

INDUCED PESTICIDE RESISTANT CELL LINES IN CLUSTER BEAN THROUGH TISSUE CULTURE

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Five days old aseptically grown seedlings of cluster bean were excised as source of explants and inoculated them on B5-medium, containing 1.25 mg/l, 2,4-D and 1.0 mg/l kinetin and vitamins (myoinositol) for inducing callus. After the 6 weeks of growth period, fresh and friable callus were transferred to MS-medium containing various concentrations of Anthio (80, 100, and 120ppm) to induce resistance. The stressed callus were isolated at 100 ppm following direct selection procedure of biochemical variants. The cultures were maintained and growth index, cell variability, fresh and dry weights were found to be enhanced at 100 ppm concentration.

Keywords : Cluster bean; Pesticide resistance; Tissue culture.

Morphogenetic response in plant tissue cultures varies from plant to plant or even within the plant species¹. Pesticide resistance is of interest to plant scientist for several reasons for basic and applied study. Increasing interest of the herbicide resistance to crop plants can broaden the range of crops for which the particular pesticide is useful. One of the important ways of obtaining a pesticide resistance in plant system is through cell culture technique.

The certified seeds of cluster bean, a local cultivar Pusa Nacabhar, were obtained from National Seeds Corporation. The seeds were surface sterilized with freshly prepared 0.1% of HgCl₂ solution and thoroughly washed with sterile water. Explants were excised from five days old seedlings and cultured on B-5 medium² and MS basal medium³ with kinetin and other combinations of auxins (NAA, BAP & IAA). The excised explants of cluster bean cotyledons, epicotyledons, hypocotyledons, imma-

ture leaf, shoot base and roots were used separately. Friable and well grown callus was induced from the hypocotyle and cotyledonary explants. The same medium was incorporated with various concentrations of the Anthio (40, 60, and 120ppm) after autoclaving under aseptic condition to induce pesticide stress on the callus. After 4 week's growth period the surviving cell lines have been isolated from callus cultures grown on different test media. The callus growth was measured as fresh and dry weights, and cell viability with other morphogenetic aspects were also recorded.

In both MS and B-5 media various phytohormones responded variably in induction of callus. *Cyamopsis tetragonoloba* (L.) Taub. required low level of auxins (IAA, BA, NAA) and high level of 6-furfuryl aminopurine (2.25 mg/l) for good growth. Among various explants used in the present study, the morphogenetic activity of

Table 1. Selection of Anthio tolerant cell lines in cluster bean.

(Mean value of 10 replicates)				
Aspects observed	Controls	40 ppm	80 ppm	120 ppm
Percent viability	83.60	89.50	76.50	58.30
Growth index	12.07	14.53	10.32	08.38
Fresh weight	00.850	00.950	00.870	00.480
Dry weight	00.630	00.680	00.620	00.215

hypocotyl was more, followed by cotyledon, leaf, shoot base and root.

Highest percentage of callusination was observed with the hypocotyl explant. The static cultures were more vigorous and friable. The maximum (14.53) growth index was recorded after 9 weeks of growth period with 40 ppm of Anthio treatments. While working with same species Ramulu and Rao⁴ reported the similar results with regard to nutritional and growth requirements. Mitotic index was significantly enhanced while sub-culturing the static cultures with 1.75mg/l of NAA and 1.25 mg/l kinetin. For this PNB genotype a more uniform callus growth was observed on B- 5 medium with low level of 2, 4-D with Kinetin.

Differentiation may be due to auxin/cytokinin ratio responsible for morphogenesis in the excised plant tissue and cells. In the callus cultures of the clusterbean embryoid like structures have been observed enormously.

Table 1 shows that lower concentrations of pesticide, 40 ppm, were found to be promotory in to its activity with regard to growth index.

Maximum growth index and cell viability were recorded in the 40 ppm of Anthio stressed callus. A tendency of gradual retarding effect of the pesticide was noted. The total reduction in the other aspects were recorded at higher concentrations of Anthio treatments. This effect may be due to the severe influence of pesticide on metabolic activities. This is encouraging prospects of improving agronomically useful traits for selection system *in vitro*. The significant level of tolerance has been produced by an alternative selection by Oswald *et al.*⁵

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