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In vitro propagation of Arid zone tree species (*Acacia nilotica and Acacia senegal*) in Maiduguri, Nigeria

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ABSTRACT

Trees are important for various reasons in the arid environment, the arid environments have less diversity and abundance of tree species. In reforesting arid environment for their various ecosystemic roles, conventional methods of propagation have been found to be inadequate. Hence there is need for the in vitro growth of plant species found in Arid zone to ameliorate the problem. The objective was to evaluate the effects of growth regulators Benzyl Adenine (BA) and Naphthalene Acetic Acid (NAA) and determine the potentials for regenerating plants (in vitro) and having superior genetic traits such as high quality need, disease free planting materials and tolerance to environment of the arid zone tree species. The cotyledonary node explants were exercised from the juvenile (0.5-1cm) explants and inoculated on Murashige and Skoog medium, enhanced with various concentration of BA (1.0-5.0mg/L^{-1}) singly and in combination with NAA (0.5-2.5 mg/L^{-1}) for the Acacia species mentioned above. The rateat which cotyledonary nodes regenerate shoot was influenced by diffrentratio of BA and NAA. Acacia nilotica produced the most shoot (1.77 ± 0.27) from medium concentration BA (1.0 mg/L^{-1}) plus NAA (0.1 mg/L^{-1}) for Acacia nilotica, (1.44 ± 0.41) was obtained from BA (3.0 mg/L^{-1}) mix with NAA (0.3 mg/L^{-1}) Acacia senegal and (1.33 ± 1.09) were obtained from BA (5.0 mg/L^{-1}). After four weeks on the rooting medium, the plantlet failed to induce root. Result from histological study, revealed that plantlets exhibited fairly similar anatomical structures and normal organisation of epidermis, cortex and vascular bundles and also the pericycle did not show any sign of rooting. Given that callusing developed at the explants bases, this could be the cause of the unsuccessful rooting. It is recommended that further research is required to understand more about the biochemical and anatomical factors of these species to further improve both shooting and the rooting success.

Keywords: *In vitro, Propagation,Arid zone, auxin, cytokinin*

INTRODUCTION

Plant tissue culture is an effective method for the in vitro cultivation of plant cells, tissues, or organs under controlled conditions, typically on a nutrient-rich medium supplemented with essential minerals, vitamins, and plant growth regulators [1]. This approach plays a vital role in modern plant biotechnology, offering a means for the mass propagation of elite genotypes, genetic transformation, and conservation of plant genetic resources [2]. Micropropagation, a widely used form of plant tissue culture, is particularly beneficial for producing disease-free, genetically uniform, and high-yielding plants [3]. It enables large-scale propagation of commercially important crops, medicinal plants, and forestry species, including Acacia, which is valued for its ecological and economic significance [4]. Acacia species are known for their adaptability to harsh environmental conditions, including extreme drought and poor soils, making them ideal candidates for afforestation, agroforestry, and reforestation programs in tropical and subtropical regions [5]. Moreover, the utilization of in vitro propagation methods in Acacia species has facilitated the rapid multiplication of superior genotypes with desirable traits such as high biomass production, nitrogen-fixing ability, and its ability to withstand both biotic and abiotic stresses [6]. In addition to commercial propagation, plant tissue culture techniques contribute to fundamental research in plant physiology, genetic engineering, secondary metabolite production, and conservation of endangered species [7].

Advances in somatic embryogenesis, organogenesis, and cryopreservation have further expanded the applications of plant tissue culture in agriculture, horticulture and forestry [8].

Methodology

Study Area

The research was conducted in the Tissue Culture Laboratory, North Eastern Biotechnological Centre, University of Maiduguri (Lat.11°27' 30" N and 11° 33' 30 N and Long. 13°2' 30" E and 13° 9' 10" E), Nigeria. Maiduguri is located on altitude of 354mm above sea level with mean relative humidity ranges from 30% to 45% [9]. However, three seasons have been identified, harmattan i.e., dry/cold (October-January), hot/dry (February-May) and wet (June-September) with a varying temperature ranging from 20°C during dry/cold season to 44°C during hot/dry season. Moreso, relative humidity go as high as 30%-45% in August and to about 5% in December and January and day length also varies from 11-12 hours [10].

