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Full Length Research Paper

IN VITRO regeneration of *ACACIA NILOTICA* (L.) Willd. ex Del from nodes on B5 medium

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***ACACIA NILOTICA* (L.) Willd. ex Del epicotyls buds were initiated in a medium supplemented by a combination of naphthalene acetic acid NAA (0.1 mg/L), benzyl amino purine BA (1.5 mg/L) and gibberellic acid GA (1.5 mg/L) were then used to produce a factorial experiment on B5 medium supplemented with auxin indole-3-butyric acid (IBA) and cytokinin BA with five levels (0.0, 0.1, 0.5, 1.0 and 1.5 mg/L) of each. At 0.5 mg/L level BA produced the highest number of nodes (5.8) and longest stem length (55.83 mm). However, the interaction between the two hormones (IBA and BA) had a negative effect on the growth and rooting of the shoots. High level, on the other hand, had a shortening effect on the internodes. BA used only with high doses has an effect of shortening on plants. Level 1.0 mg/L of IBA produced 100% rooted plants with short roots. Shoots are thin and have general growth retardation.**

Key words: *Acacia nilotica,* regeneration, microcutting, rooting, node.

INTRODUCTION

Acacia nilotica (L.) Willd. ex Del is a multipurpose leguminous tree of arid and semi arid zones. This species has a great nitrogen fixation potential then it is used in banks protection and soil restoration (Toky et al., 1992; Faye et al., 2007; Bargali and Bargali, 2009). Moreover, it offers a good resource of income to rural populations as it acts as a source of tannin, timber and arabic gum. According to Arbonnier (2002), fruit pods are used in tanning leather and skins. *A. nilotica* may also be used for medicinal purposes, as a demulcent or for conditions such as gonorrhea, leucorrhoea, diarrhea, dysentery, and diabetes. It is styptic and astringent (Arbonnier, 2002). In traditional medicine, the gum is used to consolidate watery semen (Mahesh and Satish, 2008). Bargali and Bargali (2009) have demonstrated its capacity to resist temperature above 50°C and to air dryness, but very sensitive to freezing especially when it is still young. Also Yang et al. (2006) demonstrated that it could not survive in extreme low minimum temperature of -18°C. The extreme hardness of the seed coat makes the germination process difficult. Also some seeds may conserve their germinating power potential even after a period of 15 years. *A. nilotica* can play an important ecological role with a relatively rapid growth rate, which possess an important biological fixation potential of nitrogen and restoration of degrading soils (Toky and Bisht, 1992). In Mali, the number of plant tree significantly reduced as a result of the wildfire, the uncontrolled cutting and urbanization, so according to Haider (2010), *A. nilotica* became threatened. However, though to introduce this species in China it is facing difficult problems with natural propagation (Bargali and Bargali, 2009) the presence of *in vitro* culture serves as an

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Abbreviations: BA, benzyl amino purine; **IBA**, indole-3-butyric acid; **NAA**, Naphthalene acetic acid; **GA**, gibberellic acid, **B5**, basal medium.

opportunity for rapid organogenesis under the influence of different combinations of growth regulators. In the continuity of the development of a serial of protocols with different types of culture medium this paper develops a protocol for reproducible *in vitro* production of plants well-rooted plant on B5 medium (Gamborg et al., 1968) with this multifunction tree.

MATERIALS AND METHODS

Plant materials and sowing conditions

Some mature seeds of *A. nilotica* were used as explants. To start with, seeds were scarified by opening the skin. Then seeds were surface sterilized with 2% (w/v) sodium hypochlorite for 20 min and with 70% (v/v) ethanol for 1 min. In sterile distilled water, seeds were rinsed five times. 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. All the plant and materials were incubated at 25 ±1°C under 16 h in light by day photoperiod.

Buds initiation

The epicotyl of one month old seedlings was sliced into nodes of 0.5 to 1 cm and subcultured in different initiation media composed of the basic medium MS supplemented with different dosages of NAA (0.0 ; 0.1 ; 0.5 and 1.0 mg/L), of BA (1.5 mg/L), and of GA (1.5 mg/L).

