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Full Length Research Paper

Shoot Proliferation Using Cotyledonary Nodes of Locally Conserved *Parkia biglobosa* (Jacq.) Benth Seeds

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ABSTRACT

The study was conducted to establish a protocol for *in vitro* multiplication of shoots from cotyledonary nodes obtained from locally conserved seeds of *Parkia biglobosa* for genetic improvement and germplasm conservation. The objectives include determination of plant hormone that induces shoot proliferation and the explant that shows rapid shoot multiplication. Axillary and apical nodes of 8 and 12 weeks old seedlings of *P.biglobosa* were used as explants on Woody Plant Medium (WPM). NAA, KIN and BAP were supplemented at 0.0, 0.5, 0.75, 1.0 mg/l for inducing shoot. Parameters measured include number and length of shoots and the number of nodes of the cultured explants. The design is a 2x2x3x4 factorial experiment in a Complete Randomized Design (CRD). Data obtained were analysed using Analysis of Variance and significant means were separated using Least Significance Difference (LSD) at 0.05 and 0.01 respectively. It was observed that for 8 weeks old axillary node, shoot length increases as the concentration of hormone. Age and age-hormone interaction had significant effect on the number of nodes (P=0.05) with the highest mean, 7 obtained among 8 weeks apical nodes of 0.75mg/l and 1.00mg/l KIN. It was observed that organogenesis using juvenile cuttings would be effective for this species.

Keywords: Cotyledonary, Hormones, Nodes, *Parkia*, Woody.

INTRODUCTION

Parkia biglobosa (Jacq) Benth popularly called African Locust Bean is a multipurpose tree belonging to the family Leguminasae, a light demanding species of open sites and parkland savannah forming pure stands in some areas but often also associated with other trees, such as *Vitellaria paradoxa* (Sina and Traore, 2002: Aleiro, 2004). The tree is widely distributed on the sandy loam soils of Sudan and Guinea savannah areas of Nigeria and known to spread across the semi arid zone of sub-saharan Africa from Senegal to Sudan. (Aliero *et al*., 2001: Sacande and Clethero, 2007). It is a key economic multipurpose fruit tree species that has played a vital role in poverty alleviation and food security in Nigeria and with a wide range of value with main product as seeds extracted from the pods for condiments in stews and soups, as sweetener and various parts of the tree species is used as fodder for livestock, medicine, dye, and timber for fuelwood and construction as well as for soil improvement in agroforestry practices (Aliero *et al*., 2001). Trends have also shown that it offers investment opportuinties for people in the rural areas (especially women) (Shao, 2002), for shelter belts and will help in combating the current menace of climate change globally (Nair, 1993).

The *Parkia* tree is threatened and has been listed among the trees for *in situ* conservation which constitute African genetic resource priority (Izquirdo and Roca, 1998). Other factors that affected the natural regeneration of this species include abuse of seeds, bush fires and the pastures. (Sambe *et al*., 2010; Sacande and

Clethero, 2007). *In vitro* propagation of plants holds tremendous potential for the production of quality plant-based medicines and used in conservation of valuable indigenous germplasm threatened with depletion (Murch *et al*., 2000).

Attempts to propagate conventionally and biotechnologically have been impressive (Tecklehaimanot, 2000; Sacande and Clethero, 2007; Orkpeh, 2008; Sambe *et al*., 2010) but there is the need to expand the propagation to the traditionally conserved seeds while using different plant parts as explants. The aim of this study is propagating *in vitro* Parkia tree seedlings using explants nodes at different parts for multiple shoot induction from locally preserved seeds so as to determine the plant hormone that induces more shoot proliferation. It will also determine explant node that shows rapid shoot multiplication of this woody species. This study is relevant as it will fill the gap of knowledge of expanding the scope of mass propagating *Parkia* using long time preserved seeds but there is also the need to further expand the scope to various parts of the plant using the woody plant medium which is widely known to induce the growth of woody plants (Razdan, 2003) than other culture medium.

MATERIALS AND METHODS

Study Location

The study was carried out in the Tissue Culture Laboratory of National Centre for Genetic Research and Biotechnolgy, (NACGRAB), Moor Plantation, Ibadan.

