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Full Length Research Paper

In vitro regeneration of *Acacia nilotica* from nodes on MS medium

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In the regeneration of *Acacia nilotica,* the effect of cytokinin (BA) and auxin (IBA) in factorial experimentation on the MS medium was determined with different dosages each. Starting from the epicotyls of germinated seeds, a combination of benzylaminopurin, naphtaleneacetic acid and gibberelic acid (BA*NAA*GA3) served as a good initiator of adventitious buds. However, the best production of nodes was obtained with BA for 0.1 mgl^{-1} dosage. The rate of node production was equal to 6.767%, while the longest stem was 53.33 mm. The best and high rate of rooting was 93.33 and 83.33%, respectively with 0.1 and 1.0 mg/l of IBA. The interaction between the two hormones had a negative effect on the growth and rooting of the shoots, whilst high dosage had a shortening effect on the nodes.

Key words: Acacia nilotica, regeneration, microcutting, rooting, growth, cotyledonary node.

INTRODUCTION

Acacia nilotica is a multipurpose leguminous tree of arid and semi arid zones. This species has a great nitrogen fixation potential. That is why it is used in banks protection and soil restoration (Faye et al., 2007; Kiran et al., 2009). Moreover, it is generally considered as a source of tannin, timber and Arabic gum, while for the rural populations as an important source of income. According to Arbonnier (2002) also, it is used in tanning leather and skins in fruit pods.

Nilotica which is a styptic and astringent would be used for medicinal purposes, as a demulcent or relieve pain for the following diseases: Gonorrhea, leucorrhoea, diarrhea, dysentery or diabetes. In traditional medicine, the gum is used to consolidate watery semen (Mahesh and Satish, 2008).

Kiran et al. (2009) have demonstrated its capacity to resist, both to temperatures above 50°C and to air dryness, but very sensitive to freezing especially in it juvenile period. The extreme hardness of the seed coat makes the germination process difficult, so 95% of seeds do not germinate. The seeding was decimated by bush fire and or perpetual passage of animals. The seeds can conserve their germinating power after a period of 15 years, the limit and the insufficiency of its natural regeneration and propagation. The population of this specie was considerably reduced because of growing urbanization and excessive cutting for firewood. *A. nilotica* can play an important ecological role because of its relatively rapid growth rate and biological fixation potential, making it useful in the restoration of degraded soils (Toky et al., 1992). Unlike seeds, plantlets regenerated *in vitro* had rapid growth and can be produced in wide quantities defying the constraints of season. The applicability *in vitro* culture of this specie is a great opportunity for rapid organogenesis under the influence of different combinations of growth regulators.

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Abbreviations: BA, Benzylaminopurin; IBA, indol-3-butyric acid; NAA, naphtalenacetic acid; GA, gibberelic acid.

This study aimed to develop a protocol for reproducible in vitro production of plants well-rooted tree with multiple uses

MATERIALS AND METHODS

Plant materials and sowing conditions

Some mature seeds of *A. nilotica* were used as explants. Firstly, seeds were scarified by opening the skin. Then, seeds surface were sterilized with 2% (w/v) sodium hypochlorite for 20 min followed by 70% (v/v) ethanol for 1 min. The seeds were also rinsed five times, in sterile distilled water. Seeds so treated were sown on solidified Murashige and Skoog (MS) (1962) medium readably adjusted to pH 6.30 before autoclaving at 121°C for 20 min (after autoclaving pH will be 5.7). All the plant and materials were incubated at 25±1°C under 16 h in light by day photoperiod.

Buds initiation

The epicotyls about one month old seedlings were sliced into nodes of 0.5 to 1 cm. They sub-cultured in different initiation media composed of the basic medium MS supplemented with different dosages of NAA (0.0; 0.1 mgl^{-1}; 0.5 and 1.0 mgl^{-1}), BA (1.5 mgl^{-1}) and GA (1.5 mgl^{-1}). All media contain 30 $g.l^{-1}$ of sugar and 6 $g.l^{-1}$ of agar. For the four combinations, the cells were proliferated and nodes were swollen with a whitish green color similar to callus formation. After three weeks of incubation, the swollen nodes like callus were transferred on MS medium without hormone for two weeks. Transferred nodes have initiated adventitious buds. The regenerated shoots were excised at the base into nodes of 1 cm after a growth of two weeks.

