CALLUS INDUCTION FROM EPICOTYL AND HYPOCOTYL EXPLANTS OF *PARKIA BIGLOBOSA* (JACQ.) BENTH

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Abstract

Epicotyl and hypocotyl explants of *Parkia biglobosa* (Locust bean) were cultured *in vitro* to investigate their callogenic capacity. Established cultures were obtained and maintained on MS medium supplemented with either 2,4-D or NAA, each of concentration range of 0.4-1.0 mg/L. In general, while higher concentrations of 0.8 and 1.0 mg/L NAA appeared to have favoured callus production from these explants, the same concentrations of 2, 4-D appeared to have caused inhibition. The incubation period has an additional effect on callus production with respect to the epicotyl explants.

It is concluded that both epicotyl and hypocotyl explants of *Parkia biglobosa* can serve as sources of callus production, a starting point for a range of *in vitro* studies including micropropagation and conservation of this endangered species.

Keywords: Callus, epicotyl, hypocotyl, *Parkia biglobosa,* auxin, micropropagation.

1. Introduction

Parkia biglobosa commonly known as African Locust bean is a perennial, deciduous tree, indigenous to Africa, and it belongs to the sub-family Mimosaceae in the family Leguminosae or Fabaceae. It is generally found in diffuse pattern within the guinean and sahelian savanna (latitude 7° – 13°N) of West Africa.

Parkia biglobosa, a multipurpose agroforestry tree, plays vital ecological roles in cycling of nutrients from deep soil. Its bark, leaves, seeds and pulp are of medicinal value, being used in the treatment of more than forty ailments (Quedraogo, 1995). Lemmich *et al.* (1996) found the leaves effective as molluscicide in the control of schistosomiasis. The fermented seeds produce a protein-rich condiment and the fruit pulp serves as fodder for livestock (Alabi, 1993).

Parkia regeneration is, however, affected by over-exploitation of land, particularly in densely populated and dry areas. Furthermore, its seeds have been found to suffer from dormancy (Etejere *et al.*, 1982; Tsamani, 1991). *Parkia biglobosa*, despite its economic importance, is uncultivated and yet it is a constant victim of uncontrolled burning which is a regular feature of the savanna, the ecological zone where it is mostly populated. According to Okafor (1993), *P. biglobosa* is an endangered species and the need to preserve it from imminent danger of extinction has been highlighted by Alabi (1993).

Conventional propagation through seeds certainly cannot meet the demand for this valuable tree in time because of the dormancy of the seeds. *In vitro* propagation methods offer powerful tools for plant gernplasm conservation as well as for rapid multiplication. It has been shown that shoot organogenesis from callus cultures can be used as an effective method for multiplication of medicinal plants (Sarasan *et al.*, 1994; Lusia and Rojas, 1996; Ahroni *et al.*, 1997). Plant regeneration via embryogenesis has been reported in calli initiated from explants of *Swainsona formosa* (Sudhersan and AboEl-Nil, 2002), *Anarcadium occidentale* (Jha, 1988) and *Theobroma cacao* (Esan, 1977).

Central to plant regeneration via these techniques is the production of callus. This paper reports the results of experiments carried out to determine the basic procedures for successful callus induction from epicotyl and hypocotyl explants of *Parkia biglobosa*. This is the first report on callus production by seedling explants of *Parkia biglobosa*, to the best of our knowledge.

2. Materials and Methods

(a) Source of plant material and germination

Ripened, mature fruits of *Parkia biglobosa* were collected from Ilorin in savanna belt of North Central zone of Nigeria and the seeds were extracted. Extracted seeds were scarified with concentrated sulphuric acid for 15 minutes, and rinsed in four changes of sterile distilled water before they were

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germinated aseptically in 250 cm^3 Erlenmeyer's flask containing 1% agar.

(b) Preparation of Media and Culture of Explants

The basal medium consisted of Murashige and Skoog (MS) (1962) macro-and micro-elements, vitamins (Nitsch and Nitsch, 1965), 3% sucrose, 10 mg/L Ascorbic acid, 0.1 g/L myo-mositol, 0.08 g/L Adenine sulphate and 0.02 g/L cysteine. The pH of the medium was adjusted to 5.7, gelled with 0.8% agar and autoclaved for 15 minutes at 121 °C and 108 kPa.

The effects of two auxins, Naphthaleneacetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) on callus induction were investigated. In each treatment, four different concentrations (0.4, 0.6, 0.8 and 1.0 mg/L) were employed. The basal medium without any auxin served as the control.

