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IN VITRO RESPONSE OF GENOTYPES IN PEARL MILLET

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ABSTRACT

The present investigation was undertaken to study the *in vitro* response of various explants viz., seed, leaf, inflorescence and immature embryo from four different genotypes of pearl millet viz., PT 3095, ICMS 7703, 732B and 81B. Among the four basal media used for callus induction, MS medium was found to be better than N6, LS and whites medium, for the explants seed, leaf and immature embryo. For the inforescence explants, N6 medium suited better than N6 for regeneration. Among the four genotypes studied the morphogenic potential was high in ICMS 7703 followed by PT 3095, 732B and 81B. The maximum response for callus induction and regeneration was obtained from inflorescence followed by leaf.

Key words: Pearlmillet, tissue culture, immature embryo, inflorescence, leaf.

The assembly of genetic variability is vital to any plant breeding programme. Plant celi culture has often been hailed as one of the most significant ways to plant improvement. Considering the need for further genetic enhancement and improvement in pearlmillet, studies were initiated to standardize an effective method for *in vitro* culture of pearlmillet from callus tissue derived from different explants.

MATERIALS AND METHODS

The donor genotypes selected in the present study are four inbreds of pearlmillet PT 3095, ICMS 7703, 732B and 81B. PT 3095 is a good combiner in hybrid combinations. ICMS 7703 is a composite variety popularly cultivated by farmers. 732B and 81B are the maintainers for 732A and 81A respectively.

The seed materials of inbreds were obtained from the Millet Breeding Station, School of Genetics, Tamil Nadu Agricultural University, Coimbatore. Four basal media used for callus induction were MS, N6, LS and Whites. 2, 4-D (0.5 mgl^{-1}) in combination with kinetin (0.1 to 2.5 mg l^{-1}) was used. 3.0% sucrose and 0.8% agar were included in all the callus induction media combinations. For regeneration the media used was MS with different combinations of kinetin, zeatin, BAP with NAA and IAA.

Seeds were surface sterilized with 70% alcohol for 40 seconds and then with 0.1% mercuric chloride for 10 minutes. Surface sterilized seeds were cultured in callus induction medium. Young shoots with several unfurled pale yellow leaves were collected from 30 to 33 days old plants grown in glass house, cut into pieces of 1.5 cm long, surface sterilized and inner most tightly unfurled cream coloured leaves were cut and inoculated. Immature inflorescences of 2-6 cm length were collected, surface sterilized cut into segments and cultured. The inflorescence at milky stage was used for culturing immature embros. Culture were incubated in dark at 25 ± 2°C for callus induction. Through frequent subcultures on regeneration medium plantlets were regenerated from calli of different explants. The *in vitro* derived plants were thoroughly washed and transferred to plastic cups filled with sand, soil and vermiculite mixture and kept in mist chamber. After 18-20 days well established plants were transferred to mud pots and kept in glass house.

RESULTS AND DISCUSSION

Seed: Direct callusing of seeds and callusing after plumule emergence was also obtained. Callus induction was highly significant at 5.0 mgl^{-1} of 2, 4-D.

Leaf: The basal portion of the leaf nearing the node was found to be the best responsive region. The callus induction frequency was significant at 2.5 mg l^{-1} of 2, 4-D. Nodular calli after transfer to regeneration media, produced somatic embryos, developed into plantlets, within 12-14 days. The response of leaf tissue was low when compared to the inflorescence of all the genotypes studied. This is due to presence of mitotic inhibitors in mesophyll cells [1].

Table 1. The response for callus induction and regeneration in different genotypes

Genotypes / Tissue explant	PT3095		ICMS7703		732B		81B	
	CI	Rn%	CI	Rn%	CI	Rn%	CI	Rn%
Seed	45	35	51	39	48	33	45	30
Leaf	60	48	73	50	59	40	50	38
Infloresence	73	52	80	54	65	45	50	43
Immature embryo	63	39	70	42	55	37	50	35

CI: Callus induction percentage; Rn: Regeneration percentage

Inflorescence: Callus induction was highly significant at 2, 4-D 2.0 mg l^{-1} and kinetin 1.0 mg l^{-1} concentration. Higher regeneration percentage (80%) was obtained at 1.5 mg l^{-1} of BAP (Fig. A). The best media for regeneration of inflorescnece callus was MS + BAP 1.0 mg l^{-1} + NAA 1.5 mg l^{-1} (54%) (Table 2). Inflorescence gave the best response for callus induction and regeneration in all the four genotypes [2].