The cells were proliferated while nodes were swollen. After three weeks of incubation, the swollen nodes were transferred to MS medium (Murashige and Skoog, 1962) without hormone Transferred nodes 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

These nodes of two weeks bearing, a leaf and an apical bud were used to investigate the effect of two growth regulators BA and IBA in a complex factorial experiment. In this experiment the basal medium of B5 was enriched with different dosages of BA (0.0; 0.1; 0.5; 1.0 at 1.5 mg/L), and IBA (0.0; 0.1; 0.5; 1.0 at 1.5 mg/L), thus 25 treatments carried out in a randomization mode.

Thirty shoots were cultured in the different media explained above. The number of formed nodes, the length of stem and the number of rooted plants were scored in each medium after thirty days of culture. The obtained data were processed using the SPSS 16.0 inch software.

RESULTS

Initiation of auxiliary buds

The benefit of NAA in the MS medium stimulates the buds initiation. The buds emerged after two weeks of culture. The best initiation rate was obtained with 0.1 mg of NAA and an average of 8 buds during the first two harvests and of 4 buds in the third harvest. We also noted that, using 1 g/L of polyvinylpyrrolidone (PVP) with the use of knots incised only at the base the rate of browning was almost zero.

Proliferation and root induction

The analysis of the variance results (Table 1) show that interactions IBA*BA are highly significant. From Figure1, using only the cytokinin BA or IBA auxin and at a low level produces a highly significant effect respectively on nodes production and rooting. The greatest production of nodes was observed at 0.5 mg/L BA with 5.8 nodes/plant followed by a dose of 0.1 mg/L BA with 5.13 nodes (Figure1). High levels (1.0 and 1.5 mg/L) did not only produce few nodes but also a short internodes height (Figures 1 and 2).

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). The rooting was obtained mainly only by using auxin (IBA). The best levels were 1.0, 0.5 and 0.1 mg/L respectively for 100, 90 and 83.33% of rooted plants (Figure 3). The roots on 1.0 mg/L were longest, most thick and robust. In addition, the combination of these two hormones is not favorable to the growth of *A. nilotica* on the B5 medium. This was noticed with the dosages of 1.0 and 1.5 mg/L.

Acclimatization and transplantation

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

DISCUSSION AND CONCLUSION

The *in vitro* regeneration techniques are useful tools for the production of seedlings with a lower risk of genetic variability (Rao et al., 1986). Dewan et al. (1992) obtained good growth of *A. nilotica* with the level 1.5 mg/L of BA, after two to three months of growth in the B5 medium. Sascha et al. (1998) obtained 85% initiation with 2 mg/L BA and 75% of rooted plants with 1.0 mg/L IBA after 30 days of incubation on MS medium in the micro propagation of *Acacia mearnsii.* Rout et al. (2008) observed the highest number of shoots (4.21) from nodal cuttings of seedlings of *Acacia chuandra* grown on MS medium with 1.5 mg/L BAP*0.05 mg/L IAA. Mathur and Chandra (1983) also observed the development of multiple shoots from nodal explants of *A. nilotica* on auxin

Table 1. Analysis of variance of data collected.

