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Full Length Research Paper

Induction of callus and somatic embryogenesis from cotyledon explants of *Parkia biglobosa* (Jacq.) Benth

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To explore the potential for *in vitro* rapid regeneration of *Parkia biglobosa*, an endangered multipurpose woody angiosperm, cotyledon explants obtained from 7-day old aseptically germinated seedlings were cultured on Murashige and Skoog (MS) basal medium supplemented with 0.4-1.0 mg/L naphthalene acetic acid (NAA) or 2,4-dichlorophenoxyacetic acid (2,4-D). Cotyledon explants were placed either with the abaxial surface facing up or down on the media. Induced calli were subcultured on media containing 0.8 mg/L Kinetin combined with different concentrations (0.2, 0.6 and 1.0 mg/L) of 2,4-D. Unlike in NAA, successful calli induction was observed in all concentrations of 2,4-D investigated. Callus production was only at the cut edges and on the abaxial surface of the explants. Induced calli turned friable, more nodular and with small protuberances on media containing 0.8 mg/L Kinetin combined with either 0.2 mg/L or 0.6 mg/L 2,4-D. The protuberances eventually developed into somatic embryos in an auxin-free suspension culture medium. The developed protocol established the potential to produce plantlets from cotyledon explants through somatic embryogenesis. It also offers itself as a highly efficient method for mass clonal propagation and conservation of *P. biglobosa*.

Key words: *Parkia biglobosa*, callus induction, somatic embryogenesis, cotyledon explants.

INTRODUCTION

In Nigeria today, deforestation is resulting in substantial loss of plant diversity of indigenous germplasm. As a result of over-exploitation and lack of purposeful management in the past, *Parkia biglobosa*, commonly called African locust bean has become one of the indigenous trees threatened with extinction. It is a multipurpose tree that is not naturally cultivated. Okafor (1993) and Adeofun (1993) have already listed it as an endangered species. Etejere et al. (1982) observed that only a small percentage of the seeds produced germinated in the field leading to low population of the crop in the savannah region. The dormancy of these seeds has been attributed to the presence of endogenous inhibitor as well as the impermeable seed coat (Fasidi et al., 2000).

To prevent extinction and derive maximum benefits from the indigenous plants of a nation, it is necessary to preserve the germplasm. Vegetative propagation by means of cell and tissue culture techniques is a powerful tool for plant germplasm conservation and rapid clonal multiplication as well as for reforestation and tree improvement (Reddy et al., 2001; Farnum et al., 1983; Biondi and Thorpe, 1982). *In vitro* vegetative propagation via somatic embryogenesis has been successful in some non-woody horticultural plants and its feasibility has been demonstrated in several woody plants (Tautorus et al., 1991). Esan (1997) achieved somatic embryogenesis using embryo axes of mature seeds and cotyledons of immature embryo of cocoa. Somatic embryogenesis has

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Table 1. The effect of MS medium supplemented with 2, 4-D on *P. biglobosa* cotyledon explants.

2,4-D concentrations (mg/L)	Callus induction	Order of callus size	Order of callus initiation
0	---	---	---
0.4	+	4	IV
0.6	+	3	III
0.8	+	2	I
1.0	+	1	II

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

also been reported on many medicinal plants (Sahrawat and Chand, 2002; Rao and Narayanaswamy, 1972). Plantlets through somatic embryogenesis are reported as true type (Jyanthi and Mandal, 2001; Ishii et al., 1998).

A review of literature has shown that information on *in vitro* studies of *P. biglobosa* is scanty. Up to now, no somatic embryogenesis has been reported on this multipurpose, endangered woody angiosperm. This study describes the basic procedures for the establishment of callus culture and induction of somatic embryogenesis from cotyledon explants of *P. biglobos*a as part of a study outlined to explore the potential for *in vitro* regeneration of this woody plant species.

Materials and Methods

Plant material and germination

Seeds were extracted from ripened, mature fruits of *P. biglobosa* which were collected from Ilorin in savanna belt of North Central zone of Nigeria. The extracted seeds were chemically scarified using concentrated sulphuric acid for 15 min, rinsed in four changes of sterile distilled water and then germinated aseptically in 250 cm^3 Erlenmeyer's flask containing 1% agar.