Proliferation and rooting

The nodes were obtained after two weeks bearing a leaf and an apical bud; which were used to assess the effect of two growth regulators BA and IBA in a complex factorial experiment. The choice of this node type is justified by his youth and nearly zero rate of browning. In this factorial experiment, the MS medium was enriched with different dosages of BA (0.0, 0.1, 0.5 and 1.0 at 1.5 $mg.l^{-1}$) and IBA (0.0, 0.1, 0.5 and 1.0 at 1.5 $mg.l^{-1}$), thus, 25 treatments carried out in a randomization mode. Thirty shoots were cultured in the different media mentioned earlier. Thirty (30) tubes containing a node each were tested with whole combination. The number of busted buds, the average size of stems formed and the number of rooted plants were recorded for each medium after thirty days (Tables 2 and 3). Note that after producing a sufficient number of apical nodes, test factorial was installed one day for thirty days of incubation. A margin of five test tubes (5 nodes) by additional treatment was added to prevent possible contaminations. The data collected were statistically analyzed using SPSS16. inc. Excel software was used to plot histogram graphics.

RESULTS

Buds initiation

The presence of NAA in the MS medium has the benefit to stimulate buds initiation. These buds present an enlarged meristematic dome and were surrounded by leaf primordia widened. The same domes were developed into shoot primordial, which became shoots later. The buds emerged after two weeks of culture. 0.1 mg of NAA induced the best rate of buds with an average of 8 buds during the first two of harvests and 4 buds in the third of harvest. Using 1 $g.l^{-1}$ of polyvinylpyrrolidone (pvp), the rate of browning was almost zero.

Proliferation and rooting

The variance results analysis show that using only the cytokinin (BA) and auxin (IBA) with low dosage produces a highly significant effect on nodes production (Table 1). The effect was also significant on the internodes height and the percentage of plants bearing at least one root. The greatest production of nodes was observed at 0.1 $mg.l^{-1}$ with an average of 6.77 nodes per plant (Figure 1). The average size of the highest (53.33 mm) was obtained on treatment (0.1 $mg.l^{-1}$ BA without auxin). High dosages (1.0 and 1.5 $mg.l^{-1}$) did not only produce few nodes but also a short internodes (Figures 1 and 2).

The rooting was obtained only by using auxin (IBA). The best dosages were 0.1 and 1.0 $mg.l^{-1}$.These dosage induced respectively, 93.33 and 83.33% of rooted plants for a total of 30 plants per treatment (Figure 3). Long roots were obtained with a dosage of 0.1 $mg.l^{-1}$, while the most thick and robust roots (Figure 4) was obtained with a dosage of 1.0 $mg.l^{-1}$. The rooted plants with a dosage of 1.5 $mg.l^{-1}$ were the shortest. Also, for the simple effects of the studied factor, the analysis of variance revealed a highly significant effect of the interaction of auxin* cytokinin on nodes production (Figure 1), the plants height (Figure 2) and plants rooting (Figure 3). In addition, the combination of these hormones is not favorable to *A. nilotica* growth on the MS medium. This was noticed with the dosages of 1.0 and 1.5 $mg.l^{-1}$. In general, a dosage of 0.1 mg/l of BA combined with different dosages of IBA produced the best results in terms of number of nodes.

Acclimatization and transplantation

The rooted plants were acclimated for two weeks and then transplanted into pots containing soil in the greenhouse. The rate of recovery after 30 days of transplantation was 100% (Table 3).

DISCUSSION

This study enables us to notice that the initiation of adventitious bud is observable when combining 0.1 mg NAA*1.5 mg BA*1.5 mg GA. Furthermore, they revealed the effects of shortening of internodes and slower growth for high doses of 1.0 and 1.5 mg.l-1BA. Sometimes swelling of the base nodes on MS medium was remarked.

