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TISSUE CULTURE INDUCED GENETIC VARIABILITY IN CERTAIN FOOD LEGUMES FOR IMPROVEMENT OF AGRONOMIC TRAITS

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Plant cell and tissue culture comprises the various culture objective of developmental biology and crop modification. Various parameters like cell viability, inhibition of growth studies, plating efficacy and growth index were studied at different growth intervals. Certain cell lines of these food legumes were selected against Anthio, Monocrptophos and Atrazine.

Keywords : Agronomic traits; Genetic variability; Legumes; Tissue Culture.

Tissue culture has made tremendous progress in plant biotechnology for crop improvement by manipulating genetic material at cellular level. It involves picking up useful characters dispersed in numerous individual and putting them together to produce a variety that containing many desirable attributes. In conventional approach of crop improvement, establishment of static culture is a primary step in the tissue culture practice. Grain legumes are very important for their economic value as food and fodder, for their role in biological fixation of nitrogen and a raw material for industrial use. Increased awareness of the nutritional value of legumes has brought modern agriculturist to reconsider their strategies for genetic manipulation of these crops. Plant cell and tissue culture is considered in wide sense, which comprises the various culture objective of developmental biology and corp modification.

For the last one-decade new approaches were developed to produce cultures capable of regeneration into fertile plants in recalcitrant legume crops by either organogenesis or embryogenesis. The suspension culture system facilitates an experimental approach with a large variety of objectives for crop modification, cellular selection and transformation. Though legumes have been extensively used in plant cell and tissue culture system, regeneration of whole plants from *in vitro* experiments not amicable like other systems.

The certified seed material of three important food legumes of Chickpea (Annigiri), Cluster bean (Pusa Navabhar) and Cowpea (Local cultivar) obtained from Acharya N. G. Ranga Agriculture University, Research Station, Warangal (A.P.). The seed material were surface sterilized with 70% Ethanol followed by 0.1% Hg Cl_2 for about 6 min and germinated them on half strength MS medium[1] without any growth regulators. Depending upon the species and either MS or B5[2] medium was used with various concentration and combinations of plant growth regulators such as 2, 4-D, IAA, BA, Kinetin etc., for initiation of unorganized static cultures and morphogenetic studies.

The callus cultures were initiated successfully from seedling explants and 0.5g of tissue was transferred to a liquid M S medium for growth of suspension culture to perform cell line selection experiments. Due to very poor response to the liqid medium for growth of suspension cultures in chickpea and cluster bean the static cultures were used for selection studies on agar solidified petridishes containing herbicide in the medium. Observations on various parameters like cell viability, inhibition of growth studies, plating efficiency and growth index were recorded at different growth intervals. For morphogenetic studies and induction of multiple shoot from direct seedlings, especially from cotyledons and cotyledonary node, were used with various combinations of growth regulators. During the course of investigation, certain cell lines of these food legumes were selected against Anthio, Monocrptophos and Atrazine.

Freshly established suspension culture system on a liqued MS medium was used to study the influence of Glyphosate on gene amplification in chick pea. In the selected cell lines against 10 mM of Glyphosate, increased level of the target gene product EPSP synthase enzyme was observed in *Cicer arietinum* due to amplification of DNA[3].

In chick pea the static cultures have been established on higher levels of auxin (3.25 mg/l 2,4-D and 2.0 mg/l of NAA) on B5 medium with various seedling explants. Three different species of food legumes viz. *Cicer, Cymopsis* and *Vigna* were used. In cluster bean various aseptically grown one week seedling explants were cultured on B5 medium[2] with 1.50 mg/l 2,4-D and 0.50 mg/l kinetin. Root tips and immature leaf explants poorly responded for callusination with brownish clumps of cells proliferated at wounded sites. High phenolic secretions in

Table 1. *In vitro* growth response and selection studies for herbicide resistance.

Species	Medium	Explants	Callusination response of Explants in culture			Nature of cultures	Regeneration	Selection agents applied (ppm)
			F.W. %	D.W.	G.I.			
Cicer	B5	EC	6.30	2.83	11.60	74.30±1.03	Organogenic	MCP 90 ppm
	mg/l	Cot.	3.20	1.34	5.40	42.60±0.98(C)		
	2, 4-D	IL	2.90	1.65	4.80	39.40±0.68		
	B5.	Cot	5.20	1.93	9.40	70.35±2.01	--	Anthio
	3.25	RT	4.90	1.80	8.80	37.80±1.01.50		
		HC	*6.40*	*2.50*	*11.8*	*82.50±1.03(C)*	*Nil*	
Cyamopsis	6 mg/l	IL	2.30	0.80	3.60	40.60±1.09		
	2, 4-D	RT	1.90	0.90	2.80	21.40±1.30	–	–
	MS	Cot.	4.80	1.10	8.60	60.82±1.32	Embryogenic	Atrazine.
	2.75	HC	5.30	1.80	9.60	69.30±0.32 (C)		40 ppm
Vigna	mg/l	IL	1.80	0.95	2.60	34.20±1.39		
	2, 4-D	RT	1.56	0.84	2.12	40.30±0.69		

Cot : Cotyledons; HC : Hypocotyledons; EC : Epicotyl; IL : Immature leaf; RT : Root tip; IP : Immature pods; G.I : Grwoth index; MCP : Monocrptophos.

the medium caused browning of the callus and low callusination frequency[4]. High frequencies of callusination (82.50) with highest growth index (11.8) were observed in hypocotyl explants followed by cotyledons where growth index was 9.4 and percentage callusination was 70.35. In this case endogenous level of growth regulators were optimized with external supplementation in the medium which was responsibile for the cell proliferation.

The friable callus was subcultured on the same medium and used for selection against Anthio. This organophosphorus pesticide was able to induce tolerance at 60 ppm concentration on sensitive cluster bean static cultures and further using this we have slected Anthio resistant calli clones in *Cyamopsis tetragonoloba* (L.) Taub. These results were in conformity with other experimental studies on legumes with different species[5].

Cluster bean is a recalcitrant species among the food legumes, several attempts were made to induce somatic embryogenesis and *in vitro* shoot bud induction in the PNB cultivar. This cultivar is not amecable to tissue culture experiments, but few reports were available in literature about morhphogenetic ability of protoplast isolated from cotyledons[6, 7].

For callus cultures, the seedling explants are very efficient in producing optimum friable whitish morphogenic callus. An unorganized friable static cultures of *Vigna sinensis* were exposed to Atazine stress and isolated resistant cultures at 40 ppm concentrations[8]. In glyphosate resistant carrot cell suspension cultures the increased EPSP synthase activity and amplification of the target DNA confers the herbicide resistance[9]. In cowpea direct shoot formation was induced in cotyledonary explants on the same medium with BAP.

Using unorganized static culture system, derived from seedling explants, has been utilized for genetic modification of some important food legumes including beans[10].

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