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SOMATIC EMBRYOGENESIS IN THE SUSPENSION CULTURES OF *VIGNA RADIATA* L.

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Abstract

Suspension cultures established from hypocotyl derived calli pretreated with AS (adenine sulfate) and $(NH_4)_2SO_4$ (ammonium sulfate) stimulated somatic embryogenesis in addition to root morphogenesis. All stages of embryos were produced but shoot formation was not observed. Control cultures exhibited rooting response only.

Introduction

Mungbean (*Vigna radiata* L.) is an important pulse crop in the Indo-Pakistan subcontinent. Tissue culture methods for callus induction and organ differentiation from seedling, root, shoot, leaf (Choudhury *et al.*, 1983; Singh & Singh, 1984; Eapan, 1988), embryonic axis with hypocotyl (Illahi & Masood, 1985) and epicotyl segments and cotyledonary nodes with and without cotyledons (Sharma & Jha, 1989; Singh *et al.*, 1984) have been reported. Somatic embryogenesis which is common in other plant species appears to be less frequent in leguminous plants especially in *Vigna radiata*. The present paper describes the development of somatic embryos in hypocotyl derived suspension cultures of *V. radiata* L.

Materials and Methods

Seed of *Vigna radiata* L., after surface disinfection with 0.1% mercuric chloride containing 1-2 drops of Tween-20 and washed 4-5 times with sterile water were used. Murashige & Skoog (1962) medium (MS) containing 3% sucrose, adjusted to pH 5.5 and gelled with 1% agar for callus induction and maintenance was used. Growth hormones and other additives were added before autoclaving the medium. Suspensions were shaken on a rotary shaker at 100 r.p.m. Conical flasks and culture bottles were used as culture containers. All cultures were kept at $25 \pm 2^{\circ}C$ under 16h light at 2.6 W/m^2 provided by cool fluorescent tubes. Cultures were periodically observed under the Stereomicroscope.

For the determination of ploidy level, the root tips excised from the regenerants were stored at 2°C and fixed in acetic alcohol (1:3) for 24 h in dark at room temperature. Fixed root tips were transferred to 2% acetocarmine for at least 24h and squashed in 45% acetic acid. Chromosomes were counted under compound light microscope using 10x40 optics. For making permanent mounts slides were passed through ethanol grades and mounted in Eupral (Dyer, 1979).