Table 1. Interaction of IBA*BA on nodes number.

Dependent variable	IBA (mg/l)	BA	Mean	Standard error	95% confidence interval	
					Lower bound	Upper bound
Nodes number	0.0	0.0	5.133	0.121	4.896	5.371
		0.1	6.767	0.121	6.529	7.004
		0.5	3.800	0.121	3.562	4.038
		1.0	2.533	0.121	2.296	2.771
		1.5	3.000	0.121	2.762	3.238
	0.1	0.0	4.367	0.121	4.129	4.604
		0.1	5.767	0.121	5.529	6.004
		0.5	5.200	0.121	4.962	5.438
		1.0	2.567	0.121	2.329	2.804
		1.5	2.033	0.121	1.796	2.271
	0.5	0.0	4.500	0.121	4.262	4.738
		0.1	1.233	0.121	.996	1.471
		0.5	1.033	0.121	.796	1.271
		1.0	1.667	0.121	1.429	1.904
		1.5	1.467	0.121	1.229	1.704
	1.0	0.0	4.700	0.121	4.462	4.938
		0.1	5.133	0.121	4.896	5.371
		0.5	4.400	0.121	4.162	4.638
		1.0	1.733	0.121	1.496	1.971
		1.5	1.433	0.121	1.196	1.671
	1.5	0.0	4.267	0.121	4.029	4.504
		0.1	5.267	0.121	5.029	5.504
		0.5	2.533	0.121	2.296	2.771
		1.0	2.167	0.121	1.929	2.404
		1.5	1.367	0.121	1.129	1.604

Table 2. Interaction of IBA*BA on stem length (mm).

Dependent variable	IBA (mg/l)	BA	Mean	Standard error	95% confidence interval	
					Lower bound	Upper bound
Stem length (mm)	0.0	0.0	40.167	1.006	38.191	42.142
		0.1	53.333	1.006	51.358	55.309
		0.5	17.900	1.006	15.924	19.876
		1.0	12.233	1.006	10.258	14.209
		1.5	13.233	1.006	11.258	15.209
	0.1	0.0	26.867	1.006	24.891	28.842
		0.1	39.867	1.006	37.891	41.842
		0.5	27.167	1.006	25.191	29.142
		1.0	13.700	1.006	11.724	15.676

Table 2. Contd.

		1.5	12.133	1.006	10.158	14.109
		0.0	29.800	1.006	27.824	31.776
		0.1	11.200	1.006	9.224	13.176
	0.5	0.5	11.033	1.006	9.058	13.009
		1.0	12.167	1.006	10.191	14.142
		1.5	11.433	1.006	9.458	13.409
		0.0	35.267	1.006	33.291	37.242
		0.1	34.633	1.006	32.658	36.609
	1.0	0.5	27.400	1.006	25.424	29.376
		1.0	13.900	1.006	11.924	15.876
		1.5	12.900	1.006	10.924	14.876
		0.0	27.367	1.006	25.391	29.342
		0.1	32.967	1.006	30.991	34.942
	1.5	0.5	17.533	1.006	15.558	19.509
		1.0	11.733	1.006	9.758	13.709
		1.5	11.767	1.006	9.791	13.742

Table 3. Interaction of IBA*BA on rooted plants.

