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IN VITRO PLANT PROPAGATION AND PARTIAL ORGANOGENESIS IN PALO DE BALSA [*OCHROMA PYRAMIDALE* (CAV. EX LAM.) URBAN.]

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ABSTRACT: 'Palo de balsa' or 'Balsa wood' [*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, because is a source of the lightest commercial wood, which is widely used in the aerospace industry, manufacture of boats and rafts, and as a substitute for cork. The aim of this study was to optimize the cultivation conditions for *in vitro* micropropagation and root regeneration of callus of *O. pyramidale*. For micropropagation, 30-day-old seedlings developed on full MS medium were used to prepare stem node explants. In the treatments with BAP, KIN o 2iP an average shoot length of 4.72 cm and an average number of nodes per shoot of 3.2 was recorded in the treatment supplemented with 2.0 mg/L 2iP after 60 days in the culture medium. In the rooting process, the most significant values were obtained with the treatments supplemented with 0.01 mg/L IBA and 0.01 mg/L GA_3, and 0.01 mg/L IBA giving a rooting rate of 100% and an average number of roots of 6.7 and 6.3, respectively. Of the three types of explants tested, the cotyledons calli were the most responsive in terms of roots regeneration followed by the root calli, reaching up to 60% in cotyledon calli (+++, > 20 roots formed) in the treatment with 2.0 mg/L NAA. Well-rooted plants were succesfully established in a greenhouse with a survival rate of 70%.
Key words: Callus induction, micropropagation, rooting, root regeneration, *Ochroma pyramidale*.

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INTRODUCTION

Ochroma pyramidale (Cav. ex Lam.) Urban belongs to the family Bombacaceae, order Malvales. The order Malvales consists of 5 families (Malvaceae, Sterculiaceae, Tiliaceae, Elaeocarpaceae and Bombacaceae) and about 3000 to 3500 species, cosmopolitan in distribution, but best represented in the tropics (Cronquist, 1988) [1]. In the system proposed by the Angiosperm Phylogeny Group, the family Bombacaceae is incorporated into the family Malvaceae (APGII, 2003) [2], and is included with Bixaceae, Muntingiaceae, and others into the order Malvales, Eurosids II (APGIV, 2016) [3].

The Bombacaeae (= Malvaceae) is a pantropical family of 20-30 genera and 200 species; from these, 14 genera and 47 species have been recorded in Peru. The family is very close to Malvaceae which shares the fused staminal filaments and other characters; however the latter are usually herbs or small shrubs and have distinctive spinous pollen grains (Pennington et al., 2004) [4]. The genus *Ochroma* includes only 1 species, *O. pyramidale*, which is commonly known as 'palo de balsa', 'topa', 'pau de balsa' or 'balsa Wood', and is distributed from Mexico and the West Indies to southern South America.The plant is a tree of 30 m high and 1.8 m d.b.h with smooth grey lenticellate bark; the lightweight timber (specific gravity 0.13) is used internationally for model making and locally for rafts, and the kapok for stuffing cushions (Pennington et al., 2004; Pennington and Sarukhan, 2005) [4,5].

Baobab (*Adansonia digitata*), a native deciduous tree from African savannas, has been reported to have interesting properties with ethnomedicinal uses in the treatment of fever, diarrhoea, dysentery, smallpox, measles, asthma, general fatigue, kidney and bladder diseases, amenorrea, toothache, stomachache, insect bites, inflammations, Guinea worms, and is diaphoretic, expectorant, anti-histaminic, substitute of *Cinchona* for malaria patients, to smooth skin in babies and as an antidote for *Strophanthus* poisoning (Sugandha et al., 2013; Refaat et al., 2014) [6, 7]. The parts most commonly used are roots, bark, pith, leaves, fruits, and seeds.The baobab fruit pulp has a particularly high antioxidant capability mainly due to its high natural vitamin C (300 mg/100 g corresponding to the content of six oranges), flavonoids, α-linolenic acid and provitamin A (Shukla et al., 2001;Vertuani et al., 2002) [8, 9].

