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TISSUE CULTURE INDUCED HERBICIDE RESISTANCE AND SHOOT MULTIPLICATION IN CERTAIN FOOD LEGUMES

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Tissue culture technique has been used for crop improvement by manipulatiing genetic material at cellular and molecular level. During the present investigations effects of auxins were studied on shoot multiplication, callusination, morphogenesis and herbicide tolerance in cenrtain food legumes.

Keywords : Herbicide resistance; Food legume; Shoot multiplication; Tissue culture.

Introduction

Grain Legumes are very important for their economic value as food, fodder for their role in biological fixation of nitrogen. Tissue culture has made tremendous progress in Plant Biotechnology for crop improvement by manipulating genetic material at cellular and molecular level. For the last one decade new approaches were developed to produce tissue and cell culture techniques capable of regeneration in to fertile plants in recalcitrant Legume crops *via* 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. The importance of plant cell and tissue in applied and fundamental research has out lined by many workers. Though Legumes have been extensively used in plant cell and tissue culture system regeneration of whole plants from *in vitro* experiments are not amicable like other systems. This approach is tested in the food Legumes by comparing auxin and cytokinins requirements of callus tissue derived from different genotypes. The objective was to identify normal and plant types with distinct auxin and cytokinin response to be used in studies of genetic regulation of hormonal function and metabolism. This paper reports the results obtained from the study of auxins response to shoot multiplication, callusination, morphogenesis and herbicide tolerance in certain food Legumes.

Material and Methods

The certified seed material of three important food Legumes of Cowpea (Pusa), Chickpea (Annigiri), Cluster bean (Pusa Navabhar) were obtained from Acharya N.G.Ranga Agriculture University Research Station , Warangal (A.P). Aseptic seedlings were raised by surface sterilization of seeds with 70% ethanol for about 2min followed by 0.1% aqueous Mercuric chloride solution for about 4 min. The seeds were thoroughly washed with sterile water and then place aseptically on MS[1] or B5[2] medium containing sucrose and 0.8% agar-agar. All the media were adjusted to pH 5.8 before autoclaving at 1.04 kg/cm^2 for 15 min. The seeds were allowed to germinate at 25±2 ^{0}C under 16h photoperiod and light intensity of 2000 lux. Cotyledons from 3day old seedling were excised and cultured on MS, B5 media supplemented with different concentrations of BA (0.1-2.0 mg/l), Ads (0.1-2 mg/l) and KN (0.1-2 mg/l) to investigate the morphogenetic potentiality and regeneration by production of multiple shoots.

After subsequent establishment of callus cultures, 0.5g of tissue was transferred to a liquid MS medium for growth and maintenance of suspension cultures to make selection of cell line experiments. In the case of chickpea and cluster bean the static cultures were used for selection studies on agar solidified petridishes containing herbicide in the medium. Parameters like, inhibition of growth studies, plating efficiency and growth index were studied at different growth interval and transferred them to fresh nutrients medium. To investigate the morphogenetic studies andmultiple shoot induction from seedling explants, we have used mainly cotyledons and cotyledonary nodal explants inoculating them on to the MS and B5 medium. For the last one and half decade experimental studies[3-10] were carried out under *in vitro* and *in vivo* interaction of pesticides and herbicide and their tolerance at cellular level using tissue culture system. During the course of these investigations certain cell lines selected against Atrazine, Anthio, Monocrptophos and Glyphosate.

Results and Discussion

The establishment of an efficient plant tissue culture is a basic step in static culture system to study the morphogenesis. We investigated on three different

Table 1. Tissue culture response of various explants on MS and B5 media of grain legumes for selection and propagation.

Species	Media	Explants used	Callusination response of explants in cultures				Narture of cultures	Regne-reations.	Selection agents applied (ppm)
			F.W.	D.W.	G.I.	Percent			
	MS	Cot.	5.40	1.24	9.80	69.30±0.32		---	----
	2.50	HC	5.70	1.38	10.4	84.82±1.32	(C)	Emb-	Atraz-
Vigna	mg/l	IL	3.80	1.25	6.60	34.20±1.39		ryog	ine.
	2,4-D.	RT	2.56	0.84	4.12	40.30±0.69		enic.	90 ppm
	B5.	EC	7.30	3.83	15.60	79.30±1.03		----	----
Cicer	2.25	RT	5.90	2.80	10.80	57.80±1.06		Orga-	MCP.
	Mg/l	Cot.	4.30	2.34	7.6	42.60±0.98	(C)	noge	60ppm
	2,4-D.	IL.	3.90	2.65	6.80	39.40±0.68		nic.	Conc.
	B5.	Cot.	4.90	1.63	8.80	60.35±2.80		---	Anthio.
	2.0	HC	6.20	2.40	11.4	84.50±1.03	(C)	NR	40 ppm
Cyamopsis	mg/l	IL	2.10	0.90	3.90	30.60±1.09			
	2,4-D.	RT	1.70	0.70	2.40	2.40±1.30	---	---	----

Cot: Cotyledons HT: Hypocotyledons. EC: Epicotyl. IL: Immature leaf.
RT: Root tip. IP: Immature pods. G.I: Growth index. MCP: Monocrotophos.
NR: Not responded C: Callus. S: Suspension M.S Medium

cultivars of food legumes, *Vigna, Cicer* and *Cymopsis* local varieties. 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 were poorly responded for callusination with brownish clumps of proliferated tissue wounded sites. Due to high secretion pressure of phenolic compounds on to the medium callus cultures were turned to brownish and to the callus and low frequency of callusination.For induction of callus cultures, the seedling explants are found to be very efficient in producing friable whitish efficient morphogenetic callus in *Vigna sinensis.* Somatic Embryogenesis and multiple shoot induction were made from *Vigna* coty ledonary explants with MS medium 1,5 mg/l BA where highest shoots (6.2) forming ability was observed and 69% of differentiation observed in the cultures (Table 2). Clonal multiplication of cotyledonary explants by induction of multiple shoots facilitates the *in vitro* genetic transformation and transgenic plant production of Cowpea.