Dependent variable	IBA (mg/l)	BA	Nbr rooted plants	Mean	Standard error	95% confidence interval	
						Lower bound	upper bound
Rooted plants	0.0	0.0	13	.433	0.036	0.363	0.504
		0.1	00	1.500E-17	0.036	-0.070	0.070
		0.5	00	1.500E-17	0.036	-0.070	0.070
		1.0	00	1.500E-17	0.036	-0.070	0.070
		1.5	00	-2.290E-17	0.036	-0.070	0.070
	0.1	0.0	28	0.933	0.036	0.863	1.004
		0.1	00	3.741E-18	0.036	-0.070	0.070
		0.5	00	1.114E-17	0.036	-0.070	0.070
		1.0	00	1.114E-17	0.036	-0.070	0.070
		1.5	00	-2.429E-17	0.036	-0.070	0.070
	0.5	0.0	18	0.600	0.036	0.530	0.670
		0.1	00	-2.559E-17	0.036	-0.070	0.070
		0.5	00	-2.620E-17	0.036	-0.070	0.070
		1.0	00	-2.620E-17	0.036	-0.070	0.070
		1.5	00	7.147E-17	0.036	-0.070	0.070
	1.0	0.0	25	0.833	0.036	0.763	0.904
		0.1	00	-2.313E-17	0.036	-0.070	0.070
		0.5	00	-1.542E-17	0.036	-0.070	0.070
		1.0	00	-1.542E-17	0.036	-0.070	0.070
		1.5	00	1.898E-16	0.036	-0.070	0.070

Table 3.Contd

		0.0	18	0.600	0.036	0.530	0.670
		0.1	00	-6.680E-17	0.036	-0.070	0.070
	1.5	0.5	00	-8.130E-17	0.036	-0.070	0.070
		1.0	00	-8.130E-17	0.036	-0.070	0.070
		1.5	00	1.905E-16	0.036	-0.070	0.070

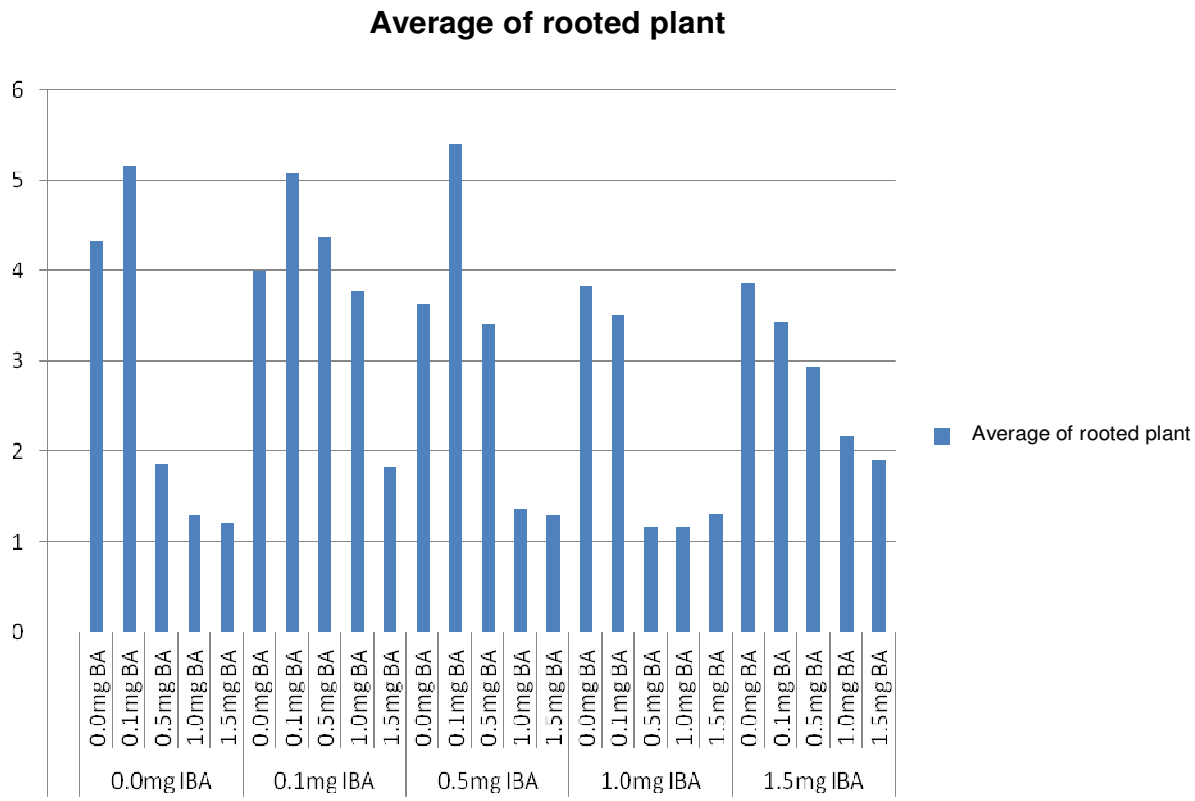


Figure 1. Average of nodes number after 30 days of incubation on MS medium.