Seed Source and Germination

Parkia biglobosa seeds were preserved in woven sack stored in earthen pots in a cool dry area for 3 years. Batch of the seeds stored was collected from Lafiagi, Kwara State, Nigeria at December, 2010. The seeds were identified as those of *Parkia biglobosa* R. Br Ex. Don. at the Herbarium of National Centre for Genetic Research and Biotechnolgy, (NACGRAB), Moor Plantation, Ibadan, Nigeria. The seeds were scarified using absolute H_2SO_4 for 1 hour and placed in a Petri-dish for 24 hours. The seeds had 98% germination.

Seed Scarification and Disinfection

In order to chemically break the seed dormancy and reduce the testa toughness, the procedure of Amoo and Ayisire (2005) were carried out with little modification in time interval (30 minutes duration in absolute H_2SO_4). The scarified seeds were surface sterilized by soaking in 70% ethanol for 5 minutes after which the chemical was decanted and rinse. The seeds were disinfected in 15% solution NaOCl, (Jik, liquid whitener, Uniliver product) with two drops of teepol detergent as a wetting agent for 5 min, followed by 4 successive rinsing and left in the fourth rinse to soften. Only seeds that settled at the sterilization jar bottom were used to ensure viability.

Explant preparation

3000ml Woody Plant Medium (WPM) with pH 5.7 was solidified in agar 18.0gL^{-1}. The media was distributed in 200 sigma test tubes of culture/treatment, at a rate of 15 ml/test tube. The test tubes were sterilized at 110ºC for about 20 min. They were then incubated in the growth room at 27 ±1ºC. Seeds germinated were counted for a period of 8 and 12 weeks. All instruments used were autoclaved at 121ºC for 30mins. Proper flaming of instruments during dissection of seeds and handling of explants was done under laminar flow hood and instruments dipped in 70% ethanol. Two hundred (200) of the treated scarified *Parkia* seeds were sown in the Woody Plant Medium (WPM) at one seed per test tube.

Plant Growth Media

Three different hormones namely 1-naphthaeneacetic acid (NAA), 6-benzylaminopurine (BAP) and Kinetin (KIN) were added to Woody Plant Medium (WPM) containing 3% sucrose at different concentrations {0.00 (control), 0.50, 0.75, 1.00mgL^{-1}} per testube per node (axillary or apical) and each treatment concentration replicated four times. Two types of 1-2 cm length explants of cotyledonary nodes were taken from 1 weeks plantlets in uniform WPM and transferred to treatments test tube with a single explant per culture tube for the apical and the axillary nodes respectively.

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 weekly interval for a period of four weeks of incubation was taken for all the treatments. The experiment was repeated twice.

Data Collection and Analysis

The experimental design is a 2x2x3x4 factorial experiment in a Complete Randomized Design (CRD) at 4 replicates each. The factors are The Age of explants, The plant parts, Hormones, and Concentrations of the Hormones. The number and length of the shoots, and the number of nodes of the cultured explants on different treatments were collected and subjected to statistical

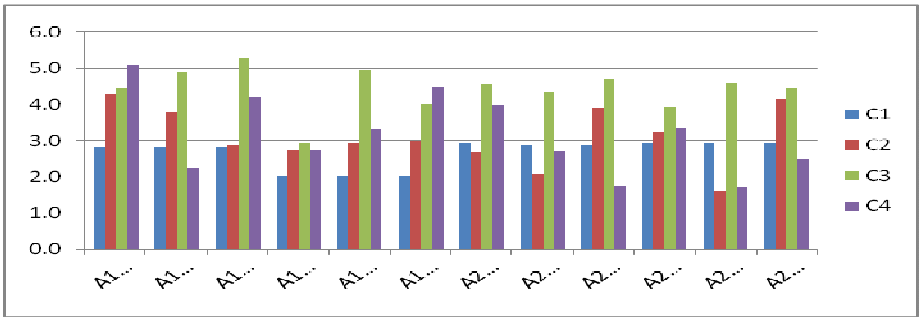

Figure 1. Shoot length from the explants in response to the treatment factors. X-axis represents treatment factors. A1= 8 weeks old explants, A2= 12 weeks old explants, P1= Axillary nodes, P2= Apical nodes, H1= NAA, H2= KIN, H3= BAP. Y-axis represents concentration levels. C1= 0.00mg/l, C2= 0.50mg/l, C3= 0.75mg/l, C4= 1.00mg/l

Figure 2. The 8 weeks old explants at different concentrations of the hormone.