An unorganised friable static cultures of *Vigna sinensis* were exposed to Atazine stress and isolated resistant cultures at 90 ppm conc. In glyphosate resistant carrot cell suspension cultures the increased EPSP synthase activity and amplification of the target DNA confers the herbicide resistance[11]. 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. In cowpea direct shoot bud formation was observed in low frequency while inoculating the cotyledonary explants on the same medium with BAP. Very recently we were able to establish the suspension culture system on a liquid MS medium and studies the influence of Glyphosate[9] on gene amplification. 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 corresponding DNA.

A high frequency of callusination (84.50) with highest growth index (11.4) were observed in hypocotyl explants followed by cotyledons where growth index was 8.8 and percentage (60.35) (Table 1). In this case endogenous level of growth regulators were optimized with external supplementation in the medium responsible for the cell proliferation. The friable callus was subcultured on the same medium for selection against Anthio. This organophosperous 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 defferent species.Cluster bean is a recalcitrant species among other food legumes, we have made several attempts to induce somatic embryogenesis and *in vitro* shoot bud induction in the PNB cultivar. This cultivar is not amicable to tissue culture experiments, but few reports are available in current literature about morhphogenetic ability of protoplast isolated from cotyledons of *Cymopsis.* We

Table 2. Morphogentic potential and induction of multiple shoots in Grain Legumes.

Plant Species	Media	PGR	Conc mg/l	Average No. Shoots	%Callus forming Shoots	%Cultures in Defferentiation.
Vigna	MS	BA	0.1	2.4±0.21	39	34
			0.5	*4.8±0.32	57	43
			1.5	*6.2±0.40	71	69
Cicer	B5	BA	0.1	*3.6±0.43	36	47
			0.5	*5.2±0.30	52	59
			2.0	5.0±0.42	58	52
Cyamopsis	B5	KN	0.1	1.3±0.32	34	27
			0.5	2.7±0.56	37	18
			2.0	*2.9±0.26	34	16

Mean values 7 replicates. * Significant at 1% level.

performed several experiments related to the *in vitro* studies of cluster bean and made documentation of several aspects related to tissue culture and morphogenesis[3,5,6].

The excised cotyledons from 3 days old seedling of four legume sps cultured on MS, B5 supplemented with BA, Ads and Kinetin to investigate their morphogenetic behaviour in production of direct multiple shoots and also from callus cultures and percentage of differentiation. Maximum number of shoots per explants 7.2±06with 59 % of differentiation was observed in *Glycine max* fallowed by 6.2±0.40, no shoots, with 69% differentiation in *Vigna* with Adenine sulphate 0.5 mg/l. Reduced number of shoots 2.9±0.26 with poor differentiation abilities 16% from cotyledon explants were observed in *Cicer* and *Cyamopisis* (Table 2) a recalcitrant species due to presence of low endogenous plant growth regulating substances.

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References

1. Murashige T and Skoog F 1962, A revised medium for rapid growth and bioassay with tobacco tissue cultures. *Physiol. Plant.* **15** 473-497.
2. Gamborg O L, Miller R A and Ojima K 1968, Nutrient requirements of suspension cultures of soybean root cells. *Exp.Cell.Res.* **50** 151-158.
3. Ramulu C A and Seeta Ram A 2004, Tissue culture induced genetic variability in certain food legumes for improvement of agronomic traits. *J.Phytol.Res.* **17**(2) 201-203.
4. Ramulu C A 1997, A study on EPSP synthase gene amplification in herbicide selected cell suspension cultures. International Food legume Research Conference III, Aldelaide Southern Australia, Sept,22-26,1997.
5. Ramulu C A 1989, *Experimental and Tissue culture studies in Cyamopsi stetragonoloba* (L.). Ph.D thesis Kakatiya University, Warangal-9 (A.P).
6. Ramulu C A and Rao D 1996, Tissue culture derived genetic modification of *Cicer arietinum* using pesticide. *Role of Biotechnology in Pulse Crops:* Page.155-158. Edited by ZafarNizam, Eukaz Publications, Hyderabad.
7. Ramulu C A 1996, Induction of Genetic tolerance in certain grain legumes using tissue culture methods (P-1052). World Congress on *in vitro* Biology (IAPTC) June, 22-27, At San Fransisco,CA, USA.
8. Seetha Ram A, Ramulu C A and Rao D 1991, Endosulfon induced resistant calli clones in Chick pea through tissue culture. IAPTC Conference at Anaheam, CA, USA.
9. Seetha Ram, Ramulu C A and Rao D 1991, Endosulfon induced pesticide resistant calli clones in chick pea through tissue culture. IAPTC Conference at Anaheim CA ,USA.
10. Srinivas B, Ramulu C A and Rao D 1987, Selection for amplification EPSP synthase gene in Glyphosate tolerant cell lines of Chickpea. Congress on *in vitro* Biology (P-1145), Washington.D.C, June 14-18, 1987.
11. Widholm J M, Ramulu CA, Hesook Song and Jeff Brotherton 1996, Glyphosate selected gene amplification in several species. American Society for Plant Physiologist (ASPP), Meeting at Texas, (U.S.A).