A semi-fluid gum, obtained from the baobab bark, is used to treat sores, and it also contains the alkaloid adansonin which has been used for fever treatment, especially when 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 (Sugandha et al., 2013; Shukla et al., 2001) [6, 8]. Other species of the family Malvaceae-Bombacoideae as *Bombax ceiba* (silk cotton tree or locally know as semal), has been found to posses strong antiinflamatory, antibacterial, antiviral, analgesic, hepatoprotective, antioxidant, oxytocic, hypotensive, hypoglycaemic, antiangiogenic, antimutagenic, as well as fibrinolysis enhancing activities (Saleem et al., 1999; Gupta et al., 2004) [10, 11]. An appraisal of this species was recently published by Jain *et al*. (2009) [12]; however, other species of the *Bombax* genus such as *B. malabaricum*, *B. malabarica* and *Ceiba pentandra* (= *B. pentandrum*) have also shown ethnomedicinal properties (Refaat et al., 2014) [7].The few reports corresponding to *Ochroma lagopus* belong to the Guyana and the Amazon region, where it is used as emetic and to expel the cow placenta after childbirth, and flowers are responsible for high mortality of insects (Pérez-Arbelaez, 1953; Hueck, 1972) [13, 14].An important review showing the results of various pharmacological and toxicological studies conducted on different Bombacaceae plants in light of their ethnomedicinal uses was recently published by Refaat et al. (2014) [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-strength MS medium containing 2.0 mg/L IBA (Chand and Singh, 1999) [15]. In *Adansonia digitata*, an African multipurpose tree species, shoots were obtained from nodal segments of *in vitro* germinated seeds cultured on half-strength QL médium (Quoirin and Lepoivre, 1977) [16] containing 10 µM BAP, and rooted plantlets were regenerated on half-strength WPM (Woody Plant Medium) (Lloyd and McCown, 1981) [17] containing 3.5 µM IBA and 0.32 µM NAA (Ishii and Kambou, 2007) [18]. In this same species, 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 (Singh et al., 2010) [19]. In another species, the *in vitro* propagation of *A. digitata* was carried out from different types of explants taken from twenty-day aged sterile seedlings such as cotyledonary nodes, axillary nodes and terminal apex (N'Doye et al., 2012) [20]. In the case of *Ochroma pyramidale* the literature only reports the study carried out by our working group on the *in vitro* seed germination, callus induction and germplasm conservation (Morante-Alarcón et al., 2014) [21].

Despite the considerable advance in rooting of cuttings and tissue culture of several tree species as *Eucalyptus* sp. (de Assis et al., 2004) [22], *Acacia mangium* (Shahinozzaman et al., 2012) [23], *Cedrela montana* (Díaz-Quichimbo et al., 2013) [24], *Prosopis pallida* (Minchala-Patiño et al., 2014) [25] and others, there still is no asexual propagation technique for *Ochroma pyramidale* which could be suitable for production of massive quantities of clones that would be required for comercial scale plantations. The present study was carried out to develop a simple, reliable and efficient protocol for micropropagation and root organogenesis of *O. pyramidale* using seed explants.

MATERIALS AND METHODS

Plant materials and seed disinfestation

Elite 50-year-old O.*pyramidale* tree was selected from Refugio de Vida Silvestre Laquipampa (Lambayeque, Peru), and the selection was based on the trees anatomical features: straight trunks reaching at least 12 m in height and 80 cm in diameter. The protocol of seed disinfestation was described in a previous work (Morante-Alarcón et al., 2014) [21].