Previous studies have got the same results. The observations made in these studies are focused on the number of shoots produced and not the number of nodes obtained per plant. Mathur and Chandra (1983) observed the development of multiple shoots from nodal explants of *A. nilotica* on auxin (0.5 to 1.0 mg.l^{-1} IAA) container. Dewan et al. (1992) research showed that a higher number of shoots (6.3 per nodal explants) are observed on B5 medium supplemented with 1.5 mg.l^{-1} BAP after 2 to 3 months.

Using other species of *Acacia*, plant regeneration was achieved by Vengadesan et al. (2000) in that case; the BAP combined with IAA was effective in inducing adventitious shoot regeneration. According to Haider et al. (2010) more (2.80) of root was recorded in the presence of 3.0 mg.l^{-1} IAA, whereas the IBA at the same concentration did not produce more than 1.20 roots per explant like our results. Rout et al. (2008) observed the highest number of shoots (4.21) from nodal cuttings of seedlings of *Acacia chuandra*, formally cultured on MS medium containing 1.5 mg/l BAP *0.05 mg/l IAA. However, Sascha et al. (1998) also obtained on MS medium with 2 mg.l^{-1} BA 85% of shoots using *Acacia mearnsii*, but 75% of rooted plants was realized with 1.0 mg.l^{-1} IBA after 30 days of growth through the micropropagation for the same species. *In vitro* the regeneration of *Tunisien chili*, by combining 5 mg.l^{-1} of BA and 1 mg.l^{-1} ANA increase the percentage of organogenesis developing explants (Arous et al., 2001), this remark is similar to that observed in this study. Kaur et al. (1998) recorded 8 to 10 shoots per explants of *Acacia catechu (Willd)* from nodal explants on MS medium containing 4.0 mg.l^{-1} BAP*0.5 mg.l^{-1} NAA. In *Acacia auriculiformis*, different auxin, including NAA at2.68 µM in