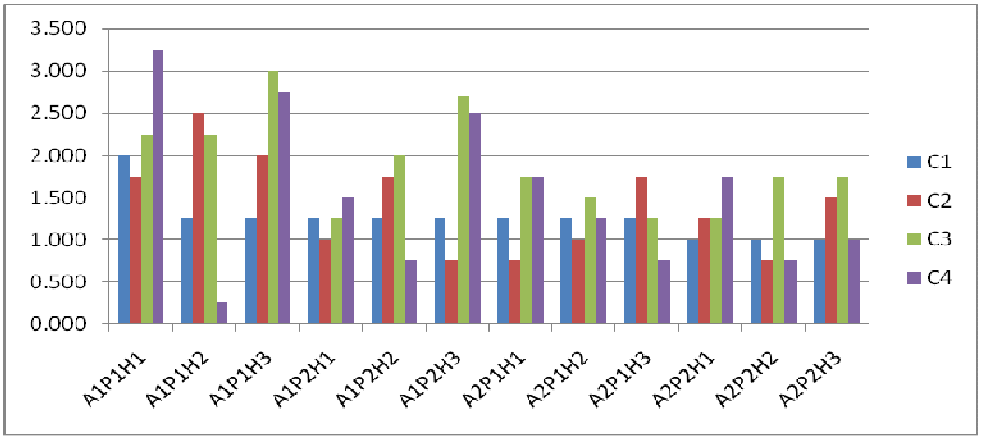

Figure 3. Number of Shoots from the explants in response to the treatment factors. X-axis represents treatment factors. A1= 8 weeks old explants, A2= 12 weeks old explants, P1= Axillary nodes, P2= Apical nodes, H1= NAA, H2= KIN, H3= BAP. Y-axis represents concentration levels . C1= 0.00mg/l, C2= 0.50mg/l, C3= 0.75mg/l, C4= 1.00mg/l

analysis. Data was statistically analyzed using Analysis of variance (ANOVA). The means were separated using Fisher's Least Significance Difference (LSD). (Software Genstat 7.0 Discovery edition).

RESULTS AND DISCUSSION

Effects on shoot length

The shoot initiation in this study was affected by a four-

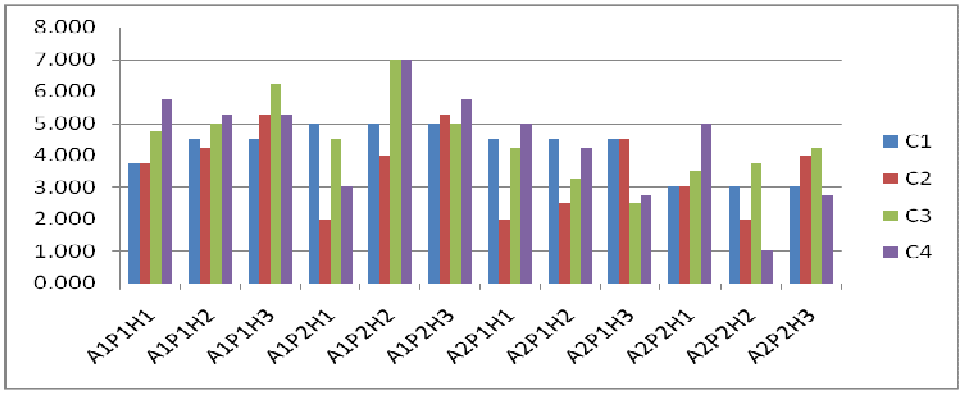

Figure 4. Number of nodes from the explants in response to the treatment factors. X-axis represents treatment factors. A1= 8 weeks old explants, A2= 12 weeks old explants, P1= Axillary nodes, P2= Apical nodes, H1= NAA, H2= KIN, H3= BAP. Y-axis represents concentration levels. C1= 0.00mg/l, C2= 0.50mg/l, C3= 0.75mg/l, C4= 1.00mg/l

way interaction which includes age of the explant (8 and 12 weeks), plant parts (axillary and apical nodes), hormones (NAA, KIN and BAP) and concentrations of the hormones (0.0, 0.5, 0.75, and 1.0 all in mg/L). Studies have shown that hormones and plant parts used affects the promotion of *in vitro* shoots from the nodal explants of *P. biglobosa.* Although many of the explants produced shoots, the shoot length varies according to treatment combination and affected its length in different ways. The results indicated that the plant parts used had a significant effect on the shoot length (Table 1) with highest mean (4.43cm) at 0.75mg/L and lowest mean (3.66cm) at 0.0mg/L (P=0.05).

