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TISSUE CULTURE STUDIES IN *VIGNA UNGUICULATA* (L.) WALP VAR. S-488.

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Callus cultures of *Vigna unguiculata* were established using seedling and field grown explants on MS medium supplemented with different auxins alone or in combination with CM/cytokinins at various levels. Profuse rhizogenesis was observed in most of the explants directly or indirectly. Regeneration of shoots/ and whole plants with multiple shoots, were obtained from cotyledonary node, shoot tips and nodal segments on various combinations of growth regulators. *In vitro* plants were transferred to soil to rear maturity. The present investigation revealed a high regenerative potential of shoot tips which is pre-requisite for clonal propagation.

Keywords : Tissue culture; Clonal propagation; *Vigna unguiculata*.

Introduction

The genetic improvement of grain legumes is essential for their immense use as human nutrition. *Invitro* plant regeneration among forage legumes has been very well established. However, success in grain legumes has been rather limited due to their restricted regenerative potential (George and Sherrington, 1984; Hamatt *et al.*, 1986). Substantial work has been carried out with respect to the genus *Vigna* (Gulati and Jaiwal, 1990). The present report deals with the study of callus cultures and organogenetic potential of different explants of *Vigna unguiculata*.

Material and Methods

Seeds of *Vigna unguiculata* Var. S-488 were obtained from University of Agricultural Sciences, Bangalore. They were surface sterilized with 70% ethanol for 30 sec followed by treatment with saturated chlorine water for 10-15 min and 0.1% mercuric chloride for 3-5 min. The seeds were throughly washed with sterile distilled water for 4-5 times, and aseptically germinated on humidified cotton/on Murashige and Skoog's basal medium (1962). Various explants like root, hypocotyl, cotyledon, cotyledonary node, leaf, stem, shoot tips and nodal segments were excised from 7 days old aseptic seedlings and field grown plants and were inoculated on MS medium containing 2% sucrose and 0.8% agar. Different growth regulators like auxins, coconut milk and cytokinins were supplemented at various concentrations based on the requirement of the experiments. The pH of the medium was adjusted to 5.8 before autoclaving. All the cultures were incubated at 25 ± 2^{0}C under 18:6 hr light and dark period. Experiments were repeated atleast thrice to confirm the results.

For histological studies, callus derived from different sources were

fixed in FAA (formalin-acetic-alcohol), dehydrated in alcohol-xylol series. Customary paraffin technique was followed. Sections at 10-12 µm thickness were cut and stained with Heidenhain's iron hametoxylin and counterstained with erythrosine or fast green. Sections were finally mounted in canada balsam or DPX.

Results and Discussion

No callusing nor rooting was observed from different explants on MS medium alone, except a slight swelling at the cut ends. Callus was initiated from root, hypocotyl, cotyledon, stem and leaf segments within 10-15 days on MS medium supplemented with different growth regulators at various concentrations (Table 1). Maximum callus growth was achieved on medium containing 2, 4-D (2mg/1) + CM (10%) (Fig.1,A). 2,4-D alone or in combinations with CM are known to produce callus on various explants of legumes (Sounder Raj *et al.*, 1991). Other auxins like IAA, NAA and IBA alone or in combination with CM (10%) are found to be less favourable for callus initiation and its growth. Callus was white/pale yellow, fribale and nodulated, they were subcultured once in 20 days for maintenance on fresh medium containing 2,4-D (1mg/1).

Explants cultured on a medium containing cytokinins like BAP, Kinetin and Adenine sulphate at different levels showed relatively poor response in terms of callus initiation. However, combination of auxins and cytokinins at various levels was much effective for callus initiation and its growth as observed in many legumes (Hamatt *et al.*, 1986).

The calli derived from different explants, when subcultured to MS medium supplemented with BAP/Ad.Sulp. (1-5mg/1) alone or in combination with NAA/IAA (0.5-2mg/1), the callus growth was resumed after 2-3 days and became green, hard and nodulated. Greening of the callus was intensified as the concentration of cytokinin increased. No caulogenesis was observed in these combinations even after prolonged incubation of 5-8 subcultures to the same medium or hormone free basal medium. However, embryoid-like structures were differentiated when hypocotyl- derived callus subcultured to MS supplemented with IAA (2mg/1) + CM (10%) (Fig.1,B). Histological studies revealed the well developed radicular region with undifferentiated shoot apical region (Fig.1,C).

