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Full Length Research Paper

Seed germination and *in vitro* plant regeneration of *Parkia biglobosa* (Jacq.) Benth

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***Parkia biglobosa* is an important leguminous forest species which is being threatened of going into extinction in Senegal. To preserve this genetic resource of great economic value, studies on germination were carried out and *in vitro* conservation option through tissue culture technique was adopted. 100% of germination rate was recorded 2 days after 5 - 6 h of concentrated H_2SO_4 treatments and incubation in a growth chamber at 27±1°C. To improve and raise *in vitro* vegetative multiplication capacities, different concentrations of growth regulators were added alone or in combination in a MS basal medium. Multiplication rate of 3.08 and 3.29 were obtained, respectively, for apex and cotyledonary nodes in a MS medium supplemented with BAP 0.5 mg.L^{-1} + TIBA 0.1 mg.L^{-1}. For axillary explants, a rate of 3.58 was recorded for the hormonal combination which consisted of BAP 0.5 mg.L^{-1} + TIBA 0.5 mg.L^{-1} + $AgNO_3$ 0.5 mg.L^{-1}. 41.66% of apex and 58.33% of cotyledonary explants were rooted after 24 h of induction treatment with IBA 2.5mg.L^{-1} whereas 41.66% of axillary bud explants developed roots after 48 h of induction with NAA 1mg.L^{-1}. During acclimatization achievement, survival rates were respectively 80% for apex and cotyledonary explants and 86.66% for axillary explants.**

Key words: *Parkia biglobosa*, scarification, germination, *in vitro* micropropagation, conservation, genetic resource.

INTRODUCTION

Parkia biglobosa, also called “néré”, “nété”, tree with flour, African caroubier (Kerharo and Adam, 1974), is the only species of *the Parkia* kind existing in Senegal. It is a significant leguminous plant characteristic of the landscapes soudano-guineens (Arbonnier, 2000). This tree belongs to the family of *Leguminosae-Mimosoideae*. Its seeds being processed after fermentation result in a condiment of high food value called "nététu" in Wolof or "soumbala" in Bambara; it is very snuffed in African kitchen and it is the subject of significant commercial transactions (Ndir et al., 2000). *P. biglobosa* is known for its potential in the improvement of agriculture (Okafor, 1980; Booth and Wickens, 1988; Sabiiti and Cobbina, 1992; Tomlinson et al., 1995). It is a species with many uses in the pharmacopeia and traditional medicine. In spite of its socio-economic importance, it belongs to the species that is being threatened of disappearance (Lykke, 2000). The natural regeneration of “néré” is weak and the actual poplar, ageing (Gijsbers et al., 1994). Indeed, in the natural environment, *P. biglobosa* multiplies primarily by way of sowing. The weakness of the natural regeneration of this species could be due to limiting factors related to seed. The abuse of seeds, the pasture and the bush fires reduce considerably the stock of seeds on the ground.

Thus, studies were undertaken on the germination of seeds and the potentialities with the vegetative multiplication *in vitro* of this species in order to preserve this phytogenetic forest resource. The major aim of this study was to develop an efficient germination and an *in vitro* regeneration system for *P. biglobosa* (Jacq.) Benth (Mimosaceae). This would make it possible to constantly layout homogeneous vitroplants capable of being distributed to producers or foresters after weaning and to renew the settlements.

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Figure 1a. Germination of seeds of *P. biglobosa* scarified to the H_2SO_4 (95%) during 5 hours, the cliché is achieved 15 days after setting in culture: all seeds germinated (G x 0.25).

MATERIALS AND METHODS

Test of viability

The seeds of *P. biglobosa*, coming from the South-west of Casamance (zone of Kandialang), were pulped and then preserved at ambient temperature in the laboratory. The evaluation of the topographic colouring of the constituent of seeds was carried out according to the protocol of Moore (1985). Batches of 50 seeds were peeled and then immersed in a solution of 1% 2, 3, 5-triphenyl tetrazolium chloride (TTC) for 9 h at 35ºC in the dark.