Culture media and culture conditions for clonal propagation

All media consisted of full- strength MS (Murashige and Skoog, 1962) [26] salt formulation containing the following ingredients: thiamine.HCl (1.0 mg/L), myo-inositol (100 mg/L), 2% sucrose and 0.6% agar-agar. The disinfected seeds were aseptically germinated in the MS formulation supplemented with two concentrations of GA_3 (0.5 and 1.0 mg/L). For micropropagation, 30-day-old seedlings developed on full MS medium were used to prepare stem node explants. All seedlings with 8 to 10 cm high were transversely cut into segments (1 to 1.5 cm) each containing one bud. The BAP – IAA – GA_3 combinations and three types of cytokinins (BAP, KIN and 2iP) were applied in several concentrations (0.1 to 2.0 mg/L), and for rooting induction several concentrations of auxins NAA, IAA and IBA with GA_3 were assessed. 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^{o}C and 1.05 kg cm^{-2}. One explant was cultured per tube. Cultures were incubated at 26 ± 2^{o}C 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. Each treatment comprised 25 explants and was performed twice. The experiments were evaluated every 60 days.

Callus induction and roots formation

Cotyledons, hypocotyls and roots of 15-d-old seedlings were used as explants for callus induction, as reported in a previous paper (Morante-Alarcón et al., 2014) [21]. These calli were subsequently subcultured in treatments with three types of auxins (2,4-D, IAA and IBA) and three types of cytokinins (BAP, KIN and 2iP) in two concentrations (1.0 and 2.0 mg/L). The callus cultures were incubated in the dark. Each treatment comprised 15 explants and was performed twice. The experiments were evaluated every 60 days.

Statistical analysis

Results were processed and analysed by analyses of variance (ANOVA) and the Duncan multiple range test ($p \leq 0.05$) in order to compare treatment means. All the statistical analyses were carried out using the IBM SPSS Statistics 20 software (IBM SPSS, 2012) [27].

RESULTS AND DISCUSSION

Plant propagation

The effect of various plant growth regulators was carried out on *in vitro* clonal propagation of *O. pyramidale*, after 60 days of culture.

In the treatments with BAP-IAA-GA_3 an average shoot length of 9.07 cm was recorded in the treatment supplemented with 2.0 mg/L 2iP (Table 1), and an average number of nodes per shoot of 1.8 was recorded in the treatment supplemented with 1.0 mg/L BAP and 0.1 mg/L IAA (Table 2). The comparison reveals non-significant differences of these treatments compared with the control. In the treatments with BAP, KIN o 2iP an average shoot length of 4.72 cm and an average number of nodes per shoot of 3.2 were recorded in the treatment supplemented with 2.0 mg/L 2iP (Table 1). The comparison also reveals non-significant differences of these treatments compared with the control. Statistical analysis showed that the values obtained in the others treatments supplemented with cytokinins BAP or KIN were significantly lower. In general, all nodal explants cultured in MS medium with different concentrations of plant growth regulators including the control, only produced one shoot per explant (data not shown).

In *A. digitata* (baobab) shoots were obtained from nodal segments of *in vitro* germinated seeds on ½ QL medium containing 10 µM BAP (Ishii and Kambou, 2007) [18]. In another study in this same species the *in vitro* propagation was carried out from different types of explants taken from twenty-day aged sterile seedlings suchs as cotyledonary nodes, axillary nodes and terminal apex, and in the presence of 0.5 mg/L BAP, a multiplication rate of 2.31 was obtained for apex explants, 1.88 for axillary nodes and 2.0 for cotyledonary nodes (N'Doye et al., 2012) [20]. As observed in *A. digitata*, where an average of 2.0 shoots per explant was induced, only one shoot per explant was observed in *O. pyramidale*, which showed a very strong apical dominance, although the formation of nodes by seedling was higher (average number of nodes per shoot of 3.2). The growing shoot apex is known to regulate a wide range of developmental processes in plants including axillary bud growth, orientation of laterals, growth of rhizomes and stolons, leaf abscission, and others (Cline, 1991) [28], and the expression of dominance by the shoot apex requires basipetal IAA transport in the subapical part of the stem (Tamas, 1995) [29].

Rooting

The results obtained after 60 days of culture are showed in Table 3. The most significant values were obtained with the treatments supplemented with 0.1 mg/L IBA and 0.01 mg/L GA_3, and 0.01 mg/L IBA giving a rooting rate of 100% and an average number of roots of 6.7 and 6.3, respectively; however, the most significant values of number nodes per shoot of 3.1 was obtained in the treatment supplemented with 0.01 mg/L NAA and 0.01 mg/L GA_3. In all treatments the rooting rate was 100%.