Seedling explants like hypocotyl, stem, and leaf segments produced numerous roots with or without intervening callus phase on MS medium containing auxins like NAA/IAA at low concentrations (0.5-2mg/1) + Ad.Sulp. (1mg/1) (Fig.1,D). Similar observations were also made in *Vigna sinensis* (Pandey and Bansal, 1989). Cotyledonary nodal segments produced elongated shoots with profuse roots on MS + NAA/IAA (0.5mg/1) + Ad.Sulp. (1mg/1) (Fig.1,E). However, on a

TABLE 1

EFFECT OF AUXINS, CYTOKININS AND COCONUT MILK ON CALLUSING OF DIFFERENT EXPLANTS OF *VIGNA UNGUICULATA* (L)

Medium + Growth regulator (mg/l)	Root	Hypocotyl	Cotyledon	Stem	Leaf
MS	S	S	S	S	S
MS + IAA (1-2)	C+	C++	C+	C++	C+
MS + NAA (1-2)	C+	C++	C+	C++	C+
MS + IBA (1-2)	C++	C+	C+	C+	C+
MS + 2,4-D (1-2)	C+++	C++	C++	C++	C++
MS + IAA(2)+CM(10%)	C++	C+++	C+++	C+++	C++
		R+		R++	R+
MS + NAA(2)+CM(10%)	C++	C+++	C++	C++	C++
MS + IBA(2)+CM(10%)	C++	C++	C+	C+	C+
MS + 2,4-D(2)+CM(10%)	C++++	C++++	C++++	C++++	C++++
MS + IAA(0.5)+BAP (1.5)	C+	C+++	GC++	GC+++	C+++
		R+		R+	
MS + NAA(0.5)+BAP(1.5)	GC++	GC++	GC++	GC+++	GC+++
		R++		R++	R+
MS + IAA(0.5)+Kin(1.5)	C+	C++	C++	C++	C+
MS + NAA (0.5)+Kin(1.5)	C+	C++	C+	C++	C++
MS + IAA(0.5)+Ad.Sulp.(1)	C++	C++	C++	C+++	C+++
	R+	R+++	R++	R+++	R+++
MS + NAA(0.5)+Ad.Sulp.(1)	C+++	C+++	C++	C++	C++
	R++	R++++	R+	R++++	R++++

Extent of Callus growth:
S - Swelling, C+ - Callus very poor, C++ - Poor
C+++ - Good, C++++ - Excellent, R - Rooting
GC - Green callus.

TABLE 2

EFFICIENCY OF PLANT REGENERATION FROM SHOOT TIPS EXPLANTS OF VIGNA *UNGUICULATA* ON DIFFERENT CONCENTRATIONS OF GROWTH REGULATORS.

Media +Growth regulators (mg/1)	No. of shoot-tipscultured	No.of Shoot tips responded	Percent response	Remarks
MS	60	15	25	Complete plant with slow growth.
MS + BAP (0.1)	75	60	80	Vigorous shoot growth + little callus at the base.
MS + BAP (0.3)	75	54	72	Short growth + Adv. shoots.
MS + BAP (0.5)	75	45	60	Stunted growth + callusing.
MS + BAP (0.7)	75	30	40	Suppressed shoot growth.
MS + BAP (1.0)	75	50	66	Only callus growth.
MS + BAP (2.0)	75	38	50	Only callus growth.
MS + Kin (0.1)	60	50	83	Complete plant with slow shoot growth
MS + Kin (0.3)	60	44	73	Slow growth with rooting.
MS + Kin (0.5)	60	38	63	Shoot growth + rooting.
MS + Kin (1.0)	60	20	33	Shoot growth + rooting.
MS + Kin (2.0)	60	31	51	callusing + suppressed shoot growth.
MS + IAA(0.5+BAP (0.1)	60	40	66	Shoot growth + callusing.
MS+NAA(0.5)+BAP(0.1)	60	48	80	Callusing + rooting.
MS+IBA(0.5)+BAP(0.1)	60	35	58	Shoot growth + callusing.
MS+2,4-D(0.5)+BAP(0.1)	60	42	70	Only callus growth.
MS+IAA(0.5)+Kin(2)	60	50	83	Complete plant with stunted growth.
MS+NAA(0.5)+Kin(2)	60	48	80	Complete plant with vigorous shoot growth
MS+IAA (0.5)+Ad.Sulp.(1)	60	32	53	Complete plant.
MS+NAA(0.5)+Ad.Sulp.(1)	60	40	66	Complete plant.