Explants were obtained from 7-day old seedlings grown aseptically. The explants were sterilized in 2.5% NaOCl + 2 drops of Tween 20 per 100 ml for 25 minutes. They were then rinsed in four changes of sterile distilled water and the edges trimmed off. They were then cut into approximately 5 mm segments.

Three or four epicotyl segments and one hypocotyl segment from a seedling were separately cultured on 25 ml sterilized medium. The cultures were incubated for eight weeks. Time of callus initiation in each medium was recorded and callus production scored (Cousins and Saenger, 2002). Each treatment had three replicates and the experiments were repeated three times.

3. Results and Discussion

A little extension growth of either the epicotyl segments or the hypocotyl segment was observed in all the treatments and their controls that lacked any growth regulator. No callus was induced in any of the media without growth regulator, though the explants remained healthy throughout the culture period. Street (1977) explained this extension growth or swelling as a wound response that is characterized by limited cell division and a rapid increase in metabolic activity.

Callus initiation was observed in all the epicotyl explants cultured on medium containing the varied concentrations of NAA. This was first observed five days after inoculation in culture containing 1.0 mg/L NAA which also produced the largest callus size of all the NAA concentration used (Plate 1). The epicotyl explants cultured on 0.4 mg/L NAA, the lowest concentration, produced the least callus size (Table 1). The induced calli were creamy coloured and compact.

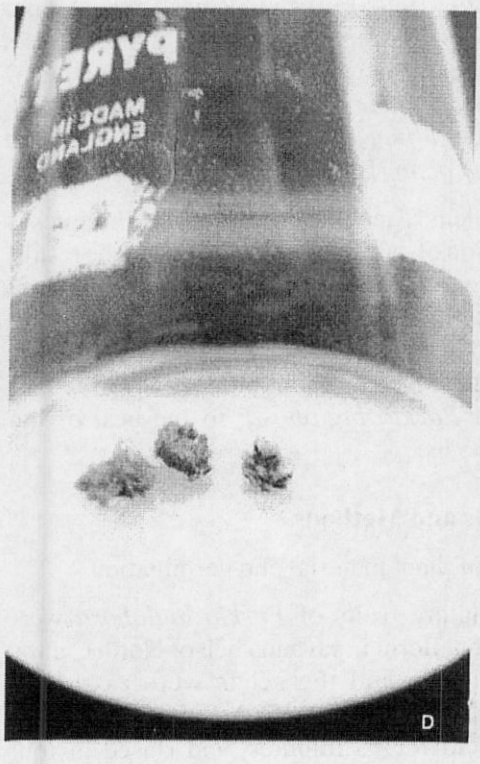

Plate 1: Epicotyl explants on modified MS basal medium supplemented with 1.0 mg/L NAA

Table 1: The effect of MS medium supplemented with NAA on epicotyl explants.

Medium	NAA concentrations (mg/L)	Callus induction	Order of callus size	Order of callus initiation
Full strength of modified MS medium	0	---	---	---
	0.4	+	1	III
	0.6	+	2	III
	0.8	+	3	II
	1.0	+	4	I

KEY TO OBSERVATIONS

+ = Callus induction
--- = No callus induction
1-4 = Order of callus size
I-III = Order of callus initiation

