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CALLUS DEVELOPMENT AND MORPHOGENESIS OF *VIGNA UNGUICULATA* UNDER NaCl STRESS

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The callus development was achieved from plumule, radicle and cotyledonary explants of *Vigna unguiculata* both under normal and saline medium. The medium supplemented with 1.5 mg/l NAA + 0.5 mg/l KN was found suitable for callus growth. The shoot regeneration was achieved in B5 basal medium supplemented with BAP. The shoots regenerated under NaCl had shorter internodes and smaller leaves compared to the shoots regenerated from sensitive calli. The number of shoots regenerated decreased with increasing concentrations of NaCl. The rooting was seen with in a week in case of sensitive callus in B5 medium, whereas the rooting was observed only after 25 days under 0.5% NaCl. The higher concentrations of NaCl inhibited the root induction.

Keywords : Callus; Morphogenesis; Salinity; *Vigna unguiculata.*

Introduction

Cow pea, *Vigna unguiculata* L. Walp a hot tropics traditional grain legume is commonly used as leafy and green pod vegetatble[1]. Cow pea variety C-152 is extensively grown in India both in saline and interior lands of Karnataka. The plant has potential for producing food under less than favourable conditions. Although, a lot of work has been carried out on *in vitro* culture and morphogenesis of *V. radiata* under saline stress[2-4], *Vigna sinensis*[5] and *Vigna aconitifolia*[6,7], not much work has been done on *V. unguiculata*. Development of callus and organogenetic potentials of *V. unguiculata* in MS medium was achieved by Sounder Raj *et al*[8]. Rina Ahmed *et al.*[9] were successful in developing the $HgCl_2$ - tolerant cell lines of *V. unguiculata* after treating them with N. methyl- N-Nitrosoguanidine (mutagen). The influence of draught stress and enzymatic degradation of polar lipids in *V. unguiculata* was studied by Sahsah *et al.*[10]. The present paper reports the development of callus, selection of NaCl tolerant cell lines and morphogenesis of *V. unguiculata*.

Materials and Methods

Aseptically grown two day old seedlings of cow pea (*Vigna unguiculata* L. Walp., cv. c-152) were used as explants which were inoculated into the culture tubes containing MS medium and incubated at 25 ± 2°C under flourescent light of 1000 lux. The growth regulators in the following combinations and concentrations were used to choose the optimum concentration for callus induction[11].

NAA mg/l + KN mg/l	2,4-D mg/l + KN mg/l
1.5 + 0.5	1.5 + 0.5
2.0 + 0.5	2.0 + 0.5
2.5 + 0.5	2.5 + 0.5
3.0 + 0.5	3.0 + 0.5

The induced calli were subjected to the medium containing different concentrations of NaCl and the tolerant calli were selected as per the flow-chart I. For the studies on morphogenesis 30-42 day old tolerant calli were inoculated into B5 medium with the following combinations of growth regulators.

BAP mg/l + KN mg/l
1.4 + 0.5
3.6 + 0.5
5.8 + 0.5
7.2 + 0.5

The well developed shoots on B5 medium with BAP were transferred to B5 basal medium and MS medium supplemented with 1.5 mg/l NAA + 0.5 mg/l KN with or without NaCl for rooting.

Results and Discussion

All the explants showed callus initiation on MS medium supplemented with different concentrations of 2, 4-D, NAA and cytokinin

Table 1. Shoot induction from sensitive and toterant calli of *Vigna unguiculata.*

Growth regulator concentrations in B5 medium		Percentage of calli responded			No. of shoots regenerated per callus	
BAP mg/1	Senstive Callus	0.5% NaCl tolerant	0.75% NaCl tolerant	Sensitive Callus	0.5% NaCl tolerant	0.75% NaCl tolerant
1.4	--	--	--	--	--	--
3.6	33	--	--	06	--	--
5.8	12	16	--	05	03	--
7.2	--	22	10	--	04	01

(KN). Among different concentrations of auxins and cytokinins, the medium supplemented with 1.5 mg/1 NAA + 0.5 mg/1 KN gave the best results (Fig.1). Callus initiation from the explants was observed on the third day and it took about 8-10 days to form a fairly well developed cell mass. The calli were spongy, light cream in colour during the first two weeks which became creamy later on. The calli developed from the plumule and the radicle were more healthier compared to those developed from cotyledons which showed lower cell mass.

