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Short Communication

Multiple shoot induction from embryo derived callus cultures of cowpea (*Vigna unguiculata* l.) Walp

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The production of whole plant of cowpea (*Vigna unguiculata)* L. Walp from calli is an efficient, reliable and rapid strategy. This provides a faster method of micro propagation. Seeds of the pea were surface sterilized, and then the embryo axes were dissected and germinated. Callus growth was induced on the cut surface of the embryo of *V. unguiculata*. Root development in the cells was promoted by the action of the auxin, naphthalene acetic acid (NAA), at low cytokinin concentration. After five weeks, multiple shoots ranging from two to four developed from the calli cultures after being subcultured on media with high concentration of cytokinin, benzylaminopurine (BAP). Percentages shoot production from calli grown on media with 1 ∝M concentration of BAP was 45.5% while calli subcultured on 4 ∝M BAP produced 87.5% shoot production. Percentage rate of survival was between 21-26% in the hardened transplanted plantlets.

Key words: Explant, Casein hydrolysate, legume, callus, cotyledon, embryo.

INTRODUCTION

Cowpea (*Vigna unguiculata*) (L) Walp. is drought tolerant grain legume which has great agronomic interest as food and fodder. The grain constitutes an important source of dietary protein and secondary staple carbohydrate. It is a semi-arid crop adaptable to a wide range of geographical and environmental conditions including poor soil and limited rainfall. The plant has well developed taproots, which can grow to a depth of about 1 m. Lateral root in the subsurface region bear numerous *Rhizobium* nodules. It has a climbing or winding stem reaching a length of 2 to -4 m. Flower colour is mostly white and in some cases pink or purple. The pods (5 to 12 cm long and approximately 1 cm wide) are straight or slightly curved and possess a small “beak” at the end. The seeds vary considerably in size and colour (white, brown and black). Cowpea cultivars are highly susceptible to certain post-flowering insects and pests such as Pod-borers (*Maruca vitrata*) and pod sucking bugs (*Clavigralla* spp.), which cause substantial losses in grain yield.

Several efforts have been recorded on tissue culture practices on beans. Kobuyana et al. (1992) reported the use of embryo culture to generate mature plants *in vitro* from hybridization of three lines of the garden bean (*Phaseolus vulgaris L.*). Numerous publications are available on the micropropagation of various *Phaseolus* species. Allavena and Rosetti (1986) examined the genotypic dependence of different *P. vulgaris* varieties under varying culture regimes. Malik and Saxena (1992) showed that BAP and Thidiazuron (TDZ) improved the frequency of multiple shoot formation in *P. vulgaris* cuttings. Mohammed (1992) developed a protocol for the induction of multiple embryo shoot formation in axillary explants from zygotic embryos of *P. vulgaris* as well as for the regeneration of shoots to fertile plants.

This work was conducted to develop a micro-propagation method in cowpea through the induction of multiple shoots from embryo-derived calli.

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Table 1. Organ development observed in Calli after 3 weeks in the presence of 1 ∝M BAP.

Cultures	No of vials	Shoot development	Shoot and root development	Callus development
A	32	10	6	16
B	22	6	5	11
C	20	7	4	9
D	16	2	3	11

Table 2. Response of Embryo cultures on MS medium after increase in cytokinin (BAP) concentration (4 ∝M).

Culture	No of vials	Single shoot development	Multiple shoot development	Average shoot produced
A	18	6	10	2.72±0.1
B	12	3	8	3.11±0.12
C	12	2	10	3.41±0.11
D	14	6	4	3.32±0.07

MATERIALS AND METHODS

Cowpea seeds (accession number IT81D-994) were obtained from the International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria. The seeds were rinsed four times in running tap water later being washed again with 70% ethanol for 2 min and then four more times in double distilled water. The seeds were finally treated with 5% sodium hypochlorite (NaOCl) for 5 min and again rinsed four times with double distilled water. The seed cotyledons were opened in a laminar flow and the embryo was removed and cut horizontally into two. Each half was then cultured separately in different culture jars (labeled A, B, C, and D). The cultured explants were incubated in the Tissue Culture laboratory of the Cocoa Research Institute of Nigeria, (CRIN). Ibadan, Nigeria. The culture jars were incubated under white fluorescent lamp at 8 h photoperiod for callus induction at 26±2°C.

The explants were cultured on Murashige and Skoog medium containing 20 ml/L each of stock solutions of both macro and micro nutrients, 1 ml/L of vitamins and supplemented with 3% (w/v) sucrose, 0.3% (w/v) agar, 100 mg/L casein hydrolysate, 1 ∝M NAA, 1 ∝M BAP for the callus induction media. The medium was adjusted to pH 5.8 and autoclaved at 121°C (1.06 kg/cm^2) for 15 min. After the development of the calli, they were subcultured in jars containing a higher concentration of cytokinin (BAP) to facilitate the development of the shoots.

RESULTS AND DISCUSSION

Callus development was noticed in each of the induction media after one week of incubation with longer period of darkness (Figure 1, Table 1). Single shoot development was observed in most of the cultures containing 1 ∝M BAP after 3 weeks of incubation. Development of multiple shoots ranging from 2 to 4 shoots in most cultures (and about 5 shoots in two cultures) containing higher concentration of BAP (4 ∝M) was observed after 3 weeks of culture after the calli were subcultured (Figures 3-5). The percentage of single and multiple shoot production was higher in cultures containing higher concentration of cytokinin (Table 1). Generally, it was observed that shoot development occurred in all the cultures with 4 ∝M BAP (Table 2).

Successful shoot regeneration of cowpea has been reported from cultured tissues like shoot and root apices (Kartha et al., 1981, Pandey and Bansal, 1989), leaves (Muthukumar et al., 1995), somatic embryogenesis (Prem Anand, 2000) . Somatic embryogenesis from leaf explant (Kulothungan et al., 1995) and smooth nodular callus from zygotic embryos (Amitha and Reddy, 1996) have also been reported. Ideally, it is expected that a shoot develops from a seedling, however, *in vitro* propagation has produced multiple shoots from a seedling by increasing the concentration of the growth regulators in the media. The results obtained from this work have shown that it is possible to develop multiple shoots from embryo axils after cutting the embryo into two through callus development. This of course, indicated the possibility of *in vitro* propagation for desired cowpea genotypes. Malik and Saxena (1992) also reported multiple shoot formation in seed cuttings of *Phaseolus vulgaris* in cultures containing high concentration of TDZ or BAP (10 ∝M).

We observed that callus development started approximately 7 days after culture (Figure 1). In the jars containing 4 ∝M BAP, the number of shoots developed varied between 2 and 5 with the highest average of 3.41±0.1 (Table 2). This is as a result of high rate of cell division induced by the high concentration of cytokinin in

the medium. The total percentage of shoot development was drastically increased with higher concentration of BAP. The initial shoot development of 59.9% recorded for 1 ∝M concentration of BAP which developed only single shoot was increased to a total percentage of 87.5% with 57.1% of the cultures producing multiple shoots and 30.4% produced single shoot. This method is therefore recommended for effective *in vitro* micro propagation of desired cowpea genotypes.

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