (+++) (Table 3). In general, from the three types of explants tested the callus induction rate (+++) was very high in case of roots explants (46.6%) in auxins and hypocotyls explants (51.6%) in cytokinins (Fig. 1). Morphologically, callus were of several colours: white, cream, light yellow and brown, especially in all explants where brown callus was only observed in the 1.0 or 2.0 mg/L IAA treatments, suggesting differences in sensitivity between the auxins used. In plants, both auxins and cytokinins are necessary for cell transition from G1 to S cell cycle phase and from G2 to M cell cycle phase [20]. Callus induction and growth of *O. pyramidale* mostly depended upon the nature of explants as well as the type and concentration of plant growth regulators, especially the auxin 2.0 mg/L NAA and the cytokinins 2.0 mg/L BAP and 1.0 and 2.0 mg/L 2iP. Auxins were reported to induce callus when they are used alone; however, the combination of auxin and cytokinin callus induction was further improved, and have already been documented for other plant species as *Holarrhena antidysenterica* [21], *Digitalis lanata* [22], and *Calotropis gigantea* [23]. Plant cell cultures have been actively studied as a potential source of high-value biological compounds [24], in that sense, the friable callus induction is a very important stage for the establishment of cell suspension cultures capable of producing medicinal compounds at a rate similar or higher than intact plants [25].In *O.pyramidale* the friable callus induction would allow the establishment of cell suspensions for use in the biosynthesis of various secondary metabolites.

Germplasm conservation

Growth of shoots in apical buds detached from the seedlings grew, cultured in medium containing 4 and 6% mannitol (Table 4) and 0.5 mg/L ABA (Table 5), was markedly suppresses when compared with those controls. After 6 months of culture, both MS full-strenght and MS half-strenght supplemented with 4 and 6% mannitol, the differences in the shoot elongation, were not significant. In these treatments the shoot elongation was 3.2 to 3.6 mm, while the control treatment was 7.7 mm; in all treatments assays with mannitol, 10 months after the culture, the survival was 0% (Table 4). Plantlets showed apical necrosis and thickened roots and oxidized, consequently, the mortality it continued throughout the experiment. In the treatments with ABA, the best treatment of conservation was MS full-strenght supplemented with 0.5 mg/L ABA; in this treatment the shoot elongation was 5.6 cm and the survival was 100% (Table 5); however, was not significantly better than other treatments with MS full-strength with 0.1 mg/L ABA, and MS half-strength with 0.5 mg/L ABA.

Table -4: Effects of mannitol on *in vitro* germplasm conservation of *O. pyramidale* after 180 days of culture.

Treatments		Shoot elongation (cm)	Number of leaves	Number of nodes	Number of roots	Survival (%)
MS	Mannitol (%)					
1	0.0	7.7 ± 2.7 b	2.7 ± 1.5 bc	1.4 ± 1.4 c	14.5 ± 9.0 a	100.0
½	0.0	7.7 ± 1.1 b	2.5 ± 1.3 b	0.7 ± 0.9 b	12.6 ± 3.1 a	100.0
1	4.0	3.5 ± 0.4 a	3.3 ± 1.3 c	0.0 ± 0.0 a	17.0 ± 5.7 ab	0.0
½	4.0	3.6 ± 0.5 a	2.2 ± 1.0 ab	0.0 ± 0.0 a	21.2 ± 8.9 b	0.0
1	6.0	3.5 ± 0.4 a	2.2 ± 0.6 b	0.0 ± 0.0 a	15.3 ± 5.7 b	0.0
½	6.0	3.2 ± 0.5 a	2.5 ± 0.8 a	0.0 ± 0.0 a	21.4 ± 12.6 b	0.0

Values with different letters in the same column are significantly different ($P \leq 0.05$)

Table – 5: Effects of ABA on *in vitro* germplasm conservation of *O. pyramidale* after 180 days of culture.

Treatments		Shoot elongation (cm)	Number of leaves	Number of nodes	Number of roots	Survival (%)
MS	ABA (mg/L)					
1	0.0	7.7 ± 2.7 b	2.7 ± 1.5 c	1.4 ± 1.4 ab	14.5 ± 9.0 a	100.0
½	0.0	7.7 ± 1.1 b	2.5 ± 1.3 bc	0.7 ± 0.9 a	12.6 ± 3.1 a	100.0
1	0.1	5.9 ± 2.3 a	1.4 ± 1.4 a	2.0 ± 2.0 bc	12.1 ± 5.5 a	100.0
½	0.1	8.8 ± 2.1 b	2.4 ± 0.9 bc	2.6 ± 1.7 c	15.8 ± 4.2 a	100.0
1	0.5	5.6 ± 1.2 a	1.7 ± 1.0 ab	1.3 ± 1.5 ab	14.0 ± 4.9 a	100.0
½	0.5	6.2 ± 1.4 a	1.8 ± 1.0 ab	1.0 ± 1.1 b	15.2 ± 5.5 a	100.0

Values with different letters in the same column are significantly different ($P \leq 0.05$)

In these treatments the plantlets were kept up to 21 months of culture (Figure 2a). In the re-growth process, after 21 months of conservation, the best treatment was MS medium supplemented with 0.02 mg/L IAA and 0.02 mg/L GA_3. (Figure 2b); in addition, the shoot elongation and the survival rate was 8.5 cm and 82%, respectively (data nos showed in table). This culture medium is widely used in the clonal propagation of *Piper* sp. [26].