In *A. digitata* rooted plantlet was regenerated on ½ WPM (Woody Plant Medium) containing 3.5 µM IBA and 0.32 µM NAA (Ishii and Kambou, 2007) [18]. In another study in *A. digitata*, after an induction period of 72 h with 5.0 mg/L NAA and a transfer into the expression medium without hormones, 50% of the apex and 75% of cotyledonary nodes rooted; in contrast, 57% of axillary nodes took root after an induction with NAA at 2.5 mg/L (N'Doye et al., 2012) [20]. In our study in *O. pyramidale*, the rooting rate was significantly high, especially in treatments without hormones, as reported by N'Doye et al. (2012) [20] in *A. digitata*, which supposes a high accumulation of endogenous auxins that lead to the apical dominance of the shoot.

Callus induction and roots regeneration

In a previous work (Morante-Alarcón et al., 2014) [21], for establishment of callus cultures, the explants were inoculated and incubated in MS media with different concentrations of auxins (2,4-D, NAA and IAA) and cytokinins (BAP, KIN and 2iP). After 60 days, the calli formed were subcultured in treatments with the same types and concentrations of auxins and cytokinins. In the case of auxins, from the three types of explants tested, the cotyledons calli were the most responsive in terms of roots regeneration followed by root calli, reaching up to 60% (+++, > 20 roots formed) in cotyledon calli for the treatment with 2.0 mg/L NAA. 2,4-D (1.0 and 2.0 mg/L) did not induce root formation in the tested calli (Table 4). In the case of cytokinins, from the three types of explants tested, the roots calli were the most responsive in terms of roots regeneration, reaching up to 60 and 30% for the treatment with 1.0 and 2.0 mg/L KIN, respectively, although root formation was very scarce (+). The other explants (cotyledons and hypocotyls) and cytokinins tested (BAP and 2iP) were not efficient (Table 5).

In *A. digitata* all explants produced exuberant callus growth *in vitro* except the root segment; however, the cotyledonary portion was the best explant for callus formation in half-strength QL medium (Ishii and Kambou, 2007) [18]. The literature does not report the induction of callus roots in any species of the family Malvaceae-Bombacoideae.

Table -1: Effect of cytokinins (BAP, KIN and 2iP) on several morphogenic responses of *in vitro* shoot elongation of *O. pyramidale* after 60 days in culture medium.

Treatments (mg/L)			Morphogenic responses			
BAP	**KIN**	**2iP**	**Shoot elongation (cm)**	**Number of leaves per shoot**	**Number of nodes per shoot**	**Number of roots per shoot**
0.0	0.0	0.0	6.33 ± 0.65^{ab}	2.2 ± 0.63^{c}	3.0 ± 0.94^{b}	8.7 ± 1.49^{c}
1.0			3.79 ± 0.72^{a}	$1.1 \pm 1.10a^{b}$	1.0 ± 1.05^{a}	0.1 ± 0.32^{a}
2.0			4.22 ± 0.44^{a}	0.6 ± 1.08^{a}	1.0 ± 0.82^{a}	0.0 ± 0.00^{a}
	1.0		3.77 ± 0.60^{a}	2.5 ± 0.71^{c}	1.0 ± 0.67^{a}	1.6 ± 1.43^{b}
	2.0		4.63 ± 0.90^{ab}	1.9 ± 0.32^{bc}	0.6 ± 0.52^{a}	0.7 ± 0.82^{a}
		1.0	4.85 ± 0.75^{ab}	2.1 ± 0.32^{c}	1.5 ± 1.27^{a}	0.0 ± 0.00^{a}
		2.0	9.07 ± 2.63^{b}	1.7 ± 1.42^{bc}	3.2 ± 1.53^{b}	0.1 ± 0.32^{a}

[a]MS + vitamins + sucrose 2.5%

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

Table-2: Effect of BAP-IAA-GA_3 on several morphogenic responses of *in vitro* shoot elongation of *O. pyramidale* after 60 days in culture medium.