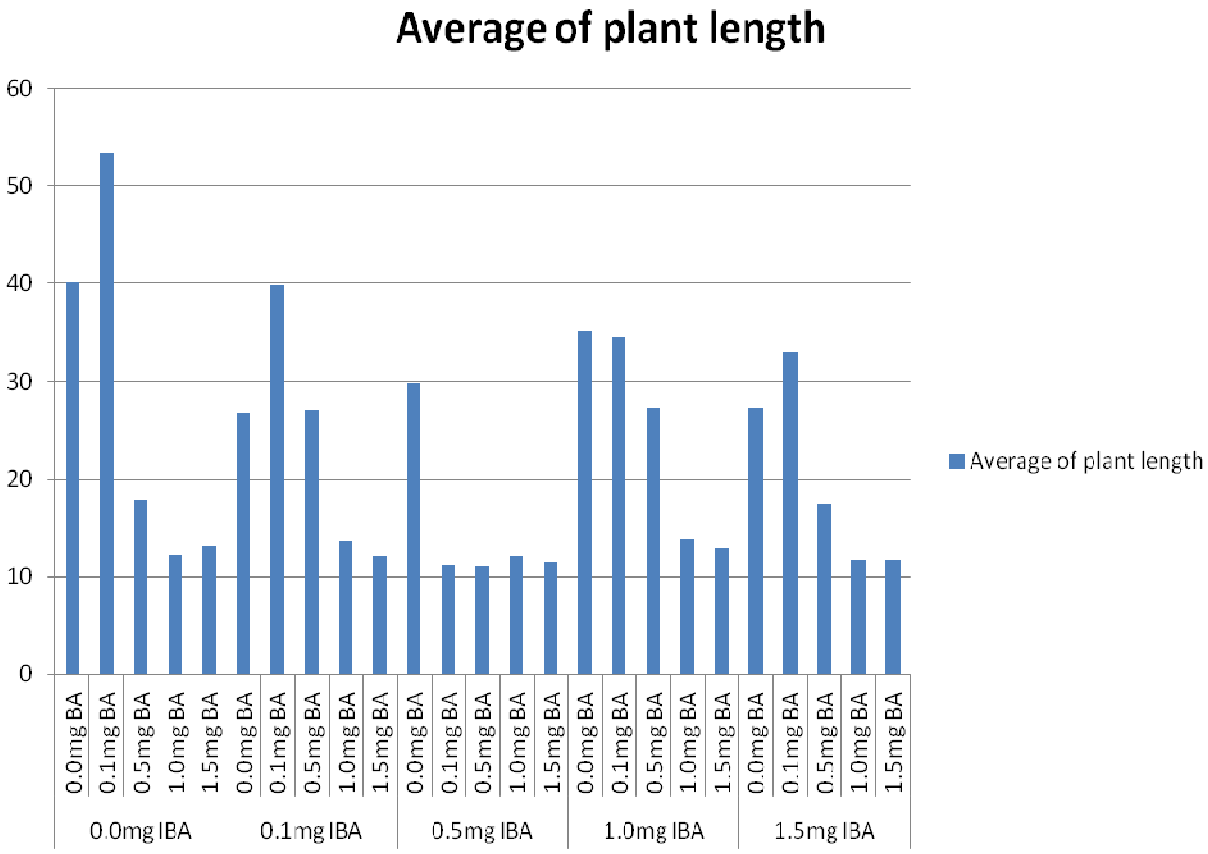


Figure 2. Average of plant length after 30 days of incubation on MS medium.

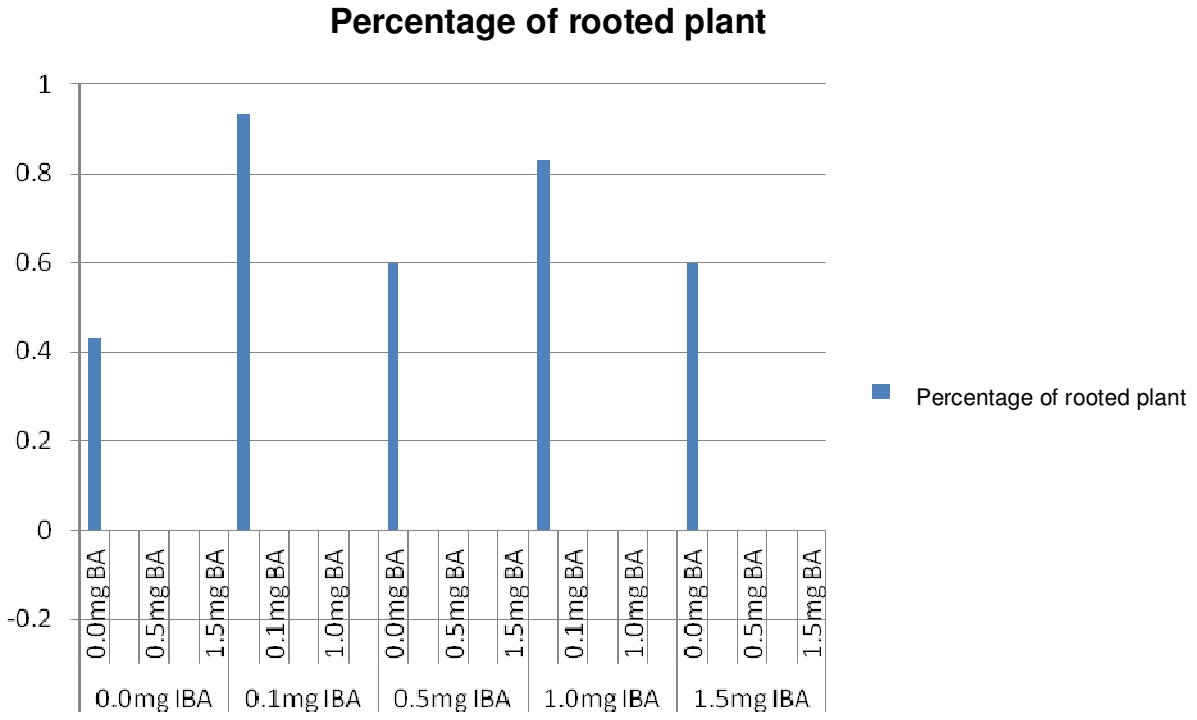
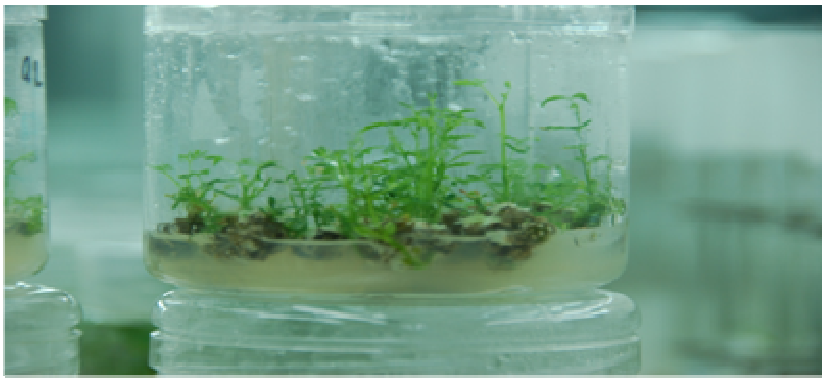