Explants Collection and Sterilization

Shoots of the three plant species (*Acacia nilotica*and *Acacia senegal*) were collected from raised seedlings of three to four months old from the Nursery of Biotechnology Centre University of Maiduguri, Borno State. Juvenile nodal explants (0.5–1cm length) was cut from shoots from seedlings of each plant mentioned above.

The explants were surface sterilised by immersing them in a solution containing 150 mg of citric acid and 100 mg of ascorbic acid for 10 minutes after being cleaned for 15 to 30 minutes under running tap water to remove surface dust, then three times with 2% Teepole (shake well). After being moved and submerged in 70% ethanol for 15 seconds, the explants were repeatedly cleaned with distilled water [11].

Culture Medium Preparation and Condition

Standard method of MS [12] was adopted supplemented with growth regulators (Auxin and Cytokinin) was used during the research. The MS [12] medium supplemented with 3% (w/v) sucrose and 7g (w/v) agar was used for the species during the research. The pH of the medium was adjusted to 5.6 - 5.8 using 1N NaOH or HCl before autoclaving. The media was distributed in 25mm x 150mm test tubes each containing 20ml of medium.

Shoot Induction and Multiplication

The Sterilized nodal segments was placed on MS medium supplemented with BA at varied concentrations (1.0, 2.0, 3.0, 4.0, 5.0mg L^{-1}), singly and in combination with (NAA) at different concentrations (1.0+0.1, 2.0+0.2, 3.0+0.3, 4.0+0.4, 5.0+0.5mgL^{-1}). The frequency of explants generating shoots, number of shoots per explants, shoot length and leaves countwas recorded after 4 weeks of culture.

In vitro Rooting

In vitro proliferating shoots, (1-2 cm) wasseperated and sub-cultured on MS basal medium augmented with NAA atconcentrations (0.5, 1.0, 1.5, 2.0 and 2.5 mg L^{-1}) was incubated for four weeks

Data Analysis

Three growth regulators at varying concentrations (MS alone, MS+BA and MS+BA+NAA) was used in the research. Each treatment involved thirty explants, and every experiment was conducted three times. One Way Analysis of Variance (ANOVA) was used to quantify and evaluate the raw data of the various treatments that was acquired. Duncan's Multiple Range Test technique was used to compare the pairwise means ($P<0.05$).

RESULTS

The results are expressed as a mean ± SE for all the experiments. The test of significance among means of different shoot multiplication between the species (*Acacia nilotica andAcacia senegal*)

1.0 Effects of growth regulator BA both indepedantly and in combination with NAA on shoot multiplication of *Acacia nilotica.*

The findings show thatBA (1.0 mg/L^{-1}) in combination with NAA (0.1 mg/L^{-1}) produced the greatest number of shoots (1.77 ± 0.27) followed by BA (3.0 mg/L^{-1}) alone with mean (1.22 ± 0.22) (Table 1.0). The result further recorded a least number of mean (0.44 ± 0.17 shoot) as obtained from BA (4.0 mg/L^{-1}) alone and BA (2.0 mg/L^{-1}) + NAA (0.2 mg/L^{-1}) (Table 1.0).

The result revealed no substantial changes among mean of shootstreated with BA (1.0, 3.0, 4.0 and 5.0 mg/L^{-1}) and BA + NAA (2.0+0.2, 4.0+0.4, and 5.0+0.5 mg/L^{-1}) (Table 1.0). However, it showed a significant difference on the mean of BA (2.0 mg/L^{-1}) of both BA treatments (Table 1.0).

As for shoot length, the highest result (0.33 ± 0.06) was obtained from BA (3.0 mg/L^{-1}) + NAA (0.3 mg/L^{-1}) subsequent by BA (1.0 mg/L^{-1}) + NAA (0.1 mg/L^{-1}) with mean (0.30 ± 0.03) shoot length (Table1.0). However, the result further showed a least mean values of (0.08 ± 0.03) shoot length from BA (4.0 mg/L^{-1}) + NAA (0.3 mg/L^{-1}). (Table 1.0). The result further showed a significant difference in the mean values (0.30 ± 0.03) shoot length of BA (1.0 mg/L^{-1}) + NAA (0.1 mg/L^{-1}) and mean value (0.33 ± 0.06) of BA (3.0 mg/L^{-1}) plus NAA (0.3 mg/L^{-1}) (Table 1.0). The means of control (MS0), BA and BA + NAA did not affect significantly on shoot length of *A. nilotica* (Table 1.0)

In plant leaf number, the result revealed the highest mean value of (1.77 ± 0.70) of treatment BA (3.0 mg/L^{-1}) + NAA (0.3 mg/L^{-1}) concentration. On the other hand, the least leaves was obtained (0.00 ± 0.00) from control (MS0) and others (Table 1.0). However, the result showed a significant difference in the meanBA 5.0 mg/L^{-1} (0.22 ± 0.22) (Table 1.0).