Treatments[a] (mg/L)			Morphogenic responses[b]			
BAP	**IAA**	**GA_3**	**Shoot elongation (cm)**	**Number of leaves per shoot**	**Number of nodes per shoot**	**Number of roots per shoot**
0.0	0.0	0.0	3.77 ± 1.10^{a}	4.4 ± 1.71^{c}	1.5 ± 0.85^{bc}	5.0 ± 2.62^{b}
1.0	0.1	0.0	4.40 ± 0.41^{ab}	2.7 ± 0.48^{b}	1.8 ± 0.63^{c}	1.3 ± 2.08^{a}
2.0	0.1	0.0	4.37 ± 0.28^{ab}	2.6 ± 0.52^{b}	0.9 ± 0.32^{ab}	0.2 ± 0.42^{a}
1.0	0.1	0.5	4.72 ± 0.33^{b}	2.2 ± 0.63^{ab}	1.2 ± 1.03^{bc}	0.2 ± 0.42^{a}
2.0	0.1	0.5	4.24 ± 0.92^{ab}	1.4 ± 1.08^{a}	0.5 ± 0.53^{a}	0.0 ± 0.00^{a}

[a]MS + vitamins + sucrose 2.5%

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

Table-3. Effect of auxins (NAA, IAA and IBA) and GA_3 on several morphogenic responses of *in vitro* rooting of *O. pyramidale* after 60 days in culture medium.

Treatments (mg/L)				Morphogenic responses			
NAA	IAA	IBA	GA_3	Shoot elongation (cm)	Number of leaves per shoot	Number of nodes per shoot	Number of roots per shoot
0.0	0.0	0.0	0.0	3.77 ± 1.10 abcde	4.4 ± 1.71 abcd	1.5 ± 0.85 b	5.0 ± 2.62 bcd
0.01	0.0	0.0	0.01	4.75 ± 1.67 e	6.8± 2.66 e	3.1± 1.52 c	3.5 ± 2.92 ab
0.1	0.0	0.0	0.01	3.5 ± 0.42 abc	4.0 ± 1.05 abc	1.5± 1.08 b	2.3± 2.11 a
0.0	0.01	0.0	0.01	3.63 ± 0.72 abcd	4.7±1.57 abcde	1.3± 0.95 b	4.6 ± 2.17 abcd
0.0	0.1	0.0	0.01	3.44 ± 0.49 abc	3.8 ± 1.14 ab	1.3 ± 0.82 b	3.4 ± 2.12 ab
0.0	0.0	0.01	0.01	3.61 ± 1.13 abcd	4.2 ± 1.87 abc	0.8 ± 0.92 ab	4.5± 2.59 abcd
0.0	0.0	0.1	0.01	3.13 ± 0.84 ab	4.5 ±1.43 abcd	1.6± 1.08 b	6.7 ± 3.53 d
0.01	0.0	0.0	0.0	2.87 ± 0.39 a	2.9 ± 0.32 a	0.2 ± 0.42 a	3.2 ± 2.74 ab
0.1	0.0	0.0	0.0	3.18 ± 0.98 abc	6.1 ± 2.38 cde	1.8± 1.32 b	2.7 ± 1.70 ab
0.0	0.01	0.0	0.0	4.22± 0.62 cde	5.6 ±2.46 bcde	1.4 ± 0.70 b	3.5 ± 2.32 ab
0.0	0.1	0.0	0.0	4.59 ± 1.42 de	6.4 ± 4.60 de	1.6± 1.78 b	4.2 ± 2.62 abc
0.0	0.0	0.01	0.0	4.75 ± 1.12 e	5.5± 1.43 bcde	1.8 ± 1.14 b	6.3 ± 2.06 cd
0.0	0.0	0.1	0.0	4.03± 1.37 bcde	3.9 ± 1.29 ab	1.0± 1.05 ab	5.2 ± 1.48 bcd

[a]MS + vitamins + sucrose 2.5%

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

Table 4. Effect of auxins (2,4-D, NAA and IAA) on roots regeneration in several callus types of *O. pyramidale* after 60 days in culture medium.