The interaction among all the treatment combinations showed no significant effect (P=0.05) on the shoot length. However, it was observed that the axillary node shoot length increases as the concentration increases and had a decrease at 1.00mg/L concentration. There was a decrease in shoot length for all treatment factors at 1.00mg/l concentration except at 8 weeks old axillary node in 1.0mg/l NAA. The shoot length means range from 5.30cm (8 weeks old apical node of 0.5mg/L KIN to 1.60cm (12 weeks apical node of 0.5mg/l KIN) for all the interactions (Figure 1-2). Hormonal specificity and concentration was evident in the study for eliciting multiple shoots and elongation. This is in line with Orkpeh (2008) and Capuana *et al.,* (2007) that observed at least 5-6 weeks seedlings are more effective for shoot elongation and multiplication and the *in vitro* responses are largely dependent on the source of the explants. Amoo and Ayisire (2005) also reported auxin specificity of *P. biglobosa* in response to treatments *in vitro.*

Effect on number of shoots

The number of shoots had a high significant effect on the hormone-concentration interaction (P=0.05) and the age-hormone-concentration interaction (P=0.01). The mean ranging from 2.18 (0.75mg/l BAP) to 0.75 (1.00mg/l KIN) and 2.50 (8 weeks old explants in 1.00mg/l NAA) to 0.75 (12 weeks old explants in 1.00mg/l KIN) for hormone-concentration interaction and age-hormone-concentration interaction respectively (Table 2 and 3). The graph (Figure 3) indicated the relationship that occurred among all the treatment factors in relation to number of shoots was not significant (P=0.05). The 0.75mg/l concentrations in all the treatments had more shoots than other concentrations and BAP hormone across all the treatments induces more shoot proliferation except at 12 weeks old apical nodes where it tends to decrease.

The highest mean 3.25 was observed at the 8 weeks old axillary node in 1.0 mg/l NAA. The elongation and multiplication of shoots increases as the concentration increases up to 0.75mg/l in most treatments and BAP treatments recording more shoots. A high cytokinin to auxin ratio leads to more shoot proliferation as reported by Ayisire *et al* (2009). This observation concurred with the study by Uddin *et al*., (2005) who reported multiple shoots and rapid shoot length increase from nodal cuttings of *Peltophorum pterocarpus* (another woody legume) using MS and WPM, Sambe *et al* (2010) also reported more number of shoots in treatments with BAP than other hormones used in the multiplication of *P. biglobosa* using MS medium.

Effect of number of nodes

The number of nodes had a significant effect (P=0.05) at the age and the age-hormone interaction (Table 1 and 2). 8 weeks node (4.86) mean is higher than the 12 weeks node in the table and the age-hormone effect had the lowest at mean 3.03 (12 weeks old explants in KIN) and highest at mean 5.28 (8 weeks old explants in BAP). The graph further illustrated the relationship that occurred

Table 1. Treatments means relationship and LSD of the parameters measured of *P.biglobosa*.

TREATMENT	SL	NS	NN	NL
AGE				
8wks exp	3.45	1.77	4.86	4.32
10wks exp	3.24	1.26	3.45	3.34
LSD	0.397	0.277	0.567	0.758
Significance	NS	*	*	*
PPRTS.				
Axi	3.54	1.66	4.28	4.02
Api	3.15	1.36	4.03	3.65
LSD	0.397	0.2770	0.567	0.758
Significance	NS	*	NS	NS
CONC.				
0.0mg/l	2.66	1.25	4.19	2.31
0.5mg/l	3.11	1.39	3.54	4.04
0.75mg/l	4.43	1.89	4.50	4.50
1.00mg/l	3.17	1.52	4.40	4.48
LSD	0.561	0.3917	0.802	1.072
Significance	*	NS	NS	*

*Significant at 0.05 level of probability **Significant at 0.01 level of probability. NS Non significant. Shoot length (SL), number shoots (NS), number of nodes (NN) and number of leaves (NL)

Table 2. The relationship between treatments means interaction of *P. biglobosa* parameters measured and its LSD.