Germination

Batches of 50 seeds (20 days after harvest) were scarified by absolute H_2SO_4 for variable durations followed by 5 rinses with sterile distilled water. Ten to seven treatments are carried out in addition to the control. The seeds were disinfected in NaOCl, (8 ºCl) for 5 min, followed by 4 successive rinsing. Batches of 10 seeds were sown in mineral basic MS medium (0)/2 (Murashige and Skoog, 1962). The medium with pH 5.7 was solidified in agar $8gL^{-1}$ and then distributed in 5 bottles of culture/treatment, at a rate of 50 mL/bottle. The bottles were sterilized at 110ºC for 20 min. They were then incubated in the dark at 27 ±1ºC. Seeds germinated each day were counted for a period of 15 days. The radicular opening of the teguments was retained as criterion for germination (Côme, 1968). The curves of germination were established according to the treatments.

Micropropagation *in vitro*

Various types of 1-2 cm length explants (cotyledonary nodes, axillary nodes and apex) were taken on sterile sowings (Figure 1a). They were transferred individually out of tubes from cultures containing 20 ml of MS solid. Benzylamino purine (BAP) or kinetin of 0.5 - 1 $mg.L^{-1}$ combined with 1-naphthaleneacetic acid (NAA) (0.1-0.2 $mg.L^{-1}$) and/or with triiodobenzoic acid (TIBA) (0.1 - 5 $mg.L^{-1}$) was added to the medium. $AgNO_3$ was also used at a concentration of 0.5 - 1 $mg.L^{-1}$. For each type of explant, a total of 24 per medium was defined. The tubes were stored to culture at a room temperature of 27 ± 1ºC under a photoperiod of 16 h day and an incidental light of 101.4 µmoles.$m^{-2}.s^{-1}$. A measurement at 15 days and another measurement at 30 days of incubation were taken for all the treatments. The measured parameters were the number and length of the growths and the number of nodes.

Rooting

Shoots of the third subculture were induced in the dark in a MS/2 solid medium in which was added NAA or IBA at 1 - 5 $mg.L^{-1}$ for 1 to 5 days before being transferred to the light in the medium from expression MS(0)/2. For each type of explant, a total of 12 was used per duration and medium of induction. Measurements were made every 15 days to evaluate the parameters of rooting.

Acclimation

The weaning of the *in vitro* produced plants was carried out in mini-greenhouse hermetically closed to maintain them in an atmosphere with relative high moisture or in mini-greenhouse with adjustable opening (3 h per days during 15 days). The miniplants were transplanted in pots containing a substrate made up of a sterile mixture of sand and compost (v/v) to which one adds carbofuran and lannate 90. A total of 10 to 15 plants was used for each treatment. The rate of survival was given after 30 days.

Statistical analysis

The treatments were differentiated by multiple comparison of the averages after variance analysis followed by the Student's t-test, Newman and Keuls with the threshold of probability of 5% (software SPSS 10.1).

RESULTS

Viability test and germination

The test with tetrazolium chloride was 100% positive (Figure 1b). All batches of 50 treated seeds belonged to group 1 of the scale of Moore. Therefore, these seeds were able to germinate when they were subjected to favourable conditions. They thus seem viable and constitute seeds with very high probability of germination. For the control batch, no seed germination (Figure 5) was recorded. As for the other treatments, at the 1st day, there was a rate germination of 76% for the treatment of 4 h sulphuric acid and 92% for the treatments of 5 and 6 h. At the 2nd day, 100% of germination was reached for the treatments of 5 and 6 h. However, this rate fluctuates between 6 and 12% for the treatments of 15 and 45 min.

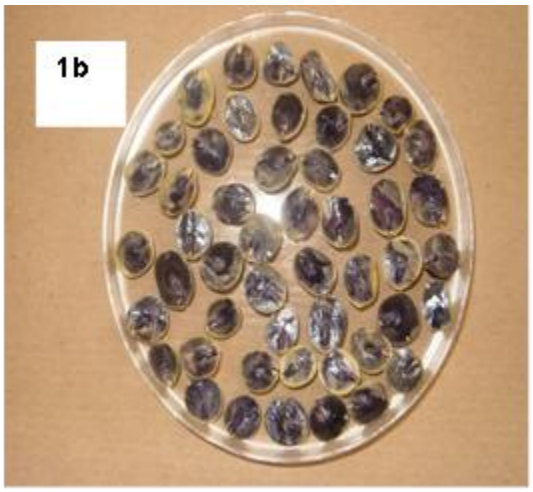

Figure 1b. Seeds of *P. biglobosa* treated to the 1% tetrazolium chloride (TTC) (G x 0.28).

Figure 1c. Influence of the medium MS + BAP 0.5 mg.L^{-1} + TIBA 0.1 mg.L^{-1} on the budding of cotyledonary explant of *P. biglobosa* after 30 days of the first subculture (G x 0.56).