Treatments[a] (mg/L)			Roots induction[b] (%)								
2,4-D	NAA	IAA	Cotyledons			Hypocotyls			Roots		
			+	++	+++	+	++	+++	+	++	+++
0.0	0.0	0.0	20	-	-	60	-	-	100	-	-
1.0	0.0	0.0	-	-	-	-	-	-	-	-	-
2.0	0.0	0.0	-	-	-	-	-	-	40	-	-
0.0	1.0	0.0	20	50	10	70	-	-	70	-	-
0.0	2.0	0.0	-	30	60	70	-	-	40	50	-
0.0	0.0	1.0	60	30	10	80	-	-	80	10	10
0.0	0.0	2.0	40	30	20	80	10	10	80	-	-

[a]MS + vitamins + sucrose 2.5%

[b]+, 1 to 10 roots formed; ++, 11 to 20 roots formed; +++, > 20 roots formed

Table-5. Effect of cytokinins (BAP, KIN and 2iP) on roots regeneration in several callus types of *O. pyramidale* after 60 days in culture medium.

Treatments[a] (mg/L)			Roots induction[b] (%)								
BAP	KIN	2iP	Cotyledons			Hypocotyls			Roots		
			+	++	+++	+	++	+++	+	++	+++
0.0	0.0	0.0	30	-	-	20	-	-	10	-	-
1.0	0.0	0.0	-	-	-	10	-	-	10	-	-
2.0	0.0	0.0	-	-	-	-	-	-	-	-	-
0.0	1.0	0.0	-	-	-	-	-	-	60	-	-
0.0	2.0	0.0	-	-	-	10	-	-	30	-	-
0.0	0.0	1.0	-	-	-	-	-	-	20	-	-
0.0	0.0	2.0	-	-	-	-	-	-	10	-	-