Figure 3. Percentage of rooted plants after 30 days of incubation on MS medium.

A



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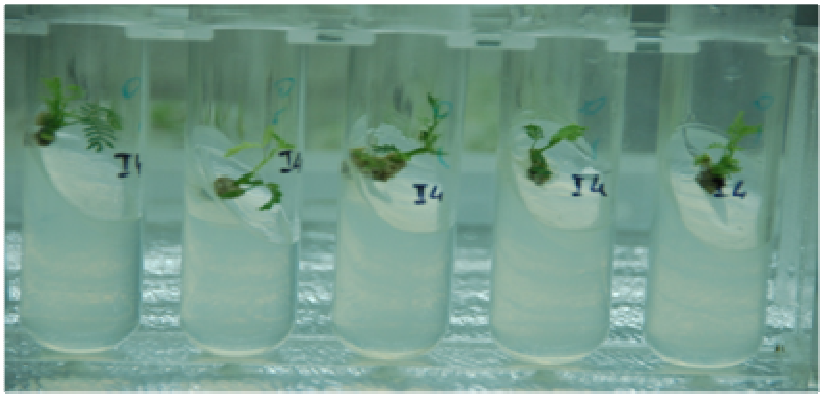

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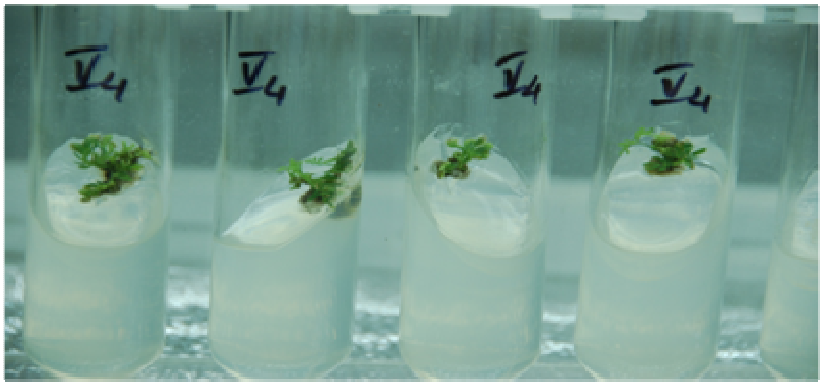