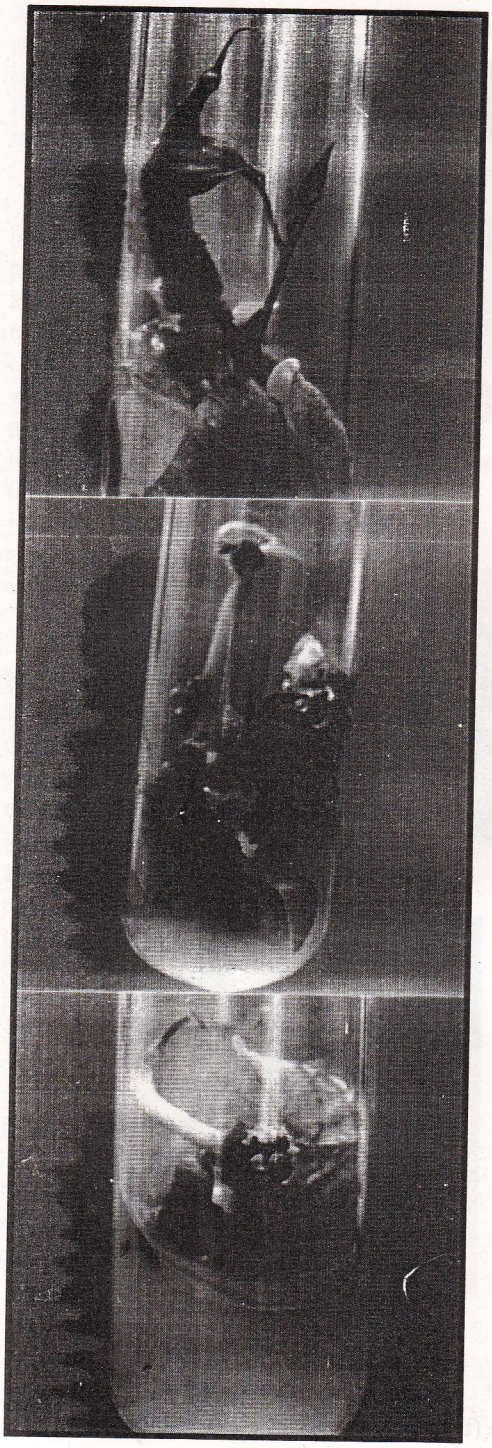
The sensitive callus in MS medium supplemented with 1.5 mg/1 NAA and 0.5 mg/l KN showed the development of torpedo shaped embryos (Fig. 2a).

The results of shoot induction from NaCl tolerant and sensitive callus lines are given in table 1. The shoot regeneration of sensitive callus was observed after 15 days of its transfer to B5 medium supplemented with BAP which was delayed with 5-10 days in tolerant lines.The shoot regenerated from NaCl tolerant callus had a shorter internodes and smaller leaves compared to the shoots regenerated from sensitive callus which had relatively longer internodes and leaves (Fig. 2b). The shoot regeneration from tolerant callus was observed only in higher concentrations of BAP . The number of shoots regenerated per callus was higher in sensitive callus which decreased with increasing concentration of NaCl in toterant calli.

The shoots developed from sensitive callus showed rooting within a week on B5 basal medium and MS medium supplemented with growth regulators (Fig. 2c).The shoots developed from 0.5% NaCl tolerant callus showed rooting only after 25 days on B5 basal medium containing NaCl.

