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INDUCTION OF CALLUS IN COWPEA

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Cowpea *(Vigna unguiculata)* is drought tolerant grain in which various concentration and combinations of different growth regulators were examined for callus formation. Callus is produced from the leaf explant *in vitro* as a result of wounding and also in response to either endogenous or supplied hormones in the medium.

Keywords: Callus; Cowpea; 2,4-D; Leaf explant.

Cowpea *(Vigna unguiculata)* (L) Walp. is drought tolerant grain legume which has great agronomic interest as food and fodder. It can withstand heat better than most other legumes, but not cold or frost[1] . The grain constitutes an important source of dietary protein and secondary metabolites staple carbohydrate. It is semiarid crop adaptable to wide range of geographical and environmental conditions including poor soil and limited rainfall[2,3]. The plant has well developed tap roots which can grow to depth of about 1 meter. Lateral roots in subsurface region bear numerous rhizobium nodules. It has a climbing or winding stem reaching a length of 2-4 meter[1]. Flower colour is mostly white, in some cases pink or purple. The pods (5-12 cm long and approximately 1 cm wide) are straight or slightly curved and possesses a small beak at the end[4].

Vigna unguiculata (L.) Walp. was taken as experimental material during the present investigation. The seeds of cowpea were collected from Agriculture Research Station, Durgapura Jaipur. Explant taken from field grown plants of variety Pusa Komal. Murashige and Skoog's (MS-medium)[5] was used for all the cultural studies. The pH of medium was adjusted to 5.8 by adding 1N NaOH / 1N HCl using Toshniwal digital pH meter before autoclaving the medium. The medium was sterilized in an autoclave at 15 lb (psi) for 20 min.

The glasswares were washed with lab detergent (Labolene, Glaxo) and then washed with dilute chromic acid solution and then rinsed thoroughly with tap water followed by distilled water. Explants taken from the vegetative/reproductive parts of the plant were kept under running tap water for 15-20 minutes to remove any soil particles adhering to the explants. They were then washed with a regular mild detergent Extran/Teepol (0.2%) (Central Drug House, India) and rinsed three-four times with sterile distilled water. Explants were surface sterilized with (0.1%) mercuric chloride ($HgCl_2$) aqueous solution (w/v) for 3-5 minutes, followed by repeated rinsing (3-4 times) with sterile distilled water. These surface sterilized explant were now ready for inoculation on solid nutrient media. All the culture were incubated in a culture chamber at 25±2°C, 55±5% relative humidity, illuminated with 16 hr photoperiod with light intensity of 1400-2000 lux provided by white fluorescent tubes (40 watts and incandescent bulbs).

In the context of *in vitro* regeneration in cowpea explants were grown aspectically and established on MS medium[5]. Various concentrations and combinations of different growth regulators *viz.* auxin (IAA, NAA, 2,4-D) and cytokinins (kinetin and BAP) were investigated for this work.

Callus was produced from the leaf explant in vitro as a results of wounding and also in response to either endogenous or supplied hormones in the medium.

Leaf explant was cultured on MS basal medium having various phytohormones (Table 1), however, better response was obtained from kinetin and 2,4-D containing medium. Brownish and blackish callus was found on MS medium. The optimal concentration of Kn is 0.5 mg/l and 0.5 mg/l 2,4-D form maximum callus which when subcultured could not further differentiate into shoot buds (Fig.1. A, B, C).

Bhargava and Chandra[6] reported regeneration of *V. aconitifolia* from dedifferentiated callus cultures using BAP/Kinetin either alone or in combination with IAA. However, Godbole *et al.*[7] and Eapen *et al.*[8] regenerated plantlets of *V. aconitifolia* on hormone free B//MS media modified variously. In all these cases plantlets developed through organogenetic pathway. The difference in hormonal requirements may be due to differences in genotypes and explants used.

Godbole *et al.*[7] regenerated multiple shoots of *V. aconitifolia* from shoot apices explants on media combinations MS and B5 without hormones and with hormone and obtained multiple shoots and observed

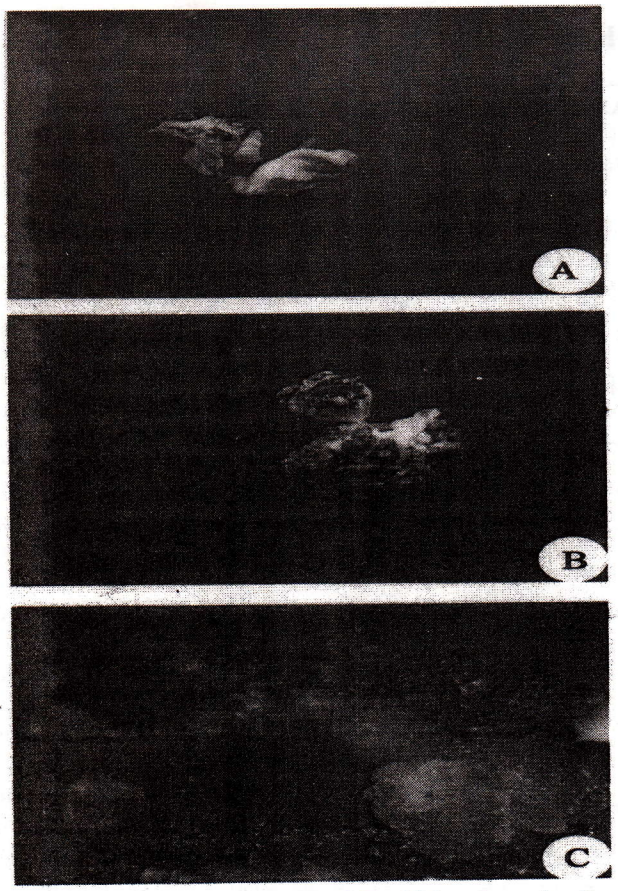

Fig.1.A, B & C. Brownish and blackish coloured callus raised on Kn 0.5 mg L^{-1} and 0.5 mg L^{-1} 2,4-D containing medium.

prolific regeneration from callus.

Present study favours the incorporation of both auxin and kinetin in callusing from leaf explant of cowpea. It can be concluded that callusing in cowpea is affected by various factors *viz.* medium constitution, hormonal ratios, temperature, age of the explant and donor plant and genotypes. Different concentration and combination of hormones play major role in the metabolic reaction and thus, affect the growth of callus cultures although environmental condition are equally effective.

Table 1. Effect of various concentrations of growth regulator on leaf explant.

Growth regulator (mgL^{-1})		Callusing
Kn	2,4-D	
0.1	-	-
0.25	-	C+
0.5	-	C++
1.0	-	C++
0.1	0.01	C++
0.25	0.01	C++
0.5	0.01	C++
1.0	0.01	C+++
0.1	0.1	C++
0.25	0.1	C+++
0.5	0.1	C+++
1.0	0.1	C+++
0.1	0.5	C+++
0.25	0.5	C+++
0.5	0.5	C++++
1.0	0.5	C+++

C+ - Low callusing; C++ - Good callusing, C+++ - Better callusing; C++++ - Best callusing

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