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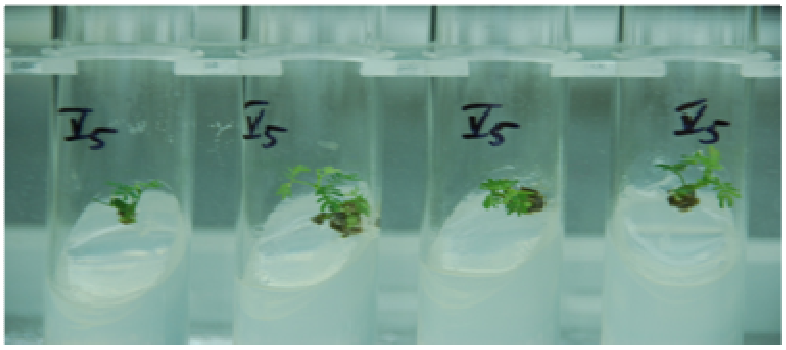

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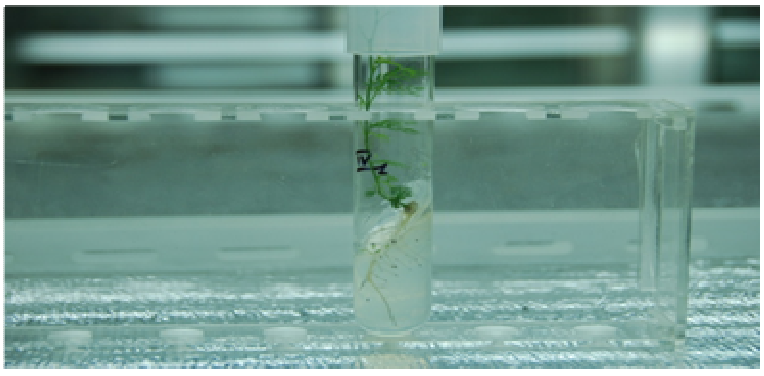

H

Figures 4. (A) Initiation and proliferation of nodes on MS medium; (B) initiation and proliferation of nodes on MS medium; (C) plant on [1.5 mg/l IBA * 1.0 mg/l BA; (D) plants on [1.5 mg/l IBA * 1.0 mg/l BA]; (E) plants on [1.5 mg/l IBA * 1.5 mg/l BA]; (F) plants on [0.0 mg/l IBA * 0.1 mg/l BA]; (G) rooted plants on [0.1 mg/l IBA*0.0 mg/l BA]; (H) rooted plants on [1.0 mg/l IBA*0.0 mg/l BA].

combination with BAP at 4.44 µM, induced organogenesis from explants on MS medium (Ranga Rao and Prasad, 1991).

The application of tissue culture methods in forestry has gained momentum because of the growing demand for biomass and other forest products, but sometime rooting is a serious problem especially with refractory plants. *Acacia* species are considered to be recalcitrant to regeneration and pose various problems during *in vitro* culture (Dewan et al., 1992). Although, successful regeneration has been reported for few *Acacia* species (Mathur and Chandra, 1983; Mittal et al., 1989; Zhao et al., 1990). In fact, Dewan et al. (1992) reported cytokinin as an obligatory part of the media for shoot differentiation, but Feng et al. (1994) showed more far away that the cytokinin BAP inhibits rooting. BAP used at dose of 0.05 mg/l with IBA or NAA induced roots significantly. They support the number of bud, not only function of the concentration of cytokinin but also by the kind of cytokinin. Shoot multiplication was restricted to BAP in addition to IBA, also, BAP combined with very low doses of IBA (0.01 $mg.l^{-1}$) induced significant shots with *Acacia mearnsii* regeneration on MS medium.

Through the outcome of various researchers mentioned earlier, it seems clear that micropropagation of *Acacia* species are obtained with either hormone, used alone or combined with two hormones which are extremely smaller than the other. Indeed, the regulators act effectively on the growth with low dosage, while few effects are obtained with high doses.

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