On the 15th day, the rates of germination extended from 48 to 96% for the treatments of 1 to 60 min.

Micropropagation

The analysis of variance revealed for apical explants a significant influence of the nature of the medium on the average number of shoots (F = 55.89; p = 0.000) (Figure 2) and their elongation (F = 286.62; p = 0.000) (Figure 3). It also revealed a significant medium effect on the average length of shoots (F = 6.395; p = 0.001) (Figure 3), the average number of nodes (F = 9.275; p = 0.000) (Figure 4) and the average number of shoots (F = 20.742; p = 0.000) (Figure 2) of the cotyledonary explants. A ratio of intensification of 3.08 in the medium MS + BAP 0.5 mg.L $^{-1}$ + TIBA 0.1 mg.L $^{-1}$ (Figure 2) was distinguished for the apex. This average value was significantly different (p = 0.000) from those of the other media. For the cotyledonary nodes, the greatest average number of growths (that is, number of shoots (3.29)) was obtained in the same medium (Figures 2 and 1c). Nevertheless, the basal appeared in these media. A fall of leaves after 20 days of culture was also detected. For the axillary nodes, the greatest average number of growths (3.58) was obtained in the medium MS + BAP 0.5 mg.L^{-1} + $AgNO_3$ 0.5 mg.L^{-1} + TIBA 0.5 mg.L^{-1} (Figure 1d). The comparison of the averages revealed a significant influence of this medium, compared to the others, that is, MS + $AgNO_3$ 0.5 + TIBA 1 mg.L^{-1} (F = 24.221; p = 0.000); MS + BAP 0.5 mg.L^{-1} (p = 0.005); MS + BAP 0.5 mg.L^{-1} + $AgNO_3$ 0.5 (p = 0.032) with regard to the average number of shoots. An average length of shoot of 5.59 cm was significantly different from the others (F = 31.516; p = 0.000) and an average number of nodes of 5.17 of significant difference (F = 22.429; p = 0.000) was also obtained.

With regard to the comparative study of the influence of the BAP and Kinetin, the statistical analysis showed that the average number of shoots resulting from the axillary nodes (1.875) in the medium MS + BAP 1 mg.L^{-1} has significant difference (F = 28.414; p = 0.000) compared to that of the other media added with kinetin. This medium (MS + BAP 1 mg.L $^{-1}$) gave the cotyledonary nodes an average number of shoots of 2.083 and an average length of shoots of 2.725 cm. All these were statistically compared to the media containing the kinetin.

Rooting

A rate rooting of 41.66%, an average number of roots of 2 and an average length of roots of 1.48 cm were obtained for the apex after an induction rhizogene of 24 h in the presence of IBA 2.5 mg.L^{-1} (Table 1). A rate rooting of 58.33%, an average number of roots of 1.43 that was not significantly different (F = 27.523; p = 0.864) from that obtained after 24 h of induction in the medium MS/2 + IBA 5 mg.L $^{-1}$ (Figure 1e) and an average length of roots

Figure 1d. Influence of the medium MS + BAP 0.5 mg.L^{-1} + TIBA 0.5 mg.L^{-1} + $AgNO_3$ 0.5 mg.L^{-1} on the budding of axillary explant of *P. biglobosa* after 30 days of a first subculture (G x 0.52).

Figure 1e. Influence of the induction time (24 hs) to the IBA 5 mg.L^{-1} on the elongation and the ramification of the roots of cotyledonary shoot of *P. biglobosa* after 30 days of incubation (G x 0.80).

of 1.91 cm were recorded for the cotyledonary nodes after an induction rhizogene of 24 h in the presence of IBA 2.5 mg.L $^{-1}$ (Table 1). The formation of roots was preceded by a basal callus whose size depended not only on the hormonal concentration of the medium but also on the duration of induction. At the cotyledonary nodes, the roots had several side ramifications. As for the axillary nodes, a rate rooting of 41.66% was calculated for MS/2 + NAA 1 mg.L^{-1} after 48 h of induction.

Acclimatization

When the *in vitro* produced plants were mended in a mini-greenhouse whose shutter was completely closed, it was observed that it began to rot at the 4th day. At the 6th day, the death rates were 40% for the apex, 10% for the axillary nodes and 30% for the cotyledonary nodes. At the 9th day, the death rate of all explants was 100%. On the other hand, when the mini-greenhouse was gradually opened, there was a rate survival of 80% for the newly formed miniplantes stemming from the apex (Figure 1f) and from the cotyledonary nodes (Figure 1g) and of 86.66% for those resulting from the axillary nodes (Figure 1h) at the 30th day.