Combinations of different auxins and cytokinins at various levels have been proved to be effective for callus initiation in legumes[12]. On MS medium supplemented with 2, 4-D alone or in combination with CM were known to produce callus in *Dolichos lablab* Var. *Lignosus* (L.)[13]. The same authors[8] reported that other auxins like IAA, NAA and IBA alone or in combination with CM were less favourable for callus initiation in *Vigna unguiculata* (L.) Walp. S-488. In the present investigation the MS medium supplemented with 1.5 mg/1 NAA + 0.5 mg/1 KN was found to be suitable for callus initiation in *Vigna unguiculata* (L.) Walp.cv. c-152. Pandey and Bansal[5] reported the callus initiation from leaf explants of *Vigna sinensis* L. on MS medium supplemented with 2, 4-D. Rina Ahmed *et al.*[14] derived the callus from hypocotyl explants of *Vigna sinensis* L. on MS medium with 2 mg/1 2, 4-D and 1 mg/1 KN. In the present study, the callus was initiated in all the explants used but better growth was observed in case of plumule and radicle compared to cotyledons.

The maximum fresh weight was observed in 0.5% tolerant callus grown in 0.5% NaCl and the maximum dry weight in 1% tolerant callus grown in 1% NaCl. The dry weight of sensitive callus grown in the



Flow Chart I : Protocol for the selection of tolerant-callus on MS Medium.

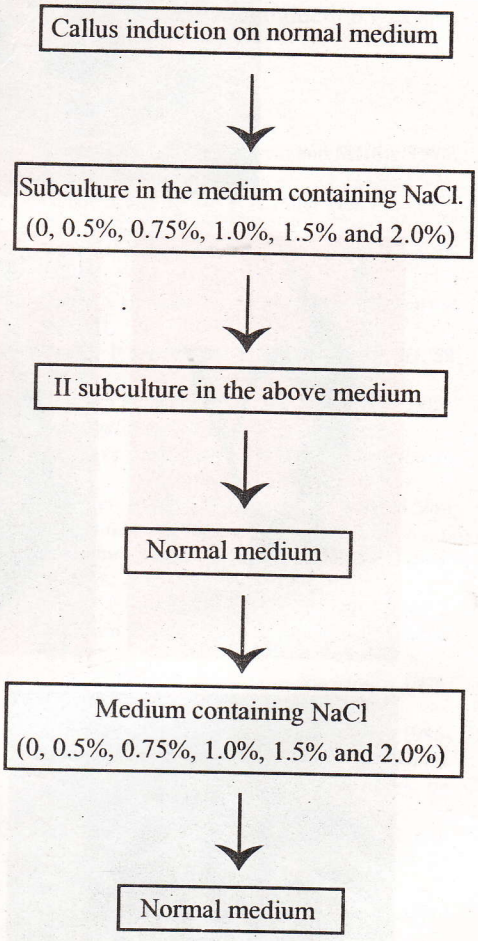


Fig. 1. Shoot regeneration from
(a) Sensitive callus
(b) 0.5% NaCl tolerant callus and
(c) 0.75% NaCl tolerant callus.