[a]MS + vitamins + sucrose 2.5%

[b]+, 1 to 10 roots formed; ++, 11 to 20 roots formed; +++, > 20 roots formed

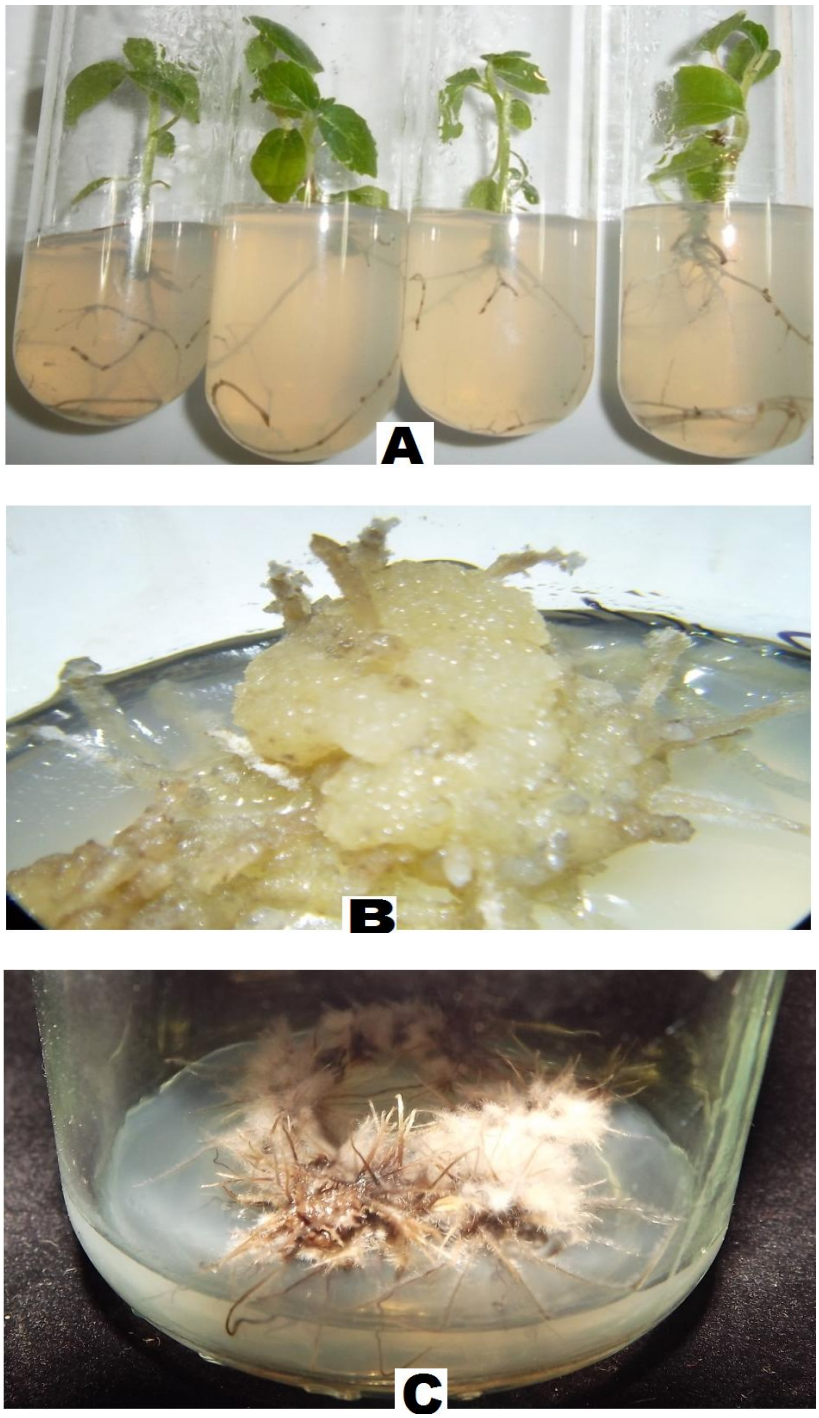

Fig-1. *In vitro* propagation, callus induction and roots regeneration of *Ochroma pyramidale*. a. Plantlets on *in vitro* propagation in MS medium supplemented with 0.1 mg/L IBA and 0.01 mg/L GA_3. b. Callus induction in roots, and c) Roots regeneration in friable callus formed in roots.

Plant cell and organ cultures have emerged as potential sources of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavors, fragrances, coloring agents, biopesticides, and food additives (Murthy et al., 2014)[30]. Although cell suspension culture is the method most commonly used method for the production of secondary metabolites, organ culture methods (e.g. root culture methods) have been developed for various plant species as an alternative for the production of secondary metabolites (Verpoorte et al., 2002) [31]. For example, natural adventitious roots have been induced in many medicinal plants via flask scale to bioreactor cultivation for the production of various bioactive compounds (Baque et al., 2012; Murthy et al., 2008a)[32, 33]. Likewise, hairy roots obtained by *Agrobacterium* rhizogenes-mediated transformation, are efficient producers of secondary metabolites; for instance, the production of withanolide A was 2.7-fold higher than that observed in nontransformed roots (Murthy et al., 2008b) [34], and the stevioside production in the hairy roots of *Stevia rebaudiana* under light and dark conditions (Pandey et al., 2016) [35].

The roots of several species of Malvaceae-Bombacoideae have shown several ethnomedicinal properties and pharmacological evidences (Refaat et al., 2014) [7]. In baobab (*A. digitata*), the methanol root bark extract displayed direct antiviral activities against Newcastle disease virus *in ovo*, and could, therefore, be useful for controlling this disease in poultry (Sulaiman et al., 2011) [36]; similarly, the same extract also showed similar effects against *Herpes simplex* 1 and 2, *Vesicular stomatitis* and *Poliovirus* (Hudson et al., 2000; De Caluwé at al., 2010) [37, 38], together with viricidal (direct inactivation of virus particles) and intracellular antiviral activity, which could indicate the presence of multiple antiviral compounds, or a single compound with multiple actions (Le Grand, 1989) [39]. The infusion of roots is also used in Zimbabwe to bathe babies to smooth skin (Masola et al., 2009) [40]. Additionally, extracts of baobab roots eliminate the motility in *Trypanosoma congolense* within 60 minutes and drastically reduce motility in *T. brucei brucei* and *T. congolense* which are unicellular parasites transmitted by the bites of tsetse fly and are the causative agents of sleeping sickness in humans and related diseases in animals (Atawodi et al., 2003) [41]. A review article on the medicinal uses of *A. digitata*, an endangered tree species, has been recently published by Sugandha et al. (2013) [6].