Figure 1f. Acclimatization of vitroplants stemming from apex of *P. biglobosa* (30 days) (G x 0.15).

Figure 1g. Acclimatization of vitroplants stemming from cotyledonary node of *P. biglobosa* (30 days) (G x 0.14).

Figure 1h. Acclimatization of vitroplants stemming from axillary node of *P. biglobosa* (30 days) (G x 0.20).

DISCUSSION

There was no germination in the control batch at the end of the 15 days of experimentation. The result got confirmed that of Aliero (2004) on seeds of the same species. The same finding has been reported by Ayisire et al. (2009) on *P. thonningii*. The absence of germination could be due to either non-viability of the seeds or to an inaptitude for germination. The first cause was isolated since the test with tetrazolium chloride appeared positive at 100%. Obtaining the rates of germination of 100% at the end of 2 days for the batches of seeds of *P. biglobosa* treated with the sulphuric acid during 5 and 6 h confirmed the existence of a tegumentary inhibition (Côme, 1970). These high rates agreed with the results of the test of viability of these seeds. Indeed, the immersion of seeds in the concentrated sulphuric acid is generally very effective to raise the tegumentary seed dormancy of woody species (Tybirk, 1991). The sulphuric acid acts by progressive corrosion of the external tegument and increased its permeability to air and water and thus supporting the inhibition of seed and the normal course of the process of germination. Concentrated sulphuric acid has been used successfully to hasten the germination of several seed species (Bensaid, 1991; Todd-Bockarie and Duryea, 1993; Muhammad and Amusa, 2003; Ayisire et al., 2009). The seeds treatment with concentrated sulphuric acid (H_2SO_4) (95%) for the 5 and 6 h made it possible to have the best rate of germination (100%) in 2 days with a latency time of 1 day when a germination of 92% was obtained. This same rate had been obtained by Dione (2001) in 5 days on seeds of *Detarium microcarpum* treated with concentrated sulphuric acid, but at a shorter duration (1 h). The rate of germination (50%) obtained after 3 min of treatment constitutes the best rate that could be obtained (Aliero, 2004) on seeds of the same species. However, the last had obtained lower rates for a pre-treatment of 5 min. Recently, seed dormancy of *P. thonningii* was successfully broken by chemical scarification using concentrated sulphuric acid for 15 min with 95% germination obtained in 6 days of germination (Ayisire et al., 2009). However, the 100% of germination at 5-6 h contrasted with the 0% obtained by Ayisire et al. (2009) at 25 min of treatment. It was probably due to the hardness of coat between the two kinds of seed (*P. biglobosa* and *P. thonningii*) and the use of concentrated H_2SO_4 for more than 15 min, especially for 25 min, which had a lethal effect on the embryos of the seeds of *P. thonningii* as mentioned by Ayisire et al. (2009). Therefore, the treatment of 5 h seems to be best appropriate since the objective was to obtain the shortest duration of treatment which would give the best rate of germination.

The neo-formation of shoots was done on the level of the zone of insertion of the cotyledons and the leaves, that is, on the level of the nodes. The formation of callus at the base of *in vitro* cultivated explants was studied by Saini and Jaiwal (2000) on *P. Harmala*; Martin (2000) on *Holostemma ada-kodien*; Ndoye et al. (2003) on *Balanites aegyptiaca* and recently Ayisire et al. (2009) in *P. thonningii*. According to Preece et al. (1991), it was frequently observed on species with marked apical predominance. It would be due to the accumulation of auxin at the base of the explants (Marks and Simpson, 1994). This auxin in the presence of cytokinin stimulated the proliferation of the cells located at the level of the zone wound of the explant. However, the callus was induced by 2,4-D and not by NAA. Amoo and Ayisire (2005) suggested that cotyledon explants of *P. biglobosa* were auxin specific.