Data obtained after 30 days of culture.

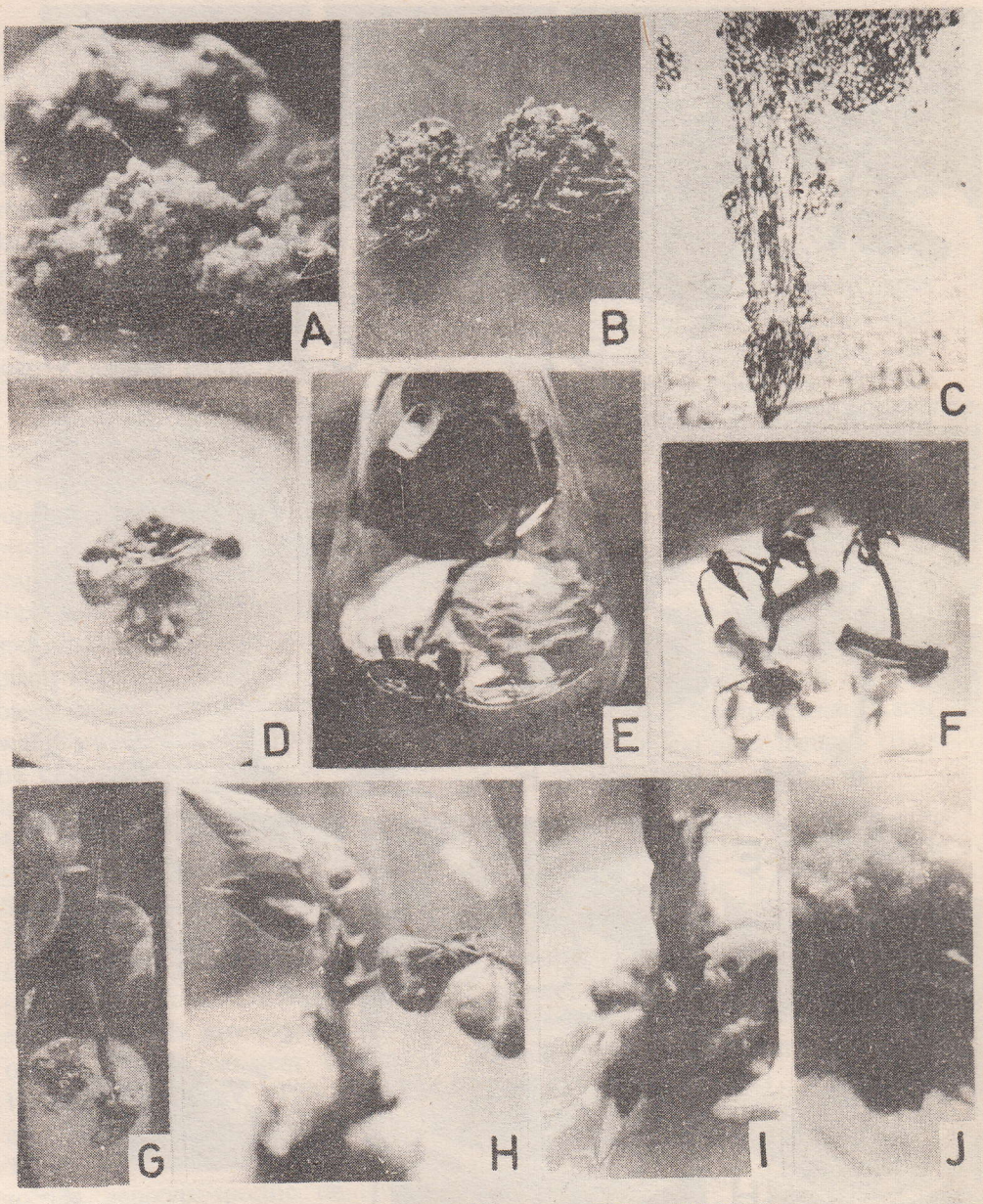

FIGURE 1: TISSUE CULTURE STUDIES IN *VIGNA UNGUICULATA* (L.) VAR. S-488.

