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CYTOVARIABILITY IN SESBANIA ACULEATE DURING CALLUS CULTURE AND ORGANOGENESIS

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ABSTRACT:
Cytological studies of callus and root of regenerated plants of Sesbania aculeata (2n = 12) with different concentrations of growth regulators in MS media showed higher instability. Some of the callus cells showed the occurrence of 6-8 celled globular somatic embryoids, some showed hypoploidy also. Plant regeneration occurs through adventitious organogenesis at diploid level of genome.

Keywords: *Cytological, embryoids, hypoploidy, MS media, callus*

INTRODUCTION:

Introduction

Plant tissue and cell cultures have been reported to exhibit genetic uniformity (Hanna etal (1984). and also genetic instability which showed variations in chromosome number and structure (Bayliss1980, D'Amato1995, Lee and Phillips1988, Pandey and Bansal 1989). Many workers perfoermed the studies and indicated that genetic structure (Larkin 1987]. Ploidy (Karp,1988) invitro culture process(Lawrence,1999), source (Ghosh,2001)and hormone composition (Nagl 1988)affect the nature and frequency of this genetic instability. Besides, many researchers (Orton,1987) have raised question to the fact that the mode of interaction of these factors creating variability and stability of chromosomes.

The present investigation is done for screening of the cytovariability of different genotypes in the callus and regenerated roots of *Sesbania aculeata*.

METHOD AND MATERIAL:

Seeds of *Sesbania aculeata* obtained from the IARI, Kanpur (U.P), India, were surface sterilized with 0.1% mercuric chloride (W/V) for 5 min followed by rinsing with sterile distilled water 4-5 times. The seeds were germinated on sterile moist filter paper in petriplates . at 22-25^{o}C on dark. Four to seven days old seedlings were taken as the source of explants.

The explants, hypocotyl (1 cm) and cotyledon (0.5 cm^{2}) were surface sterilized with 40% chlorex for 5 min followed by a thorough washed with double distilled water. Explants were inoculated onto the MS (Murashige and Skoog, 1962) basal medium containing 3% sucrose and, 0.8% agar and different concentrations cytokinin and auxins such as as BAP, IBA & 2,4-D.

Cultures were incubated at 25+2^{0}c with a photoperiod of 16 h (1000 lux) and 8 h dark. Periodical subculture of the Calli obtained from different explants was done on fresh medium at monthly intervals. Minimum of 16 replicates were taken for each treatment and treatment was repeated thrice.

For cytological analysis, fresh white and friable callus and root tips of regenerated shoots were pretreated with saturated solution of 1, 4-paradichloro benzene (PDB) for 3-4 hrs, fixed in acetic acid ; ethanol (1:3) for 7 hr, hydrolysed with 1n HCL at 60^{o}Cfor 4-5 min, stained in 2% acetoorcien:Hcl (9:1) and finally squashed in 45% acetic acid.

RESULTS AND DISCUSSION

Cytology of source plant showed that their cells were diploid with normal chromosome complement (2n=12). Cytology of cultured cells, on the other hand, showed that diploid cells were maximum during initiation stage but there was marked increase in the ratio of polyploidisation in older callus (Table-1). Aneuploid (i.e. hyperdiploid and hypodiploid) cells showing mitotic abnormalities were observed very frequently.

6 weeks old organogenetic callus grown on 1/0.1 (mg/1) NAA/BAP exhibited the occurrence of small

cells with dense cytoplasm forming cell globular somatic embryoids.

Cytological analysis of the roots of regenerated shoots revealed that out of 112,108 were diploid with 2n=2x=12 chromosome number. The remaining plantlets were predominantly diploid (80-95%) with a few cell showing euploidy (2n=4x=24) in the roots.
It has been concluded that mitotic abnormalities during in vitro culture and proliferation of callus may lead to the occurrence of polyploidy, aneuploidy and other observations (Orton, 1987)., polyploidy in the variant cells have been presumed to arise by endomitosis and endo-reduplication (Bansal and Sen,1985). According to other researchers (Bennici, 1978) variable chromosome complements resulting due to chromosome structure and number mosaicism may be carried upto maturity and progeny level in the cells and tissues of in vitro regenerated plants and can be used as raw materials for selection and evolution of populations.

Presence of large number of hyperdiploid and euploid cells in callus indicates a higher level of variations. As reported earlier (Karp, 1989) the basis of the genotypic component to unstability is not completely understood. though the results show that the genome of the Sesbania aculeata is more vulnerable to changes under in vitro situation.

Cytological investigation also exhibited the presence of globular somatic embryoids among callus cells which proves the indirect somatic embryogenesis through induced embryogenetically determined cells (IEDC) along with normally occurring adventitious organogenesis through induced organogenetically determined cells (IODC). Only IODC achieved completion resulting in formation of mature shoots. This concludes that the possibility of initial involvement of some non-optimal somatic embryogenic pathway with invitro organogenesis, out of which only the latter is selected favorably for shoot formation.

From the above experiment it was concluded that inspite of occurrence of a large number of euploid and aneuploid cells at different stages of cultures, diploidy dominated at tissue and plant level which suggests a normal level of genetic stability (Nayak, 1991
). Thus it can be summarized that the present study also supports the view that organogenesis process removes naturally the most radically variant cell lineages (Gould,1996).

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Table 1. Mitotic evaluation of 6-8 weeks old callus & regenerated roots of *Sesbania aculeata*

S.N.	Plant Growth regulators mg/L	Total Number of cells studied	Diploid cells%	Cytological anomalies (%)			Cells with embryoid formation (%)
				Hypodiploid cells	Hyperdiploid cells	Euploid Cells	
root	control	700	98%	-	-	-	-
	.5 2,4-D	800	76%	1.6	3.2	-	-
	1 2,4-D	1000	95%	7.6	3.0	15.3	-
	0.5 BAP	800	97%	2.4	2.1	4.3	-
	1 BAP	1000	91.8%	1.7	1.6	6.1	-
callus	control	700	99%	-	-	-	--
	.5 2,4-D	800	66%	.8	1.9	1.7	-
	1 2,4-D	1000	85%	5.2	4.3	3.3	-
	0.5 BAP	800	87%	0.9	1.2	-	2.5
	1 BAP	1000	92.8%	0.4	0.6	0.7	12.55