The Table also showed a significant difference in mean value (1.11 ± 0.48) of BA (1.0 mg/L^{-1}) + NAA (0.1 mg/L^{-1}) (Table 1.0). The result further revealed a significant difference in the mean value (1.77 ± 0.70) of treatment BA (3.0 mg/L^{-1}) + NAA (0.3 mg/L^{-1}) (Table 1.0). However, the results revealed no significant effect on the treatment of control (MS0), and other treament respectively ($P>0.05$) (Table 1.0).

Table 1.0: Effects of growth regulator BA alone and BA + NAA on shoot multiplication of Acacia nilotica

Medium and conc. of growth regulators (mg/L^{-1})	Mean shoot of shoot/explants (mean ± SE)	Mean length (cm) (mean ± SE)	Mean leaves/explants (mean ± SE)
MS (0)	0.50 ± 0.16 **a**	0.15 ± 0.06 **a**	0.00 ± 0.00 **a**
MS+BA (1.0)	0.66 ± 0.33 **a**	0.11 ± 0.05 **a**	0.22 ± 0.22 **a**
(2.0)	1.11 ± 0.20 **ab**	0.17 ± 0.03 **a**	0.44 ± 0.29 **a**
(3.0)	0.77 ± 0.22 **a**	0.14 ± 0.37 **a**	0.00 ± 0.00 **a**
(4.0)	0.44 ± 0.17 **a**	0.15 ± 0.06 **a**	0.00 ± 0.00 **a**
(5.0)	0.66 ± 0.16 **a**	0.12 ± 0.03 **a**	0.22 ± 0.22 **ab**
MS+BA+NAA (1.0+0.1)	1.77 ± 0.27 **c**	0.30 ± 0.03 **b**	1.11 ± 0.48 **c**
(2.0+0.2)	0.44 ± 0.17 **a**	0.07 ± 0.03 **a**	0.00 ± 0.00 **a**
(3.0+0.3)	1.22 ± 0.22 **b**	0.33 ± 0.06 **b**	1.77 ± 0.70 **bc**
(4.0+0.4)	0.44 ± 0.17 **a**	0.08 ± 0.03 **a**	0.00 ± 0.00 **a**
(5.0+0.5)	0.55 ± 0.17 **a**	0.13 ± 0.04 **a**	0.44 ± 0.29 **a**

Note: Means values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test ($P<0.05$)

2.0 Effects of growth regulator BA alone and together with NAA on shoot multiplication of *Acacia senagal*

The result from data analysis revealed that the highest mean number of shoot mean (1.44 ± 0.41) in number of shoot per explants was obtained from treatment BA (3.0 mg/L^{-1}) in conjunction with NAA (0.3 mg/L^{-1}). The result further revealed the least shoots (0.00) in number of shoot per explants was obtained from control, BA (1.0 and 5.0 mg/L^{-1}) and BA + NAA (1.0 +0.1 and 4.0 + 0.4) (Table 2.0). The results indicated no significance difference in mean shoot. However, BA (2.0 mg/L^{-1}) means (0.44 ± 0.29) and BA+ NAA (5.0+0.5 mg/L^{-1}) with means (0.22 ± 0.14) did not affect significantly the shoot number (Table 2.0). Moreover, it showed a significant difference in shoot number with BA (3.0 mg/L^{-1}) + NAA (0.3 mg/L^{-1}) mean (1.44 ± 0.41) (Table 2.0).

As for the shoot length of *Acacia senegal* (Table 2.0), the result indicated that the highest number produced in shoot length was obtained in Benzyl adenine (BA) 3.0 mg/L^{-1} with mean (0.66cm). The result further recorded least number of shoot (0.00 ± 0.00) in number of shoot length from (control, BA 1.0, 5.0 mg/L^{-1}) and BA + NAA (1.0+0.1, and 4.0+0.4 mg/L^{-1}), respectively (Table 2.0). This implied that the mean levels of shoot length did not differ much.