A- Cotyledon explants showing callus growth on MS+CM(10%)+2,4-D (2mg/1);
B- Hypocotyl-derived callus showing embryoid like structures with roots on MS+CM(10%)+IAA(2mg/1),
C- Section of a differentiating embryoid with well developed root with shoot apical region;
D- Direct rhizogenesis from leaf on MS+NAA(0.5mg/1)+Ad.Sulp. (1mg/1);
E- Regeneration of whole plant from cotyledonary node on MS+IAA (0.5mg/1)+Ad.Sulp.(1mg/1);
F- Shoot regeneration from cotyledonary nodes on MS+BAP(0.1mg/1) with little callus at the cut ends;
G- Regeneration of whole plant from shoot tips on MS alone with little callus at the base;
H&I- Shoot regeneration, with 5-6 adventitious shoot bud formation at the base of shoot tips on MS+BAP(0.1mg/1);
J- Callus formation from shoot tips on MS+BAP(0.7mg/1).

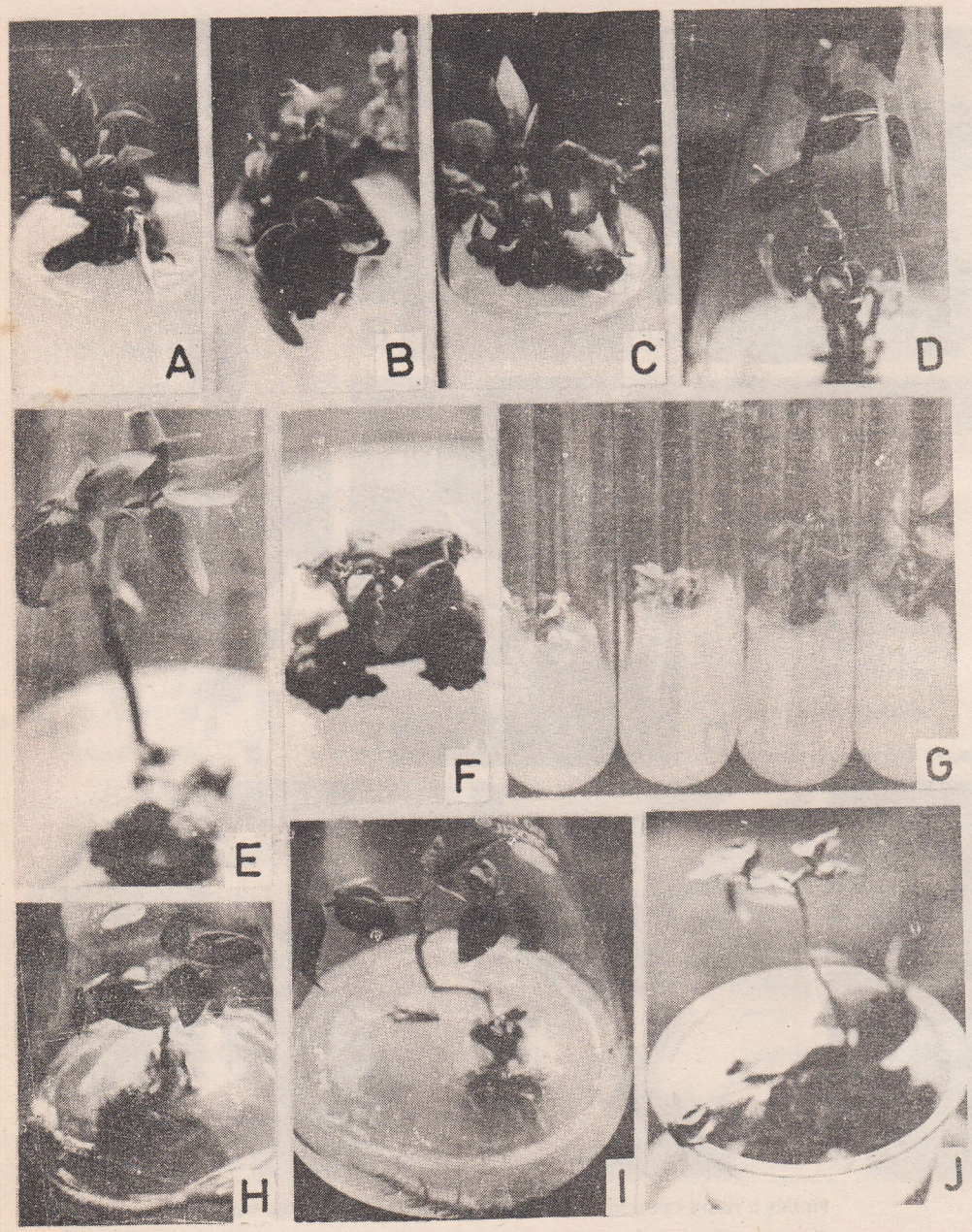

FIGURE 2- *IN VITRO* CULTURE OF SHOOT TIPS AND NODAL SEGMENTS.

A- Regeneration of shoots from shoot tip explants of *in vitro* regenerated shoots on MS+IAA (0.1mg/1) + BAP (0.1mg/1);
B- Induction of multiple shoots on MS+IAA(0.1mg/1)+BAP(0.3mg/1);
C- Multiple shoots with little callus at the base on MS+IAA(0.1mg/1) +BAP(0.5mg/1);
D- Vigorous shoot growth with multiple shoots on MS+IAA(0.5mg/1) +Ad.Sulp.(1mg/1);
E- Vigorous shoot growth from nodal explants on MS+BAP(0.3mg/1);
F- Suppressed shoot growth with callus formation at the cut ends of nodal explants on MS+BAP(0.7mg/1);
G- Shoot initiation and its growth from nodal explants on MS+Kin (0.1, 0.3, 0.5, 0.7 mg/1);
H- Regeneration of whole plants from nodal cultures on MS+NAA (0.5mg/1)+Kin(1mg/1);
I- Whole plant from nodal cultures on MS+NAA(0.5mg/1)+BAP(0.1mg/1
J- Complete plant transferred to plastic pots.

medium containing BAP alone at lower concentrations (0.1-0.5mg/1) small shoots with root initials were arise from cotyledonary nodol cultures (Fig.1,F). Complete plant formation was more pronounced on media supplemented with NAA and Ad.Sulp. than IAA + Ad. Sulp., where shoot growth was slow. Cotyledonary node of legumes are known to produce plants *in vitro* (Cheng *et al.*, 1980 ; Gulati and Jaiwal, 1990).