Culture of explants

Cotyledon explants were obtained from 7-day old seedlings grown aseptically. The explants were surface-sterilized in 2.5% NaOCl + 2 drops of Tween 20 per 100 ml for 25 min. The cotyledon explants were then wounded on all sides and cut with a sharp sterile knife into small fragments of about 5 mm × 5 mm. Three or four explants from a seedling were cultured on the prepared medium to which either naphthalene acetic acid (NAA) or 2,4-dichlorophenoxyacetic acid (2,4-D) had been added. Four different concentrations (0.4, 0.6, 0.8 and 1.0 mg/L) were investigated for each of the auxins. Also, for each hormonal treatment, the cotyledon explants were placed on the medium with either the abaxial surface facing up or down. The basal medium used comprised 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-inositol, 0.08 g/L adenine sulphate, and 0.02 g/L cysteine.

Cultures were incubated in the dark at 25 ± 2°C for duration of eight weeks for callus induction. Callus induction was visually evaluated and scored to aid a more rapid screening out of the options.

Induced calli were cultured on media containing a fixed concentration of kinetin (0.8 mg/L) combined with either 0.2, 0.6 or 1.0 mg/L of 2,4-D for callus proliferation. Calli that turned friable and nodular, following their proliferation were subcultured into liquid full strength of MS medium without growth regulator to form suspension cultures. These cultures were incubated on a flask shaker for a period of 4 weeks in a room of average temperature of 26 ± 2°C. At two weeks interval, small cell aggregates were removed aseptically from each culture, carefully separated and examined for various embryonic stages.

RESULTS AND DISCUSSION

While there was a little extension growth of the explants, no callus production was observed during the culture period in the control (that is, basal medium without growth regulator). All the treatments with NAA produced no callus throughout the duration of the culture period. However, browning of the explants was observed in all these treatments by the third week of culture.

In the treatment with 2,4-D, response in the form of callus production was observed only at the cut edges of the explants and on the abaxial surface, even when placed face down. This result shows that callus formation in a few cases is affected among other factors by orientation of the explants on the culture medium (Warren, 1991). This report agrees with the findings of Morini et al. (2001) in which they observed that callus formation occurred only on the abaxial surface of *Cydonia oblonga* leaf, which had been placed face up. Rita and Floh (1995) reported similar observation with the leaf explants of *Cuphea ericoides*. The fact that callus was induced by 2,4-D but not by NAA suggests that cotyledon explants of *P. biglobosa* are auxin specific. Zafar et al. (1995) also reported callus induction from cotyledon, hypocotyl and root explants of *Medicago littoralis* in the presence of 2,4-D alone and when it was replaced with NAA, the explants either died or showed poor differentiation. Harvey and Grasham (1969), while working on 12 species of conifers also reported species specificity for IAA, NAA and 2,4-D in their effectiveness for callus induction.