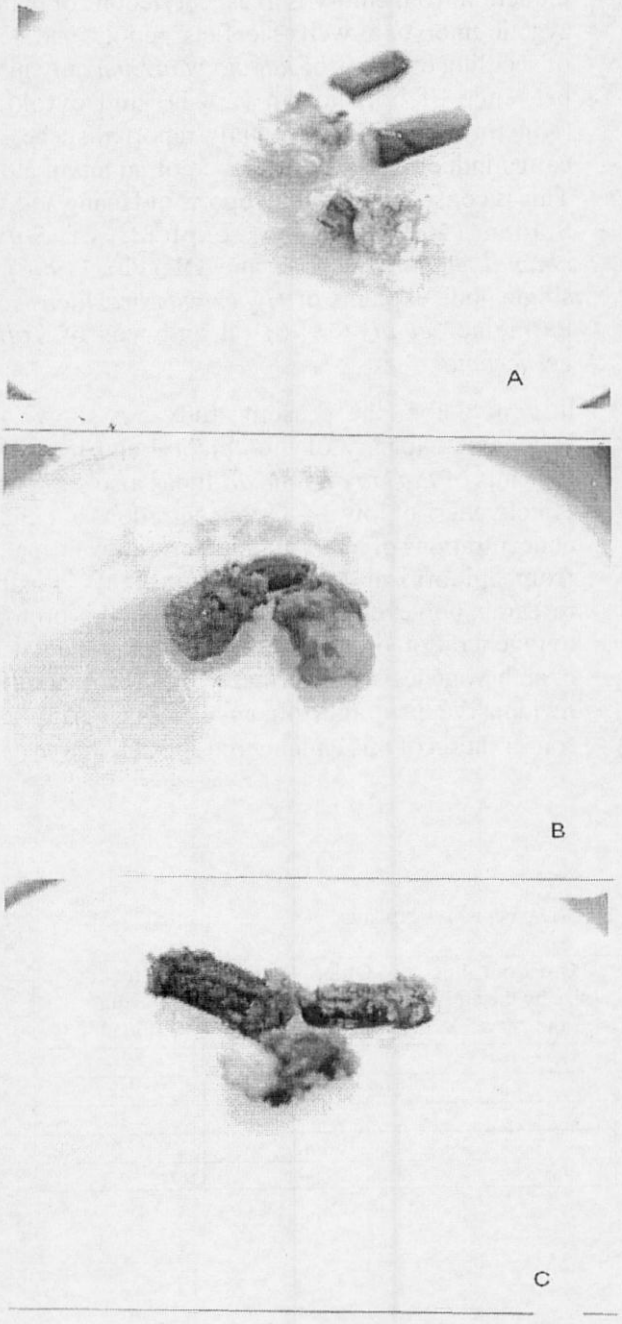

Plate 2: Epicotyl explants on modified MS basal medium supplemented qith 2,4-D concentrations.

(A) 0.4 mg/L
(B) 0.6 mg/L
(C) 0.8 mg/L

In the treatment of epicotyl explants with 2,4-D, callus induction was observed in all the concentrations investigated except 1.0 mg/L which was the highest concentration. Callus initiation became apparent on the 7th day with the medium containing 0.8 mg/L 2,4-D. The order of callus size was observed to change with incubation period (Table 2). The largest callus formation was observed in culture containing 0.8 mg/L 2,4-D and the smallest callus size was obtained in the medium containing 0.4 mg/L 2,4-D by the end of the second week of culture. By the fourth week, however, the reverse was the case (Plates 2A – C) that is, 0.8 mg/L 2,4-D induced the smallest callus size whereas the largest callus size was formed in 0.4 mg/L 2,4-D medium. Morini *et al.* (2001) reported similar results in *in vitro* cultured quince leaves where auxin concentration and induction period affected callus production.

With reference to hypocotyl explant cultured on media containing different NAA concentrations, callus formation was observed in all the treatments except the medium containing 0.4 mg/L NAA. Callus initiation was first observed on the 6th day in culture medium containing 1.0 mg/L NAA. The largest callus formation was developed on the medium containing 0.8 mg/L NAA while the smallest callus size was formed on the medium containing 0.6 mg/L NAA (Table 3).

On the other hand, all the 2,4-D concentrations induced callus formation with the hypocotyl explant. While callus formation was first observed on the 7th day in media containing higher concentrations (1.0 mg/L and 0.8 mg/L) of 2,4-D (Table 4), the medium containing 0.6 mg/L 2,4-D produced the largest callus formation by the end of the duration of the experiment (Plate 3). The size of callus produced was observed to decrease with increasing concentration of 2,4-D. This is similar to report made by Mokhtarzadeh and Constantin (1978) on Berseen clover and that of Gowda and Satyan (1984) on hypocotyl explant of *Vigna unguiculata.* The 2,4-D induced callus was compact and creamy in colour.

A statistical analysis using Cochran's Q-test revealed that callus production from both epicotyl and hypocotyl explants was dependent on auxin concentration ($Q=51.75$, $Q> \chi^2_{.05(3)}$). Generally, MS medium fortified with either low concentrations of 2,4-D or high concentrations of NAA favoured callus production. This may be because NAA is a less active auxin compared to 2,4-D. Unlike in monocots requiring a higher concentration of 2,4-D for callus induction, dicots are sensitive to 2,4-D. While concentration of 0.004 µm of 2,4-D induced germination of *Cucumeropsis edulis* seeds, 0.04 µm 2,4-D reduced hypocotyl growth of the seedlings (Ayisire *et al.*, 1997). Obembe (1995) also observed that relatively high concentrations of 2,4-D tended to inhibit viable callus formation in *Cola nitida.*