Shoot tips of about 0.5-0.7 cm length were excised from one week old aseptic seedlings and cultured on MS supplemented with different auxins and cytokinins at various levels. The response and regenerative potential of shoot tips varied depending on source and physiological conditions of the explants (Table 2). Shoot tips cultured on basal medium initiated shoot growth within a week with slight swelling or little chlorophyllous callus at the base. later roots were initiated. However, afte 4-5 weeks the plant ceased its further growth and turned brown (Fig.1,G). Similar observations were also made in *Trifolium repens* (Bhojwani, 1981), *Dolichos biflorus* and *Dolichos lablab* (Sounder Raj *et al.*, 1989, 1991). However, vigorously growing shoots were obtained from meristem culture of *Vigna unguiculata* cv. VITA 5- EXITA on MS alone (Kartha *et al.*, 1981). These differential response may be due to the genotype-specific or the endogenous hormone level of the explants.

When medium was supplemented with BAP/Kin/Ad.Sulp. at different concentrations (0.1-2mg/1) shoot tips showed variable responses. In presence of BAP at 0.1-0.3 mg/1 there was vigorous growth with 5-6 small adventitious shoots and shoot buds were emerged from callus at the base (Fig.1, H&I). At higher BAP concentrations (0.5-2mg/1) explants showed suppressed shoot growth with more callus formation at the base (Fig.1,J). Callus was white/chlorophyllous, hard and nodulated. Rhizogenesis was inhibited in all the combinations as observed by Sounder Raj *et al.*, (1989, 1991). Entire plants were also obtained on MS containing combinations of Ad.Sulp. + NAA/IAA at different levels. This confirms the synergistic effect of auxin and cytokinin for better growth of shoot and roots. Similar observations were made in *Nicotiana tobacum* (Smith and Mursashige, 1970); *Pisum sativum* (Kartha *et al.*, 1974); and other legumes (Bajaj and Dhanju, 1979).