In a similar study carried out in *Bombax buonopozense*, the total root extract demonstrated antibacterial properties against *Staphylococcus aureus*, *Bacillus subtilis*, *Klebsiella pneumoniae*, *Proteus* spp. and *Escherichia coli* (Godwin et al., 2011) [42]. In this same species, the total methanol extract of root exhibited acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities with inhibition percentages more than 80% in comparison with others protocols (Elufioye et al., 2010) [43]. In another study, the methanol extract of *B. ceiba* roots exhibited *in vivo* and *in vitro* antioxidant potential, with high levels of phenols and tannins (Jain et al., 2011) [44], and also exhibited significant dose-dependent antibacterial properties against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae* (Jain and Verma, 2012) [45]. Furthermore, a similar extract exerted substantial cytotoxicity against brine shrimp nauplii in comparison with vincristine sulphate; the hexane extract also had a significant antibacterial effect against *Sarcina lutea* and *Pseudomonas aeruginosa*, and the chloroform and carbon tetrachloride extracts of roots demonstrated important antibacterial actions against *a number* of Gram +ve and Gram –ve bacteria (e.g. *Vibrio mimicus, Bacillus megaterium* and *Vibrio parahemolyticus*), and antifungal activities against *Aspergillus niger* and *Candida albicans* (Islam et al., 2011) [46]. Recently, pharmacognostical and phytochemical studies on roots of *B. ceiba* have shown the presence of alkaloids, glycosides, flavonoids, steroids, saponins and tannins (Chaudhary et al., 2014) [47].

On the other hand, in *Ceiba pentandra* (commonly known as karpok) the methylene chloride/methanol (1:1) extract of root bark exhibited significant anti-diabetic activity in streptozotocin-induced type-2 diabetic rats; the extract significantly reduced the intake of both food and water as well as the levels of blood glucose and other compounds in comparison with diabetic controls (Dzeufiet et al., 2006; Dzeufiet et al., 2007) [48, 49]. A comparative phytochemical and proximate analysis on *C. pentandra* and *B. buonopozense* revealed the presence of tannins, alkaloids, saponins, cyanogenic glycosides, steroids, flavonoids and phenols, with 18.7 and 16.7% of glycosides in leaves and roots, respectively in *C. pentandra* and 14.2 and 12.5% in leaves and roots, respectively, in *B. buonopozense* (Iroka et al., 2014) [50]. Likewise, the evaluation of the physicochemical properties and preliminary phytochemical studies on the root of *Bombax ceiba*, used as a traditional folk medicines for its anti-dysenteric, anti-diahorreal and anti-pyretic effects, revealed the presence of glycosides, tannins, flavonoid, b-sitosterol and lupeol (Nitika et al., 2011) [51].

In general, *in vitro* and large-scale root induction and multiplication is important in species such as Malvaceae-Bombacoideae, whose roots have been shown to be of significant importance as alternative medicine.

CONCLUSIONS

A pioneer method for 'Palo de balsa' or 'Balsa Wood' micropropagation was elaborated using stem node explants of 30-day-old seedlings. In the rooting process, the most significant values were obtained with the treatments supplemented with 0.01 mg/L IBA and 0.01 mg/L GA_3, and 0.01 mg/L IBA giving a rooting rate of 100%. From the three types of explants tested, the cotyledons calli were the most responsive in terms of roots regeneration followed by root calli. This study is the first attempt to standardize the induction and proliferation of roots from various explants (hypocotyls, cotyledons and roots) of *O. pyramidale*.

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