Source	Dependent variable	Type III Sum of squares	df	Mean square	F	Sig.	Partial eta squared
Corrected model	Nodes nbr	1250.352[a]	24	52.098	111.091	0.000	0.786
	Stem Length	160602.288[b]	24	6691.762	292.950	0.000	0.907
	Rooted plant	76.267[c]	24	3.178	73.843	0.000	0.710
Intercept	Nodes nbr	7641.648	1	7641.648	1.629E4	0.000	0.957
	Stem Length	441507.745	1	441507.745	1.933E4	0.000	0.964
	Rooted plant	22.533	1	22.533	523.611	0.000	0.419
IBA	Nodes nbr	664.525	4	166.131	354.251	0.000	0.662
	Stem Length	74181.355	4	18545.339	811.871	0.000	0.817
	Rooted plant	2.627	4	0.657	15.259	0.000	0.078
BA	Nodes nbr	347.259	4	86.815	185.120	0.000	0.505
	Stem Length	48292.755	4	12073.189	528.536	0.000	0.745
	Rooted plant	67.133	4	16.783	389.997	0.000	0.683
IBA * BA	Nodes nbr	238.568	16	14.911	31.794	0.000	0.412
	Stem Length	38128.179	16	2383.011	104.323	0.000	0.697
	Rooted plant	6.507	16	0.407	9.450	0.000	0.173
Error	Nodes nbr	340.000	725	0.469			
	Stem Length	16560.967	725	22.843			
	Rooted plant	31.200	725	0.043			
Total	Nodes nbr	9232.000	750				
	Stem Length	618671.000	750				
	Rooted plant	130.000	750				
Corrected Total	Nodes	1590.352	749				
	Stem Length	177163.255	749				
	Rooted plant	107.467	749				

a. R squared = 78.60% (Adjusted R squared = 77.90%)
b. R squared = 90.70% (Adjusted R squared = 90.30%)
c. R squared = 71% (Adjusted R squared = 70%)

(0.5 to 1.0 mg/L IAA).

However, these results are not in agreement with Kaur et al. (1998), who recorded 8 to 10 shoot per explants of *Acacia catechu* (Willd) from nodal explants on MS medium containing 4.0 mg/L BAP*0.5 mg/L NAA. According to Haider et al. (2010) more (2.80) of root was recorded in presence of 3.0 mg/L IAA, whereas IBA at the same concentration did not produce more than 1.20 roots per explants.

Thus this study results also revealed the effects of shortening of internodes and slower growth at higher levels of 1.0 and 1.5 mg/L BA and sometimes swelling of the base nodes. According to our results, for better regeneration of *A. nilotica* on B5 medium, we suggest 0.5 mg/L BA to produce nodes (Figure 1) while rooting is preferable with 1.0 mg/L IBA (Figure 3).