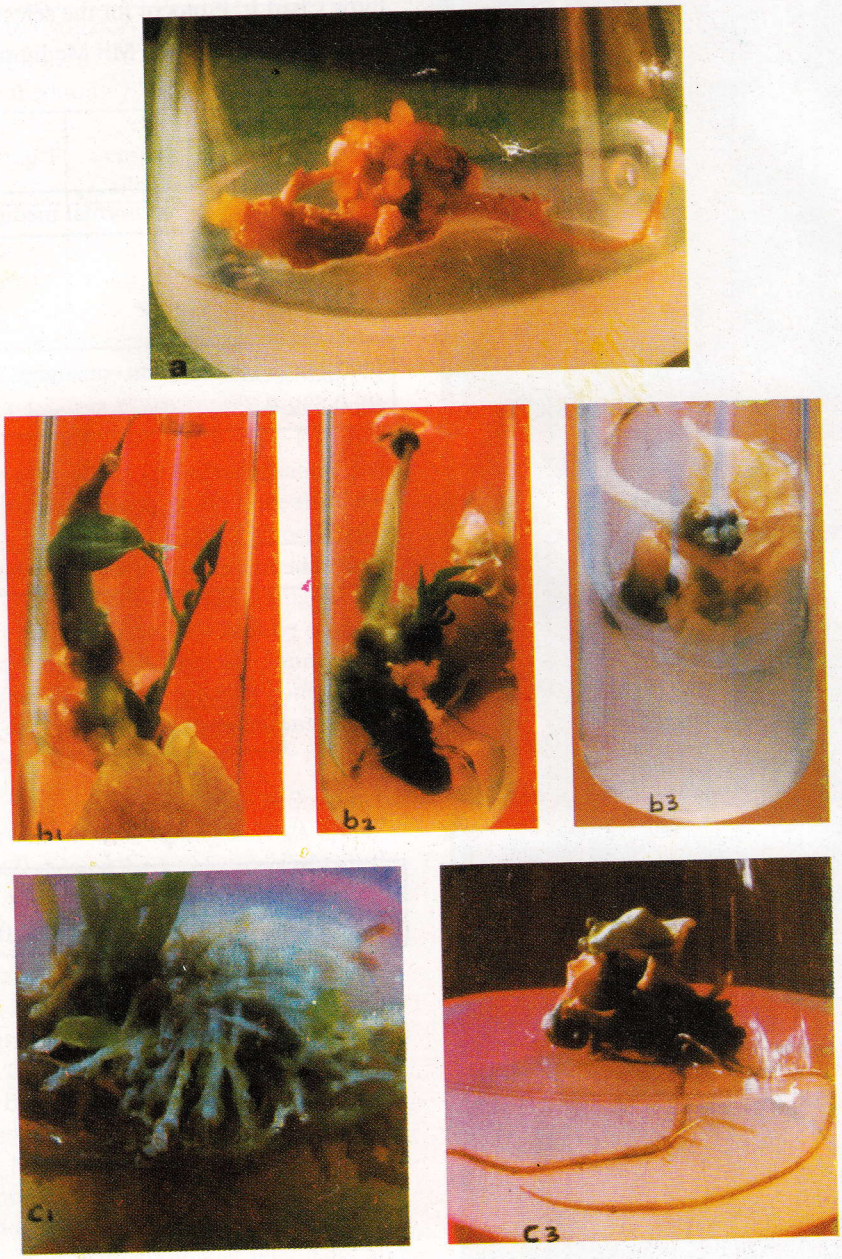


Fig. 2a. Sensitive callus showing embryos.

Fig. 2b. Shoot regeneration from sensitive (b1), 0.5% NaCl tolerant (b2) and 0.75% NaCl tolerant (b3) calli.

Fig. 2c. Rhizogenesis in the shoots from sensitive (C1) and 0.5% NaCl tolerant (C2) calli.

absence of NaCl was lower than the dry weights obtained for tolerant calli. The results of the present study corroborates with the results obtained for *Vigna radiata* (L.) Wilczek[2-4, 15].

In *Vigna unguiculata* detailed protocols have been worked out for regeneration of plantlets from somatic tissue explants[8, 14, 16]. Pandey and Bansal[5] were successful in shoot regeneration in *Vigna sinensis* in the form of green shoot buds directly from the explant with IBA and KN (1 uM : 10 uM). The growth rate, protein and proline contents of the NaCl tolerant callus was compared with the sensitive callus of *Vigna unguiculata*[11]. However, there are no reports so far, on the regeneration of plantlets of *Vigna unguiculata* under NaCl stress.

In the present investigation, it has been possible to establish the plantlets from *Vigna unguiculata* from both sensitive callus and 0.5% & 0.75% tolerant callus under normal medium. In 0.75% tolerant callus, although the multiple shoot induction was achieved it failed in rhizogenesis. The above results suggest that NaCl as a selection agent may interfere with regeneration process possibly due to hormonal balance. Gulati and Jaiwal[3] selected salt resistant plants of *Vigna radilata* (L.) Wilczek by adventitious shoot formation from cultured cotyledon explants on CB medium supplemented with NaCl (0-200 mol m^{-3}). In the present investigation, the regeneration has been possible only in the absence of NaCl from tolerant callus on B5 medium supplemented with BAP. The rooting response of shoots obtained from sensitive callus was better than the response of shoots obtained from the tolerant callus. Gulati and Jaiwal[2] developed roots from salt-tolerant shoots on MS medium supplemented with IAA (5×10^{-5}M) in the presence or absence of NaCl. The root growth and root number decreased with increase in NaCl concentrations. With increasing NaCl the root induction was also delayed.

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