Shoot tips obtained from *in vitro* regenerated shoots. when subcultured to medium containing BAP at different levels (0.1- 0.5mg/1) along with IAA (0.1mg/1) induction of multiple shoots were more pronounced (Fig.2,A-C). However, multiple shoots were also obtained on a medium containing IAA/NAA (0.5mg/1) + Ad.Sulp. (1mg/1) (Fig.2,D). Shoot tip derived calli on subculture remained friable or hard on MS medium supplemented with BAP/Ad.Sulp. (0.1-5mg/1). In presence of BAP/Ad.Sulp. the calli turned green and become nodulated. Similar observa-

TABLE 3

GROWTH RESPONSE OF NODAL SEGMENTS OF *V.UNGUICULATA* FROM TWO DIFFERENT SOURCES

Source	Field Grown Plants				*In vitro* regenerated shoots			
Media + Growth regulators (mg/l)	No. of Nodal segments cultured	No. of Nodal segments responsed	Percent Response	Remarks	No. of Nodal segments cultured	No. of Nodal segments responsed	Percent Response	Remarks
MS	30			Swelling	30	05	16	Slow growth
MS + BAP (0.1)	60	56	93	Slow growth + callusing	60	50	83	Growth + Multiple shoots.
MS + BAP (0.3)	60	50	83	Slow growth + callusing	60	46	76	Growth + Callusing.
MS + BAP (0.5)	60	42	70	Callusing	60	52	86	Suppressed shoot growth + callusing.
MS + BAP (1.0)	60	38	63	Callusing	60	40	66	Callusing
MS + BAP (2.0)	60	25	41	Callusing	60	37	61	Callusing
MS + Kin (0.1)	60	36	60	Slow growth	60	31	51	Slow growth
MS+ Kin (0.3)	60	30	50	Slow growth + rooting	60	29	48	Shoot growth
MS + Kin (0.5)	60	38	63	Slow growth + roots callusing	60	30	50	Slow growth
MS + Kin (1.0)	60	18	30	Callusing	60	20	33	Callusing
MS + Kin (2.0)	60	14	28	Callusing	60	20	33	Callusing
MS + IAA(0.5+BAP (0.1)	60	42	70	Complete Plant	60	30	50	Complete Plant.
MS+NAA(0.5)+BAP(0.1)	60	44	73	Suppressed shoot growth	60	25	41	Shoot growth.
MS+IBA(0.5)+BAP(0.1)	60	40	66	Slow growth	60	20	33	Growth + Callusing.
MS+2,4-D(0.5)+BAP(0.1)	60	38	63	Callusing	60	50	83	Callusing

Data obtained after 30 days of culture

tions were made in *Vigna aconitifolia* (Godbole and Krishnamurthy, 1987) and *Dolichos biflorus* (Sounder Raj *et al.*, 1989).

Nodal segments of about 0.5-1 cm were excised from field grown plants and *in vitro* regenerated shoots showed varied responses (Table 3). Regeneration of multiple shoots/shoot buds were more pronounced *in vitro* regenerated nodal explants than field grown explants, where it produce either single or two shoots on medium containing BAP at different levels (Fig.2, E&F). Similar observation has been made in *Glycine max* (Cheng *et al.*, 1980; Saka *et al.*, 1980) and *Vigna aconitifolia* (Gill *et al.*, 1986) where the explants were pre-conditioned on a medium containing different concentrations of BAP, Zeatin and Kinetin. The present observations showed that the preconditioning of explants with cytokinins enhance vigorous shoot growth with multiple soot formation which conforms the previous studies. Complete plants were also obtained on a medium containing NAA/IAA (0.5-1mg/1) along with BAP/Kin (0.1-2mg/1) (Fig.2,H&I). Higher concentrations of Kin (1-2mg/1) found to be inhibitory for axillary bud growth, whereas, it was more effective at low concentrations (0.1- 0.7mg/1) (Fig.2,G). The differential responses of explants depends on several factors under *in vitro* conditions have been previously reported (Bharal and Rashid, 1980; Bhargava and Chandra, 1989).

The regenerated plants were transferred to plastic pots containing autoclaved vermiculite and fed with half strength MS liquid medium with 2% sucrose and kept under high humidity (Fig.2,J) plants showed luxerient growth and later transferred to field.

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