Similarly, BA (3.0 mg/L^{-1}) with mean (0.66 ± 0.03cm), BA (4.0 mg/L^{-1}) with mean (0.13 ± 0.86), BA + NAA (2.0+0.2 mg/L^{-1}) with mean (0.33 ± 0.23) and BA + NAA (5.0+0.5 mg/L^{-1}) with mean (0.14 ± 0.08 cm) shoot length did not affect significantly shoot length (Table 2.0). However, it showed a significant difference with BA (3.0 mg/L^{-1}) and + NAA (0.3 mg/L^{-1}) with mean (0.18 ± 0.58) shoot length, (Table 2.0).

Regarding the quantity of leaves per plantlet, the outcome showed that the most leaves (4.44 ± 3.08) are obtained from BA concentration of (3.0 mg/L^{-1}) and further revealed a least number of leaves of (0.00 ± 0.00) in control and other treatemts respectively. However, the result revealed that there was no discernible variation accross the treatment (Table 2.0)

Table 2.0: Effects of growth regulator BA alone and BA+ NAA on shoot multiplication of Acacia senegal

Medium and conc. of growth regulators (mg/L^{-1})	Mean shoot of shoot/explants (mean ± SE)	Mean length (cm) leaves/explants (mean ± SE)
MS (0)	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
MS+BA (1.0)	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
(2.0)	0.44 ± 0.29 **abc**	0.11 ± 0.06 **ab**
(3.0)	0.77 ± 0.32 **c**	0.66 ± 0.03 **ab**
(4.0)	0.66 ± 0.28 **bc**	0.13 ± 0.86 **ab**
(5.0)	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
MS+BA+NAA (1.0+0.1)	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
(2.0+0.2)	0.22 ± 0.14 **abc**	0.33 ± 0.23 **ab**
(3.0+0.3)	1.44 ± 0.41 **d**	0.18 ± 0.58 **c**
(4.0+0.4)	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
(5.0+0.5)	0.33 ± 0.16 **abc**	0.14 ± 0.08 **ab**

	2.00 ± 1.41**a**	4.44 ± 3.08 **a**
	0.66 ± 0.03 **ab**	0.66 ± 0.66 **a**
	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
	0.18 ± 0.58 **c**	2.44 ± 1.04 **a**
	0.00 ± 0.00 **a**	0.00 ± 0.00 **a**
	0.14 ± 0.08 **ab**	3.55 ± 2.23 **a**

Note: Means values followed by the same letter within columns are not significantly different according to Duncan's Multiple Range Test (P<0.05)

Plate 1: Cultures of Acacia nilotica in culture medium (BA 1.0 mg/l and NAA 0.1 mg/l) regenerated at 4 weeks of growth

Plate 2: Cultures of Acacia senegal in culture medium (BA 3.0 mg/l and NAA 0.3 mg/l) regenerated at 4 weeks of growth

DISCUSSION

By ustilising tissue culture techniques, the nodal meristem can be induced to generate multiple shoots through differentiations and organogenesis by employing an adequate ratio of auxins and cytokinin [13]. This multiplication mechanism has generated a substantial quantity of plants across many families [14]. The primary challenges faced in creating an in vitro system for the regeneration of woody plants include the establishment of explants in culture, which is hindered by the oxidation of polyphenols, and the lack of juvenility in explants from mature trees which complicates in vitro establishment due to rapid necrosis of the explants.

Bud break and shoots growth were favoured when auxins and cytokinins were added to to MS medium, both separately and in different combinations. *Anogeissus sericea*has previously been shown to exhibit plant regeration from seedling explants of woody trees [15], *Prosopis cineraria* [16], *Sterculia urens* [17], and *Delbagiasisso* [18], *Syzygium cumin* [19], *Terminalia chebula* [20], *Terminalia bellirica* [21], *Boswellia ovalifolioada* [22].