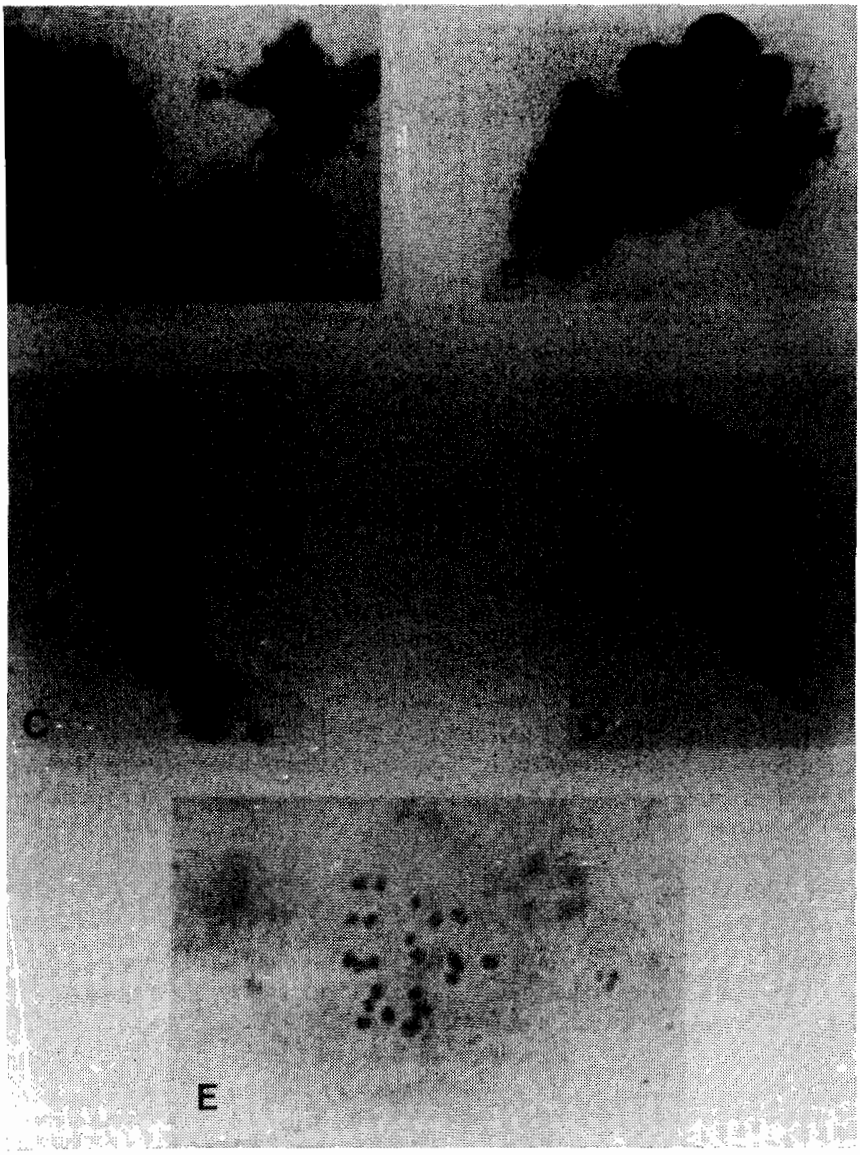

Fig. 1A-E. Stages of somatic embryo development in *Vigna radiata* L. and chromosome number in regenerated plants.

A = Proembryonal masses of cells in the suspension culture (arrows).

B = Globular and torpedo stage embryos attached to a cell aggregate.

C = A torpedo stage embryo with cotyledons.

D = A somatic embryo showing abnormal cotyledons.

E = Chromosomes in a squashed root tip cell obtained from one regenerant.

Results

Seeds cultured on MS solid medium containing 2.5 mg/l 2,4-D showed development of soft, white and friable callus on hypocotyls and root tips of seedlings in 4 weeks. The callus excised from hypocotyls when transferred to MS medium containing 1,2.5 and 5 mg/l of 2,4-D showed proliferation of the callus and produced roots in the first passage in 4 weeks. The calli which proliferated on medium containing 1 mg/l of 2,4-D were white, soft and friable whereas soft, wet and dark grey calli developed on media where 2.5 and 5 mg/l of 2,4-D was used. All the three calli formed roots on transfer to liquid medium containing 4 mg/l and 10 mg/l NAA in second passage. The roots developed in cultures containing 10 mg/l NAA also formed several laterals. No shoot morphogenesis or embryogenesis was observed (Table 1).

Callus obtained from hypocotyls of seedlings in culture grown for 2 weeks on MS solid medium containing 2.5 mg/l 2,4-D plus 20 mg/l AS showed better growth of callus than the one without AS but did not show any morphogenesis during first passage. Subculture of these calli on a medium containing AS (20 mg/l) and $(NH_4)_2SO_4$ (6.6 g/l) stimulated prolific root formation during second passage. Friable callus from these cultures when excised and transferred to liquid medium containing 10 mg/l NAA (Table 1) produced numerous roots with several laterals whereas suspensions growing in medium containing 4 mg/l NAA formed somatic embryos in the third passage (Table 1; Fig.1A-D). All stages of embryo from multicellular embryonic clumps to torpedo stage were observed in these cultures. Torpedo stage embryos exhibited elongation of roots on transfer to basal medium. Roots excised from regenerated plantlets were diploid and exhibited 22 chromosomes (Fig.1E) as reported by Sharma & Gupta (1982).

Table 1. Morphogenesis in the suspension cultures of *Vigna radiata* L., from calli of different origin.

Passage no. of callus	Callus growth medium	NAA in suspension culture medium 4 mg/l	10 mg/l	Morphogenesis
1	2,4-D (1 mg/l)	+	-	C + R
		-	+	C + R
	2,4-D (1 mg/l)	+	-	C + R
		-	+	C + R
2	2,4-D (5 mg/l)	+	-	R
	2,4-D (2.5 mg/l) + AS (20 mg/l) + $(NH_4)_2SO_4$ (6.6 g/l)	+	-	C + SE
		-	+	R

Legend + = present; - = absent; C = callus; R = root; SE = somatic embryo.

Discussion

Like other legumes *V. radiata* is also recalcitrant to shoot regeneration and in the past attempts to regenerate large number of shoots in culture have not met with much success. This has hindered the attempts of micropropagation of this species through tissue and cell culture techniques, which have been viewed as the logical approach, in the mungbean improvement programmes (Hildebrandt *et al.*, 1986; Mroginski & Kartha, 1985). In the present study, shoot morphogenesis did not occur. Formation of different stages of somatic embryos and roots from calli, pretreated with AS and $(NH_4)_2SO_4$ and transferred to the liquid medium containing 4 mg/l and 10 mg/l NAA indicated that the suspensions initiated from them possibly contained a heterogenous population of cells which under one set of conditions favoured root and under another somatic embryo development. Adenine sulfate is a purine base with cytokinin-like effects on plants which has been shown to favour the formation of buds in *Plumbago indica* (Nitsch & Nitsch, 1967) and *Nicotiana* (Skoog & Tsui, 1948). Stimulation of somatic embryo formation by NH_4^+ for other plant species is well documented (Tazawa & Reinert, 1969; Walker & Sato, 1981; Christianson *et al.*, 1983; Meijer & Brown, 1987; Trigiano & Conger, 1992).

The diploid nature of regenerated plants suggests that they can be successfully used in breeding programmes for producing new varieties with superior characteristics.

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