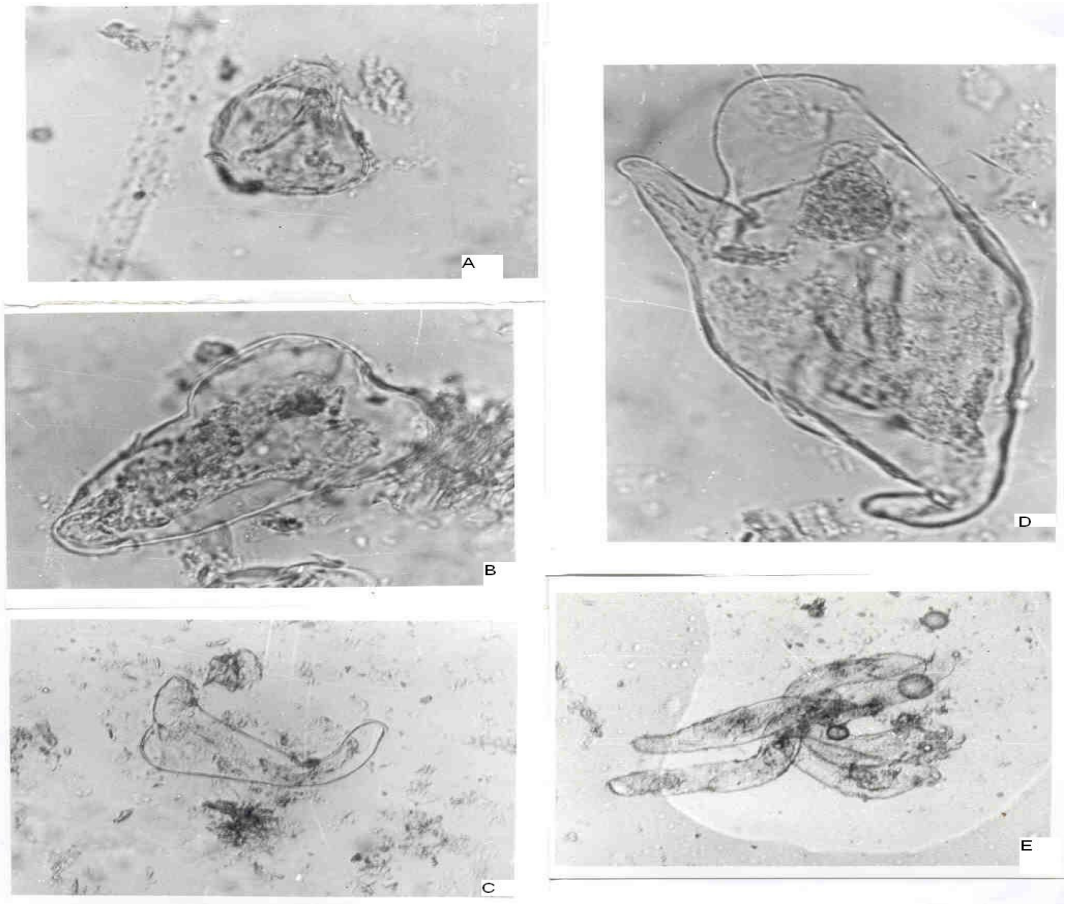

Figure 1. Development of *P. biglobosa* somatic embryos in suspension cultures. **A.** Globular stage; **B.** Heart-shaped embryo; **C.** Torpedo stage; **D.** Advanced torpedo stage; **E.** Early cotyledonary embryo.

All the investigated concentrations (0.4, 0.6, 0.8 and 1.0 mg/L) of 2,4-D showed callus production. Table 1 shows a specific order of callus initiation as well as a specific order of callus size observed during the culture period. While high concentrations (0.8 and 1.0 mg/L) were the first to induce callus production, low concentrations (0.4 and 0.6 mg/L) produced calli with a higher callus size. Callus production from cotyledon explants of *Juglans nigra* (Neuman et al., 1993) and seedling explants of *Albizzia procera* (Datta, 1987), all of which are woody trees have also been reported. In this study, callus production was successful in the presence of 2,4-D alone. This is in contrast to the observation by Xie and Hong (2001) in *Acacia mangium* where calli were reportedly induced from cotyledon explants of mature zygotic embryos in MS basal medium supplemented with both 2,4-D and Kinetin. Sondahl and Sharp (1978) also reported callus induction from *Coffea arabica* leaves in the presence of both 2,4-D and Kinetin. Cell proliferation was observed when the induced calli were subcultured on media supplemented with both 2,4-D and Kinetin. The most rapid cell proliferation was noticed on the basal medium fortified with 0.6 mg/L 2,4-D and 0.8 mg/L Kinetin and the least proliferation was observed on medium containing 1.0 mg/L 2,4-D and 0.8 mg/L Kinetin.

The calli generated, however, turned friable and more nodular on medium supplemented with either a combination of 0.2 mg/L 2,4-D and 0.8 mg/L Kinetin or 0.6 mg/L 2,4-D and 0.8 mg/L Kinetin. Small protuberances were also observed at the top of these nodular calli. Esan (1973), Obembe et al. *(*1999) and Ehsanpour (2002) all reported small protuberances on the top of growing calli and described these as certain globular or spherical morphogenic manifestations, which can actually develop into embryoids or ordinary shoots.

The transfer of friable nodular callus into suspension cultures lacking auxin facilitated the development of somatic embryos through different stages, from globular to early cotyledonary stages (Figure 1). Zimmerman (1993) observed that removal of auxin from the culture medium is a prerequisite to 'switch off ' several genes or to synthesize new gene-products that are necessary for

the successful completion of embryo development. The choice of suspension culture was informed by the work of Martin (2003), who reported the development of higher number of somatic embryos in suspension cultures than in solid medium cultures. Callus production followed by somatic embryogenesis has also been reported in cotyledon explants of *Juglans nigra* (Neuman et al., 1993), leaf explants of *Holostemma ada-kodien* (Martin, 2003) as well as in stem petioles and leaflet explants of *Swainsona formosa* (Sudhersan and Abo El-Nil, 2002).

Conclusively, this study has established the callogenic capacity of cotyledon explants of *P. biglobosa* .It has also established the possibility of inducing somatic embryogenesis from the induced calli. Further research in order to advance the embryoids to plantlets and their establishment in the field will be the next step. Meanwhile, this protocol offers itself not only as a highly efficient method for mass clonal propagation of this species but also for its conservation. It also opens up research on genetic transformation to improve this woody plant species.

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