In vitro technique used to achive medium-term conservation allow to store biological material from several months to 2–3 years without subculture, depending on the technique used and on the plant material [27]. Growth reduction is generally attained by modifying the culture medium and/or the environmental condictions. Modifications of the culture medium can include dilution of mineral elements, reduction of sugar concentration, changes in the nature and/or concentration of growth regulators and addition of osmotically active compounds [28]; however, the risks of the genetic variation increases with *in vitro* storage duration, and can lead to the loss of trueness to type. The addition of osmoticums, such as mannitol or sorbitol, reduce mineral uptake by cell through differences in osmotic pressures thereby retarding plant growth [29]. For instance, in *Capsicum chinensis* ("habanero pepper") mannitol at 2% had the better effect on minimal growth of the planlets and did not affect the plant physiology and quality; the plantlets remained small in size, turgent, with green leaves and stems and looked like normal plants until to the end of the evualation period. In this species, the addition of osmoregulators based on carbon-containing molecules that are not degraded by plant cells, like sorbitol and mannitol, induced a strong effect on minimal growth at high concentrations (sorbitol and mannitol 8%, respectively), but anatomic and physiological alterations were observed: leaf chlorosis, rolling and distorted leaves, and out-of-proportion shoot-root relationships [30]. Similar effects were observed with shoots of sweetpotato (*Ipomoea batatas*) stored in a MS medium supplemented with sorbitol (2%) and mannitol (2%) over a period of 16 months [31]; likewise, in *Vriesea reitzii*, Rech *et al*. (2005) [32] observed that the addition of mannitol also significantly improved shoot survival, although no difference was observed between 1 and 2% concentrations.

In general, the beneficial effect of mannitol for *in vitro* conservation has been previously reported in several species as *Dioscorea alata* (1.5%) [33], *Plumbago indica* (2%) [34], *Drosophyllum lusitanicum* (2%) [35], *Veronica multifida* ssp. *capsellicarpa* (3 and 6%) [36], and *Dianthus spiculifolius* and *D. glacialis* ssp. *gelidus* (0.16, 0.32 and 0.49 M) [37]; however, in other studies, has been reported the toxic effects of mannitol in *in vitro* conservation. In cassava (*Manihot esculenta*), shoot deteriorate in presence of mannitol, even at 0.1% and with a storage temperature lower than 20 ^{o}C [38]; in *Epidendrum chlorocorymbos* mannitol treatments (1, 2 and 3%) produced seedlings death [39]; in *Podophyllum peltatum* was reported that 2% mannitol was not adequate for *in vitro* conservation [40] and in *Piper hispidinervum* and *P. aduncum* was observed that 1-3% mannitol negatively affect causing low rate of recovered shoots after storage [41]. These studies demostrated that the lethal concentrations of mannitol seems to be species dependent and storage time can influence significantly [39, 41, 42]. In the present work the treatments with 4 and 6% mannitol were highly detrimental.

On the other hand, some growth retardants as abscisic acid (ABA) can be used in order to reduce the growth of shoot in several species [43]. For instance, in some grape varieties conservation on media with different concentration of ABA or cycocel (CCC) reduced the shoot number/explant and shoot lenght compared with those conserved on ABA or CCC-free medium; shoot tips explants were conserved at 10 ^{o}C under complete darkness and the results revealed that the varieties over 50% of explants, conserved on medium with 15 to 25 µM ABA for 6 to 12 months, can be able to survived [44]. In cassava, the results indicated that 20 and 30 µM ABA induced bud dormancy and delayed sprouting without affecting subsequent growth of plants after treatments [45], and in sweet potato plants grown in a medium containing ABA at 5–20 mg/L had a survival rate of 70–85% after 8 months, but showed strong genotypic effects [46]. In our study the best treatment of germplasm conservation was supplemented only with 0.5 mg/L ABA.

CONCLUSIONS

The highest percentage of seed germination (75%) was obtained in the treatment 1.0 mg/L GA_3 – 2% sucrose; however, an increase in the disinfection treatment (5.25% sodium hypochlorite) to 10 or more minutes reduced the germination less than 10%. This study is the first attempt to standardize the induction and proliferation of callus from various explants (hypocotyls, cotyledons and roots) in several auxins and cytokinins concentrations. Likewise, the present work confirmed the inhibitory effect of ABA on palo de balsa apical shoots in *in vitro* plant growth. ABA added to a MS full-strenght medium moderatly inhibited shoot elongation at 0.5 mg/L for 180 days without affecting subsequent recovery of plants in a propagation culture medium without ABA. Mannitol 4 and 6% added to the culture medium strongly inhibited the growth of plantlets and prolongs subculture time to 180 days without an appearance of dead plantlet; however, after 10 months of culture, the survival was 0%.

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