TREATMENT	SL	NS	NN	NL
AGE.PPRTS				
8wks axi	3.80	2.042	4.85	5.00
8wks api	3.11	1.500	4.87	3.65
12wks axi	3.29	1.292	3.71	3.04
12 wks api	3.19	1.229	3.19	3.65
LSD	0.561	0.3917	0.802	1.072
Significance	NS	NS	NS	*
AGE.HORMN				
8wks NAA	3.85	1.781	4.06	3.41
8wks KIN	3.22	1.500	5.25	5.34
8wks BAP	3.55	2.031	5.28	4.22
12wks NAA	3.45	1.344	3.78	3.91
12wks KIN	2.86	1.156	3.03	2.97
12wks BAP	3.41	1.281	3.53	3.16
LSD	0.561	0.4798	0.983	1.313
Significance	NS	NS	*	*
HORMN.CONC				
0.0mg/l NAA	2.67	1.375	4.06	2.38
0.50mg/l NAA	3.24	1.188	2.69	3.37
0.75mg/l NAA	3.97	1.625	4.25	4.00
1.00mg/l NAA	3.79	2.062	4.69	4.88
0.00 mg/l KIN	2.66	1.188	4.25	2.38
0.50mg/l KIN	2.61	1.500	3.19	4.44
0.75mg/l KIN	4.70	1.875	4.75	5.25
1.00mg/l KIN	2.49	0.750	4.38	4.56
0.0mg/l BAP	2.66	1.187	4.25	2.19
0.50mg/l BAP	3.48	1.150	4.75	4.31
0.75mg/l BAP	4.62	2.187	4.50	4.25
1.00mg/l BAP	3.24	1.750	4.12	4.00
LSD	0.972	0.678	1.390	1.857
Significance	NS	*	NS	NS

*Significant at 0.05 level of probability**Significant at 0.01 level of probability. NS Non significant. Shoot length (SL), number shoots (NS), number of nodes (NN) and number of leaves (NL)

Table 3. The relationship among treatments means interactions of *P. biglobosa* parameters measured and its LSD.

TREATMENT	SL	NS	NN	NL
AGE.HORMN.CONC				
8wks 0.0mg/l NAA	2.41	1.625	4.38	2.75
8wks 0.50mg/l NAA	3.53	1.250	2.88	3.38
8wks 0.75mg/l NAA	3.70	2.000	4.62	3.75
8wks 1.00mg/l NAA	3.91	2.500	4.38	3.75
8wks 0.0mg/l KIN	2.41	1.250	4.75	2.75
8wks 0.5mg KIN	3.38	1.750	4.12	5.50
8wks 0.75mg/l KIN	4.94	1.875	6.00	6.13
8wks 1.00mg/l KIN	2.77	0.750	6.12	7.00
8wks 0.0mg/l BAP	2.41	1.250	4.75	2.75
8wks 0.5mg BAP	2.94	1.875	5.25	4.88
8wks 0.75mg/l BAP	4.66	2.125	5.62	4.50
8wks 1.00mg/l BAP	4.35	1.750	5.50	4.75
12wks 0.0mg/l NAA	2.94	1.250	3.75	2.00
12wks 0.5mg/l NAA	2.95	1.875	2.50	3.38
12wks 0.75mg/l NAA	4.25	2.125	3.88	4.25
12wks 1.00mg/l NAA	3.66	1.750	5.00	6.00
12wks 0.0mg/l KIN	2.90	1.125	3.75	2.00
12wks 0.5mg/l KIN	1.85	1.250	2.25	3.37
12wks 0.75mg/l KIN	4.46	1.875	3.50	4.37
12wks 1.00mg/l KIN	2.21	0.750	2.63	2.12
12wks 0.0mg/l BAP	2.90	1.125	3.75	1.62
12wks 0.5mg/l BAP	4.03	1.125	4.25	3.75
12wks 0.75mg/l BAP	4.59	2.250	3.38	4.0
12wks 1.00mg/l BAP	2.12	1.750	2.75	3.25
LSD	1.375	0.9596	1.965	2.626
Significance	NS	**	NS	NS

*Significant at 0.05 level of probability **Significant at 0.01 level of probability. NS Non-significant. Shoot length (SL), number of shoots (NS), number of nodes (NN) and number of leaves (NL)

among all the treatment combinations with highest mean 7.00 (8 weeks apical nodes of 0.75mg/l and 1.00mg/l KIN respectively) to the lowest 1.00 (12 weeks apical nodes of 1.00mg/l KIN) (Figure 4). It was observed that 0.75mg/l and 1.00 mg/l of all the treatment had an increase in number of nodes with the exception of 12 weeks old axillary node in 0.75mg/l BAP and apical nodes in 1.00mg/l of KIN and BAP respectively.