The current investigation revealed that the medium lacking growth regulatorsstimulated shoots formation; nevertheless, the growth was inhibited relative to those cultivated on media enriched with auxins and cytokinins, as highlighted in other woody trees species regarding shoot multiplication or differentiation from seedlings [23]. Among various concentrations BA alone and BA +NAAfor *Acacia senegal*produced the highest shoot (1.44) and shoot length (0.66), (Table 1.2). There are several reports which indicated that the use of BA and NAA for shoot initiation such as *Dalbergia latifolia* [24] [25], *Bauhinia variegate* [26], *Capparis deciduas fork* [27], *Delbergiasisso Roxb*. [28] and *Salvadora L.* [29]. However, the study also revealed Callus formation from the cut end of cotyledonary node on some plantlet in the medium enriched with BA and BA + NAA . The callus reduced in BA+NAA (3.0+0.3, 4.0+0.4 and 5.0+0.5 mg/L^{-1}), the formation of callus may restrict shoot number and shoot elongation according to [29].

As for *Acacia nilotica*, the best response of shoot proliferation and growth was observed to be highest with average mean shoot (1.77). Multiple shoots developed on nodal explants after four weeks of culture. [30] reported shoot initiation occurred from nodal explants of *A. auriculiformis* even at a low concentraion of BA + NAA. Shoot growth in these combinations surpassed that observed in the medium containing BA alone. Results from A. memarmelos cotyledon [31] and hypocotyl [32] explants support this. According to the current study's findings, auxin and cytokinin are necessary for a better sprouting response. According to reports, adding slightly higher concentration of BA and lower amount of NAA to the culture medium improved the nodal culturs of *A. Senegal* [15]. cultures of *Prosopis juliflora* showed a comparable reaction [16]. This was also applicable *A. seyal* shoot tip cultures, where multiple shoot development enhanced with BA and NAA, but when either was utilised independently [34]. BA in combination with auxin was also found to be essential for multiple shoot induction on some other trees [35] [36] Tabone *et al*., 1986; [37]. Successful regeneration of plantlets using mature nodal explants [38] and cotyledonary node [39] of *A. Marmelos* has been reported.

The current investigation also showed that the cut end of explants inoculated into the majority of the tested concentrations showed a little callusing. The growth of *A.nilotica* explantsdid not appear to be impacted by the presence or lack of callus.

It was proposed, therefore, the callus should only form on explants when least 50% of them did. None of the sprouts came from the callus. The superficial callus on nodal explants is powdery, non-embryonic, and white to yellow in hue. It is commonly recognised that during *A. nilotica's* in vitro regeneration, superficial callus production on nodal stem segments is a regular occurrence [40] [41].

The observation of callus formation at the proximal ends of node explants in this research corroborates previous findings in *Peganum harmala* [42] and *Holostemmaada-kodien*[43]. [44] suggested that the accumulation of auxin at the basal cut ends, potentially synergised by cytokinins, likely triggers cell proliferation, yielding callus formation. This phenomenon is reportledly more prevalent in species exhibiting strong apical dominace, particularly when cultured on cytokinin enriched media [45].

After 28 days of culture on the rooting medium, it was obvious that the plantlets of all the three species failed to induce root from the rooting media, but established continuous shooting multiplication. The failed rooting may have been caused by callusing, which was observed at the base of the explants. The results nearly matched the study on *Jatropa curcus* by [46] which documented a half strength medium with NAA and IBA did not allow for good rooting.Similar results was also reported in*Acacia chundra* [47]. Failure of root proliferation was also observed in*Pterocarpus marsupium* shoots inoculated in MS medium (half and quarter strength) containing IAA, IBA and NAA [48]. Similar observations have been documented for other woody species specifically*Acacia koa Gray* [49], *Bauhinia vahlii* [50], *Morus nigra L*. [51], *Acacia mangium* [52].

The failure of root growth due to the formation of basal callus resulted in interfering the physiological process by trapping essential growth constituent such as plant growth regulators [46]. In the present study, the root growth is failure due to the callus formation. Therefore, it was suggested to further research for micrografting for the proliferation of root as it was found successful in the case of *A. Senegal* by [53].

CONCLUSION AND RECOMMENDATION

The research revealed that growth regulators, BA aggregated with NAA produced the best result in *A. nilotica* than *A. senegal*. It is advice that growth regulator concentrations and culture conditions be further optimised in order to optimise biomass output and secondary metabolite yield. Furthermore, incorporating this protocol into commercial propagation and conservation initiatives could guarantee the sustainable production and use of these priceless species

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