The rooting of the apexes in the MS (0) medium seemed to confirm these facts and would suggest high endogenous auxinic contents. The presence of callus constitutes, however, a limiting factor. Indeed, after one month of culture, tanning (disorganization of cell tissues) of the callus which gives a bad surface of contact of explant with the culture medium was observed on certain

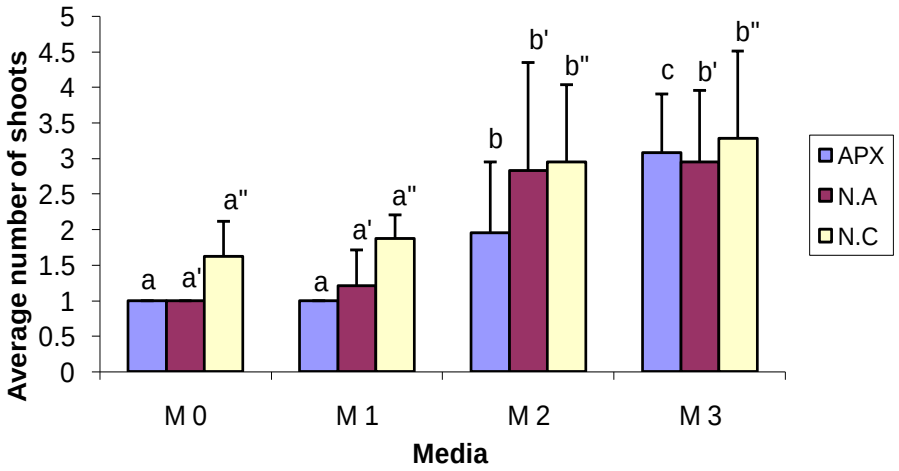

Figure 2. Effect of BAP (0.5 mg.L^{-1}) and/or TIBA (0.1 mg.L^{-1}) on shoot regeneration from different explants of *P. biglobosa* (The bars with same letter were not statistically different from the doorstep of 5% of the test of Newman-Keuls (SPSS 10.1)).

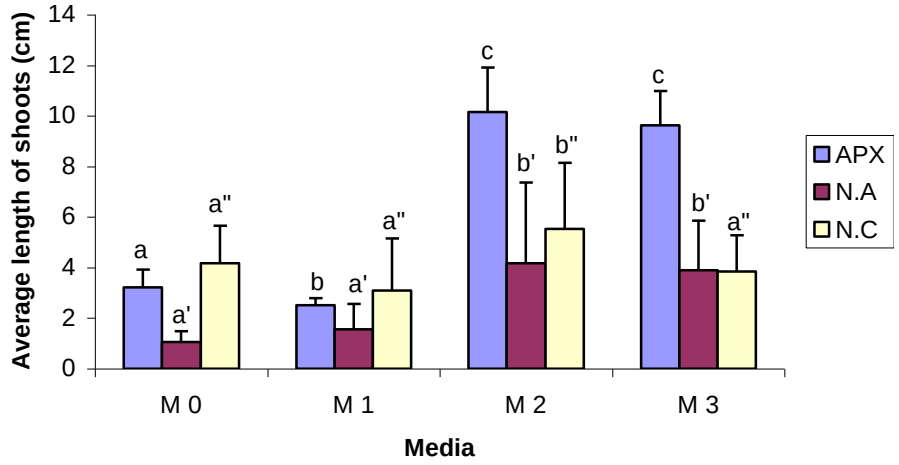

Figure 3. Effect of BAP (0.5 mg.L^{-1}) and/or TIBA (0.1 mg.L^{-1}) on shoot elongation in *P. biglobosa*. (The bars with same letter are not statistically different from the doorstep of 5% of the test of Newman-Keuls (SPSS 10.1)).

explants. This seems in this case to slow down its growth.

The increase in the concentration of BAP was accompanied by a reduction in the elongation of the shoots, which seemed to confirm the observations of Banerjee and De Lanche (1985) on *Musa*, Barghchi (1987) on *Robinia pseudoacacia* (*L*.). Indeed, Belaizi et al. (1994) had noticed on *Ceratonia siliqua L*. that the concentrations in BAP higher than 0.5mg.L $^{-1}$ caused a strong callogenesis and a vitrification of the explants. The concentrations of TIBA (2, 3 and 5 mgL $^{-1}$) seem to be more effective in the reduction of the rate and the diameter of the callus. The TIBA 5 mgL $^{-1}$ had prevented the formation of a basal callus (0%) from the axillaries and cotyledonary nodes in a medium supplemented in BAP. However, these concentrations seem to act unfavourably in the neo-formation and the elongation of the shoots. This confirmed the observations of Vissenberg et al. (2001) who showed that the TIBA with certain concentration interferes with cellular elongation. Indeed, the TIBA is known to inhibit the formation of shoot and foliar root of explant of *Nicotiana tabacum* while interfering with the endogenous auxines at the time of their implications in the organization of the cellular division (Dhaliwal et al., 2004). The concentrations of TIBA 0.1; 0.5 and 1 mg.L $^{-1}$, though do not have enough remarkable effects to reduce the rate and diameter of the callus, seem more effective to improve the neo-formation and