The BAP hormone also showed poor response to number of nodes produced in the explants which agrees with the findings of Sambe *et al* (2010) and Mao *et al* (1999) that apical nodes tends to have more node proliferation on shoots in a plantlet. However, Sambe *et al* (2010) observed that BAP tends to have more node proliferation at 0.5mg/l concurs with these findings where node was observed at 0.5mg/l BAP compared to NAA and KIN. Satyanarayan *et al.,* (2008) also reported more shoots bud initiation using BAP than KIN on *Mucuna pruiriens* but did not agree with these findings as the BAP exhibited its optimum level of shoot initiation at 0.5mg/l except 8 weeks apical nodes in KIN.

Effect on number of leaves

Results in Tables 1 & 2 showed that there are significant effects (P=0.05) for age, concentration, age-plant parts interaction and the age-hormone interaction on the number of leaves. Age and concentration had the highest mean at 4.32 (axillary nodes) and 4.50 (0.75mg/l) and the lowest at 3.40 (apical) and 2.31 (0.00mg/l) respectively. Significant effect highest mean was observed at 5.00 (8 weeks old axillary nodes) and lowest mean 3.04 (12 weeks axillary nodes) in the age-plant parts interaction. The age-hormone interaction is highly significant (P=0.01) with the highest mean 5.34 (8 weeks old

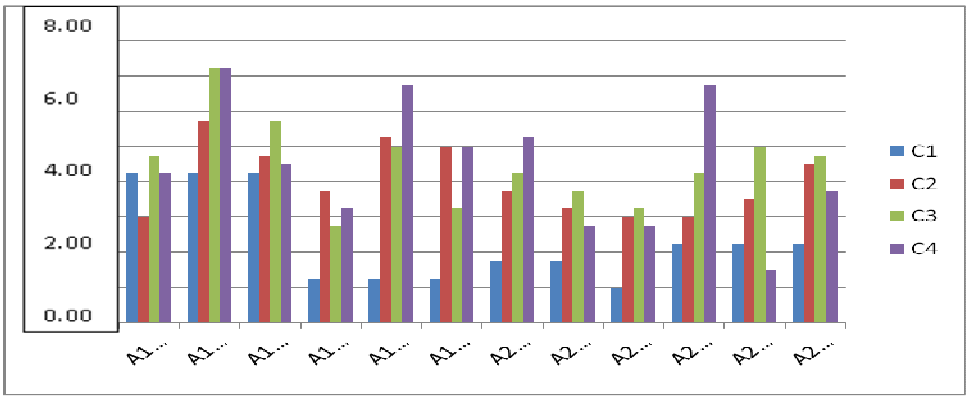

Figure 5. Number of leaves from the explants in response to the treatment factors. X-axis represents treatment factors. A1= 8 weeks old explants, A2= 12 weeks old explants, P1= Axillary nodes, P2= Apical nodes, H1= NAA, H2= KIN, H3= BAP. Y-axis represents concentration levels. C1= 0.00mg/l, C2= 0.50mg/l, C3= 0.75mg/l, C4= 1.00mg/l

explants in KIN) and the lowest mean 3.16 (12 weeks old explants in BAP).

Figure 5 illustrated the relationship that occurred among all the interactions of the treatment combinations. The number of leaves had an increase with 8 weeks old explants of axillary nodes. It was also observed that KIN had more number of leaves than other hormones except at 1.00mg/l concentration of the 12 weeks old explants. Although no literature available on the effect of hormone on number of leaves of *P. biglobosa*, Al-Bahrany (2001) reported similar results that a rise in buds and leaves of *Citrus aurantifolia* when supplemented with BAP and KIN. *In vitro* shoot multiplication and rooting of *Balanites aegyptiaca* (a multipurpose evergreen tree) has also been reported to have multiple leaves when supplemented with NAA and BAP. (Mansor *et al.,* 2003).

CONCLUSION

8 weeks old axillary nodes supplemented with 0.75mg/l BAP or less gives more shoot proliferation than other treatments in the study which implies that organogenesis using juvenile nodal cuttings at the apex from inoculated seeds would be effective in carrying out rapid clonal propagation and germplasm conservation of *Parkia biglobosa*. Also, the nutrient medium and the hormonal combinations at different concentrations used for multiple shoot induction in this study can be modified to enhance a better multiple shoot induction to increase the number of plantlets per explants culture.

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