Callus production from vegetative tissues of dicot plants especially woody angiosperms is well documented. For example, hypocotyl explants of *Albizzia richardiana* (Tomar and Gupta, 1988), as well as seedling stem explants of *Acacia mangium* (Xie and Hong, 2001) have all been found to be

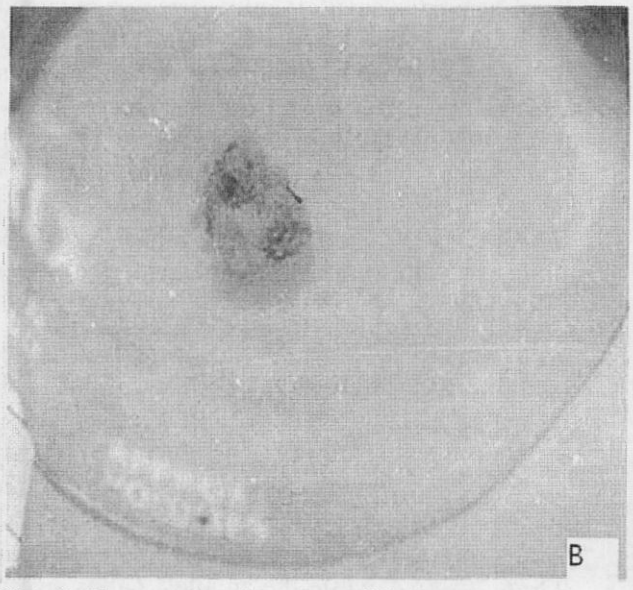

Plate 3: Hypocotyl explant on modified MS basal medium supplemented with 0.6 mg/L 2,4-D

callogenic. Rita and Floh (1995) reported successful callus production in leaf and stem explants of *Cuphea ericoides* only in the presence of both auxin and cytokinin. Xie and Hong (2001) also reported callus induction from embryos axes, cotyledons of mature zygotic embryos as well as leaflets, petioles and stems of seedling explants of *Acacia mangium* only in the presence of both auxin (2,4-D) and cytokinin (Kinetin). However, this study reported successful callus induction in the presence of an auxin alone. This is consistent with the reports of Huang and Van Staden (2002) on leaf explants of *Salvia chamalaegnea,* Cardoso and Oleveira (1996) on single node explants of *Hypericum brasiliense* and Farmelaer *et al.* (1996) on embryos of *Tulipa gesneriana.*

In conclusion, the present study has shown the callogenic capacity of the epicotyl and hypocotyl explants of *Parkia biglobosa*. It has also shown the effectiveness of low 2,4-D concentrations but higher concentrations of NAA at inducing callus formation from epicotyl and hypocotyl explants. Further research, however, is needed to extend this protocol to regeneration of plantlets by either organogenesis or embryogenesis, efforts that will no doubt manifest in massive propagation as well as germplasm conservation of this endangered species.

Table 2: The effect of MS medium supplemented with 2,4-D on epicotyl explants.

Medium	2,4-D concentrations (mg/L)	Callus induction	Order of callus size by the end of the 2nd week	Order of callus size by the end of the 4th week	Order of callus initiation
Full strength of modified MS medium	0	---	---	---	---
	0.4	+	1	3*	II
	0.6	+	2	2*	II
	0.8	+	3	1*	I
	1.0	---	---	---	---

KEY TO OBSERVATIONS
+ = Callus induction
--- = No callus induction
I-II = Order of callus initiation
1-3 = Order of callus size by the end of the 2nd week
1* - 3* = Order of callus size by the end of the 4th week

Table 3: The effect of MS medium supplemented with NAA on hypocotyl explant.

Medium	NAA concentrations (mg/L)	Callus induction	Order of callus size	Order of callus initiation
Full strength of modified MS medium	0	---	---	---
	0.4	---	---	---
	0.6	+	1	III
	0.8	+	3	II
	1.0	+	2	I

KEY TO OBSERVATIONS
+ = Callus induction
--- = No callus induction
I – III = Order of callus initiation
1-3= Order of callus size

Table 4: The effect of MS medium supplemented with 2,4-D on hypocotyl explant.

Medium	2,4-D concentrations (mg/L)	Callus induction	Order of callus size	Order of callus initiation
Full strength of modified MS medium	0	---	---	---
	0.4	+	2	II
	0.6	+	4	II
	0.8	+	3	I
	1.0	+	1	I

KEY TO OBSERVATIONS
+ = Callus induction
--- = No callus induction
I – II = Order of callus initiation
1 - 4 = Order of callus size

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