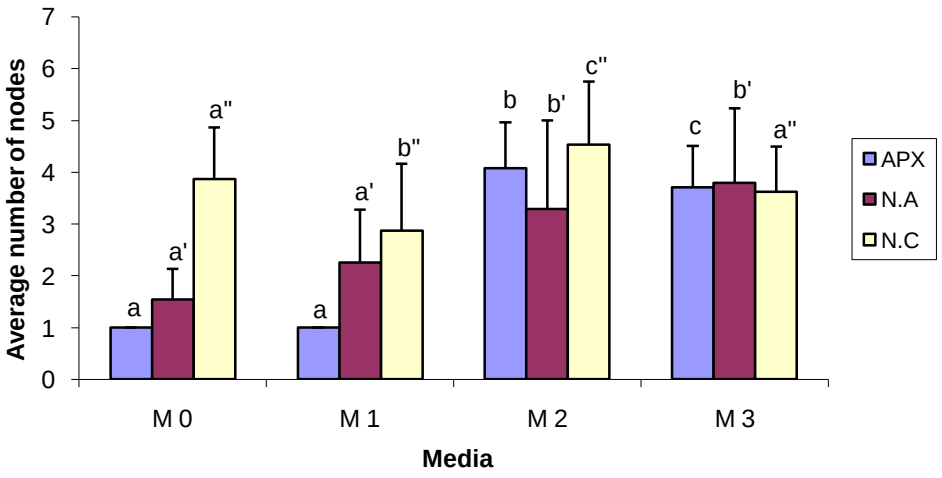

Figure 4. Effect of BAP (0.5 mg.L^{-1}) and/or TIBA (0.1 mg.L^{-1}) on nodes proliferation in shoots developed from different explants of *P. biglobosa*. (The bars with same letter were not statisticallydifferent from the doorstep of 5% of the test of Newman-Keuls (SPSS 10.1))
Each value corresponds to the average of at least 24 repetitions after 30 days of culture (1st subculture). APX = apex; NA = axillary node; NC = cotyledonary node; M 0 = MS (0); M 1 = MS + TIBA 0.1 mg.L^{-1}; M 2 = MS + BAP 0.5 mg.L^{-1}; M 3 = MS + BAP 0.5 mg.L^{-1} + TIBA 0.1 mg.L^{-1}.

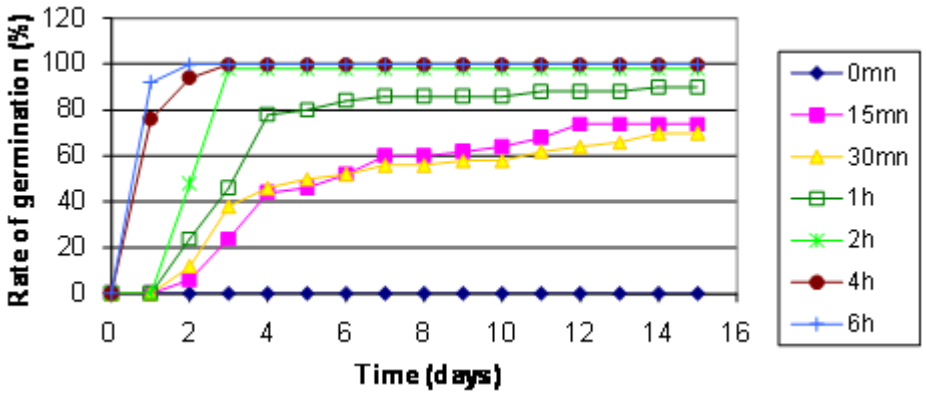

Figure 5. Effect of different times of scarification treatments with H_2SO_4 on seed germination of *P. biglobosa.* *Batches of 50 seeds have been used for each treatment, corresponding to 5 repeat per treatment. Seeds germinated carried out during 15 days.

the elongation of the shoots. This is probably by modifying the balance of auxin/cytokinin in favour of the cytokinin which improved and increased the development of shoots (Skoog, 1957). Indeed, Kavyashree (2007) found that Linsmaier and Skoog's basal medium (LSBM) fortified with the combination TIBA and BAP was the most suitable medium for initiation and multiplication of shoots of mulberry compared to other concentrations tried.