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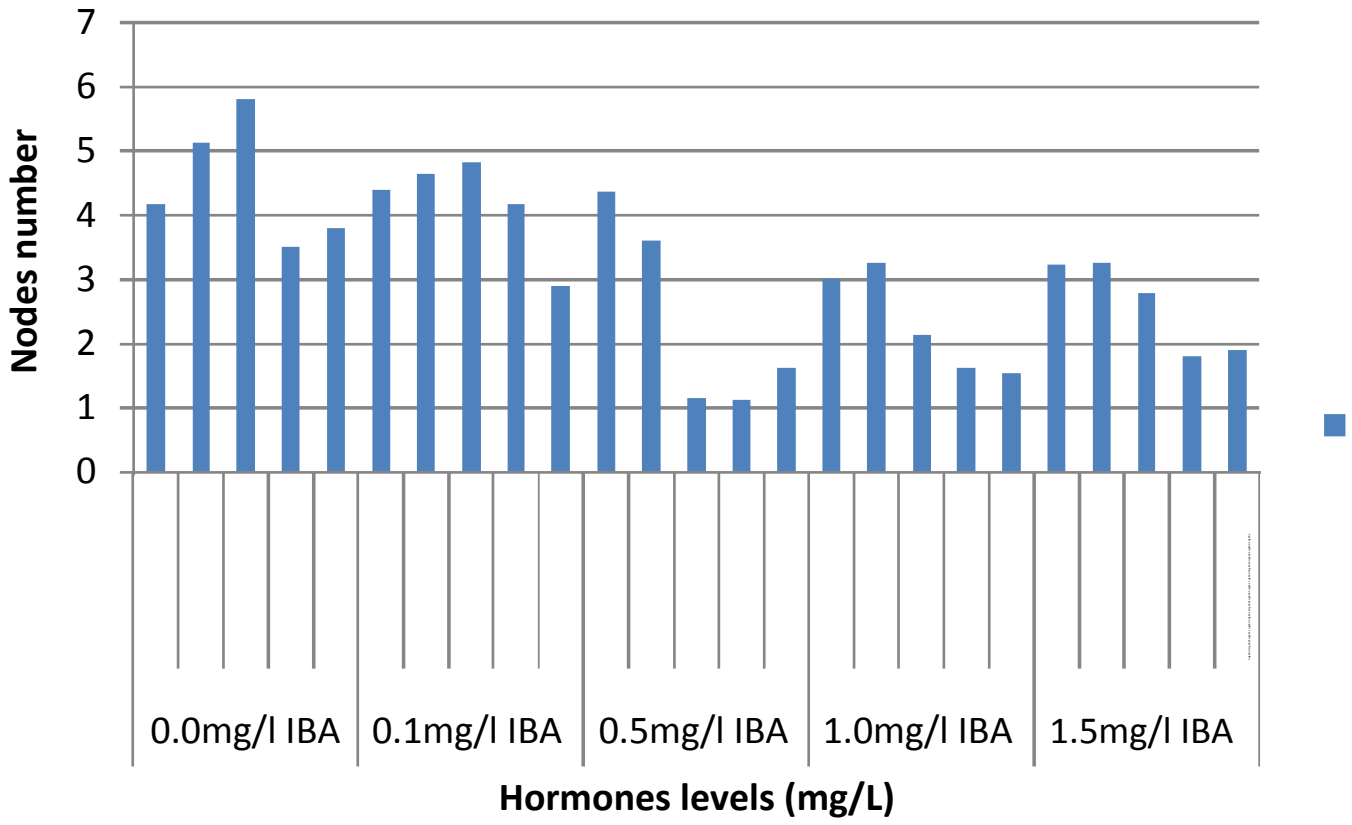

Figure 1. Means of nodes number 30 days of incubation on B5 medium.