The silver nitrate concentrations (0.5 and 1 mgL^{-1}) did not seem to be effective to fight against the fall of the leaves contrary to silver thiosulfate (0.5 mg.L^{-1}) used, for this purpose, by Lemos and Blake (1996) on *Annona squamosa*. In this experiment, the best media for the multiplication of the apex and the cotyledonary nodes seem to be the MS enriched in BAP 0.5 mg.L^{-1} and TIBA 0.1 mg.L^{-1}; with respective ratios of intensification of 3.083 and 3.29. On the other hand, for the multiplication of the axillary nodes, the best ratio of intensification (3.58) was obtained in medium MS supplemented with TIBA 0.1 mg.L^{-1} + BAP 0.5 mg.L^{-1} + $AgNO_3$ 0.5 mg.L^{-1}. These media, in addition to the medium MS + BAP 0.5 mg.L^{-1} also gave the best elongations of shoots.

The BAP seemed to be more effective than the kinetin for the regeneration and the elongation of the shoots. The same report was made by Ndoye et al. (2003) on *Balanites aegyptiaca* and Gokhale and Bansal (2009) in *Oroxylum indicum* (L.) Vent. The superiority of BAP to

Table 1. "Effect of different media and induction periods on root development from shoots of *P. biglobosa* after 30 days of culture.

Media	Induction time (days)	Explants type	Rooting rate (%)	Average root number	Average root length (cm)
MS (0)	0	APX	0	0b	0b
		NA	0	0c	0b
		NC	0	0c	0b
NAA 2.5 mg.L^{-1}	1	APX	0	0b	0b
		NA	25	1.333a	1.7a
		NC	0	0c	0b
	3	APX	0	0b	0b
		NA	0	0c	0b
		NC	0	0c	0b
NAA 5 mg.L^{-1}	1	APX	8.33	2a	1.5a
		NA	0	0c	0b
		NC	0	0c	0b
	3	APX	0	0b	0b
		NA	8.33	2a	1.5a
		NC	0	0c	0b
IBA 2.5 mg.L^{-1}	1	APX	41.66	2a	1.48a
		NA	16.66	1.5a	0.85a
		NC	58.33	1.428a	1.914a
	3	APX	0	0b	0b
		NA	8.33	4a	2.7a
		NC	0	0c	0b
IBA 5 mg.L^{-1}	1	APX	16.66	1.5a	1.5a
		NA	0	0c	0b
		NC	16.66	1.5a	2.3a
	3	APX	0	0b	0b
		NA	0	0c	0b
		NC	25	4b	2.666a

APX = apex; NA = axillary node; NC= cotyledonary node.
In the last two columns: for a same type of explant, the numbers with same letter were not statistically different to the threshold of 5% of the test of Newman-Keuls (SPSS 10.1).

kinetin has been reported for shoot bud initiation (Satyanarayan et al., 2008). On the other hand, in other studies, kinetin supported primarily the elongation of the buds. This has been shown by Chandra and Pal (1995) on *Vigna radiata* and Danthu and Bhojwani (1992) on *Gladiolus*. Induced calli from cotyledon explants of *P. biglobosa* (Jacq.) Benth turned friable, more nodular and with small protuberances on media containing 0.8 mg/L kinetin combined with either 0.2 or 0.6 mg/L 2,4-D (Amoo and Ayisire, 2005).

After one month of culture, a rate rooting of 8.33% was observed on apical explants, in the MS medium deprived of hormone.

No root was observed in the medium of expression after inductions of rhizogenes for 4 and 5 days; on the other hand significant callogenesis was detected. Therefore, these durations of induction were ineffective and even harmful in the explant rooting of *P. biglobosa*. The consistency of the medium of rooting also seemed to strongly influence the appearance of callus at the base of the explants. Indeed, Zimmerman and Brome (1980) noted that the rooting can be obtained in a solid medium or for better results, in a liquid medium. Villegas (1992) used the rootstock M106, a solid and liquid medium with paper as support and obtained an excessive development of callosity with 40% of rooting in the solid medium while the use of the liquid medium increased the rate of rooting to 80% and generated a notable reduction of the development of the callus. This significant difference would be explained by the fact that the nutritive elements would be more easily available to explants (Hammerschlag, 1982). Moreover, the agar would create a critical pressure of turgescence which puts the cells in situation of stress. This stress would induce the production

of ethylene whose concentration would tend to increase in containment that would also stimulate the production of auxin.