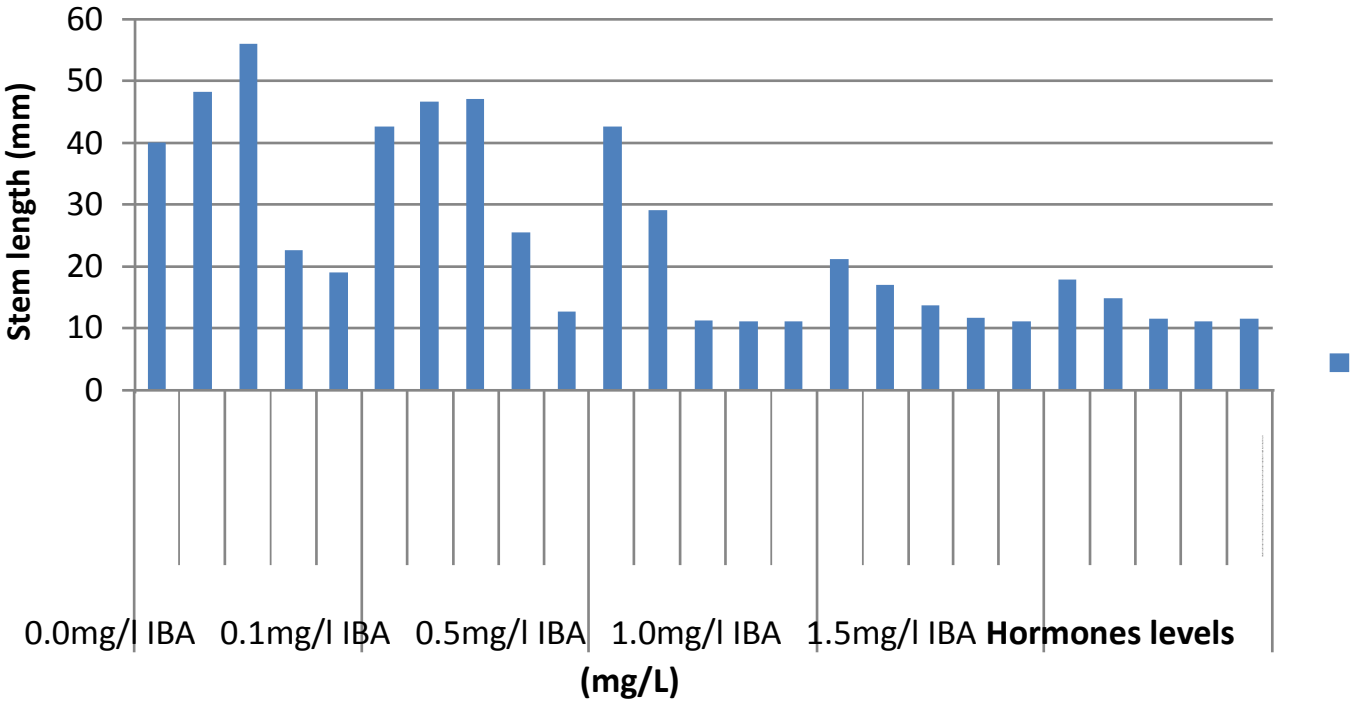

Figure 2. Means of stem length 30 days of incubation on B5 medium.

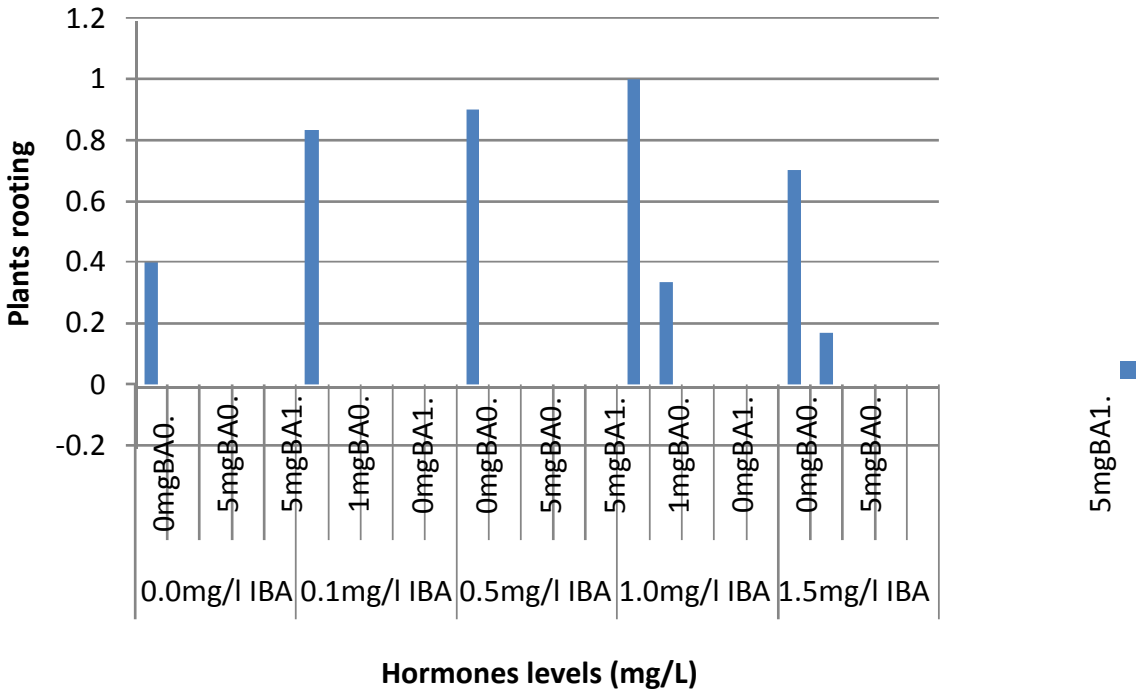

Figure 3. Percentage of rooted plant 30 days of incubation on B5 medium.

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