The best rates of rooting were obtained on the microplants resulting from the apex (41.66%) and the cotyledonary nodes (58.33%) after an induction rhizogene of 24 h in the presence of IBA 2.5 mg.L $^{-1}$. It is the same for those formed starting from the axillaries nodes (41.66%) after an induction rhizogene of 48 h with NAA (1 mg.L $^{-1}$). The best root elongations were obtained after an induction with the IBA. This hormone has always been a potential auxin that induces rooting in *in vitro* regenerated shoots (Iriondo et al., 1995; Rajore and Batra, 2005; Rajeswari and Paliwal, 2008).

Therefore, the rooting at *P. biglobosa* is dependent on the hormonal treatment and the IBA seems to be better than the NAA to induce rooting. Indeed, Sané et al. (2001) had obtained a rate rooting (80%) on *Acacia tortilis subsp. raddiana* after a treatment with IBA compared to the NAA. Altaf (2006) in Kinnow tree, Gokhale and Bansal (2009) in *Oroxylum indicum* (L.) obtained the rooting of the developed shoots in half strength MS medium with addition of IBA 2 mg/l and 4.92 µM respectively. On the other hand, the NAA is more favorable to the rooting of vitroplants of Acacia *senegal* (*L*.) (Badji et al., 1991), *Lotus alpinus* (Laberche et al., 1995) and *Gleditsia triacanthos L*. (Basbaa et al., 1993). The roots, in general, were more developed in the cotyledonary explants where more side ramifications were observed. Monteuuis and Bon (1985) could not exceed 50% of rooting at the giant *Sequoia*. This could be explained, according to Chèvre (1985), by the fact that the rooting is often more difficult at the ligneous family than at the herbaceous plants.

The results showed that the rate of survival varied according to the technique of acclimatization used. During the first test, the mini-greenhouse was completely close and all the acclimatized plants died at the end of 9 days. These *in vitro* propagated plants developed in the presence of increased moisture; their leaves had cells palisades of which some had great spaces between them and few stomates (Brainerd et al., 1981). This atypical morphology caused a greater sensitivity to water losses (Esau, 1977) and a susceptibility particular to the pathogenic attacks. These leaves have very little or no protective waxes in the cuticle that made them more prone to dehydration (Grout and Aston, 1977).

Indeed, excessive containment and the high temperature asphyxiate the young plants in an atmosphere saturated with moisture and this favoured the development of pathogenic micro-organisms. According to Mapes et al. (*In* Ndoye, 2004), the plants resulting from *in vitro* culture had a finer cuticle in general than that of the mothers plants, which caused their fast desiccation when the relative humidity was lowered by passage in greenhouse. Because of these characteristics, the control of the process of adjustment of *the in vitro* produced plants was necessary to limit the water loss and to have a good level of survival after recovery (Carreto, 1992). However, the acclimatization in a mini-greenhouse followed by a progressive contact with the ambient conditions made it possible to obtain the best rates of survival. Similar results were recorded by Belaizi et al. (1994) on microplantes of Caroubier (*Ceratonia siliqua L*.) that gradually acclimatized to the surrounding air. The substrate was also important for plant acclimatization. Rajeswari and Paliwal (2008) obtained the best rate (75%) of survival of the plant *Albizia odoratissima* L.f. (Benth.) after acclimatization on vermiculite substrate, while Ndiaye et al. (2006) reported 100% of survived plantlets of *Bambusa vulgaris* after acclimatization in pots containing sterile perlite-peat. The pre-treatment of seeds was essential for good germination. It was probably about tegumentary inhibition which was raised by scarification with the sulphuric acid, for 5 h. The callogenesis in the medium enriched in BAP revealed the existence of high endogenous auxinic contents. The TIBA with concentrations from 0.1 to 1 mg.L $^{-1}$ decreased appreciably the callogenesis while improving the rates of multiplication. The BAP seemed to be more effective than the kinetin for the neo-formation and the elongation of the shoots. The IBA induced the vitroplants rooting better than the NAA. The acclimatization in mini-greenhouse followed by a progressive contact with the ambient conditions made it possible to obtain the best rates of survival.

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