Table 2. Total number of *in vitro* derived plants

Tissue / Genotypes	Seed		Leaf		Inflore scence		Immature embryo		Total	
	T	R	T	R	T	R	T	R	T	R
PT 3095	27	14	30	15	40	25	30	20	127	64
ICMS 7703	30	18	35	21	45	27	35	22	137	68
732 B	20	10	32	12	35	20	28	18	115	60
81 B	18	8	25	10	30	14	20	11	93	43

T - Total number of plants transferred to mist chamber

R - Number of plants recovered and transferred to greenhouse

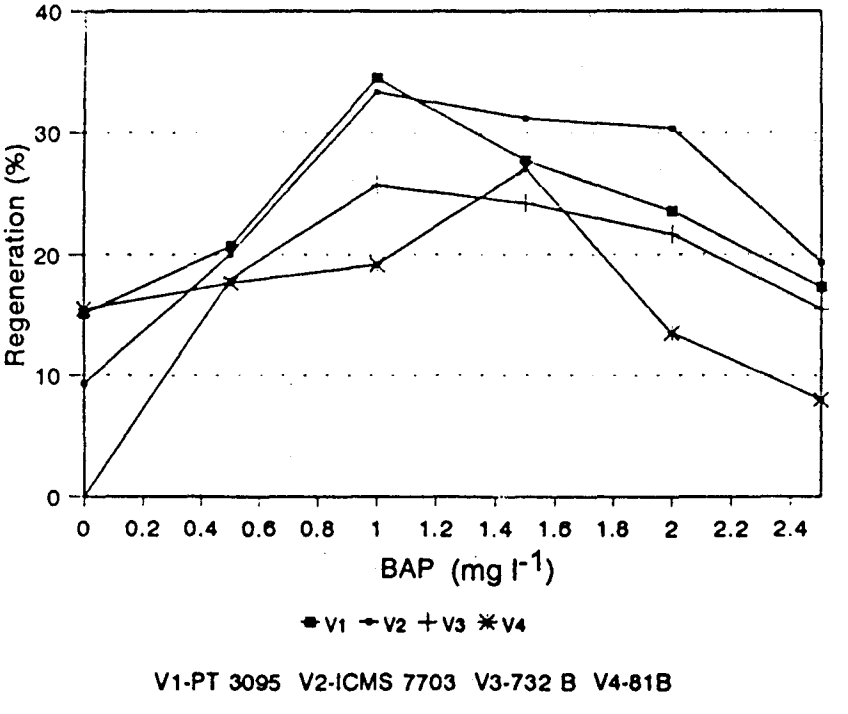

Fig. A. Effect of BAP on regeneration of plantlets in inflorescence calli of different genotypes

In immature embryo, callus induction was high (70%) at 1.5 mg l^{-1} of 2, 4-D and 0.3 mg l^{-1} of kinetin. Shoot regeneration from calli occurred after transfer to regeneration medium. Shoots were obtained at 0.5 mg l^{-1} of IAA (Fig. B) and BAP at 2.0 mg l^{-1} (42%) (Table 3, Fig. C). The response of immature embryo was low in comparison with leaf and inflorescnece but higher than seed [3-5].

Table 3. Total number of *in vitro* derived plants

Tissue / Genotypes	Seed		Leaf		Inflore scence		Immature embryo		Total	
	T	R	T	R	T	R	T	R	T	R
PT 3095	27	14	30	15	40	25	30	20	127	64
ICMS 7703	30	18	35	21	45	27	35	22	137	68
732 B	20	10	32	12	35	20	28	18	115	60
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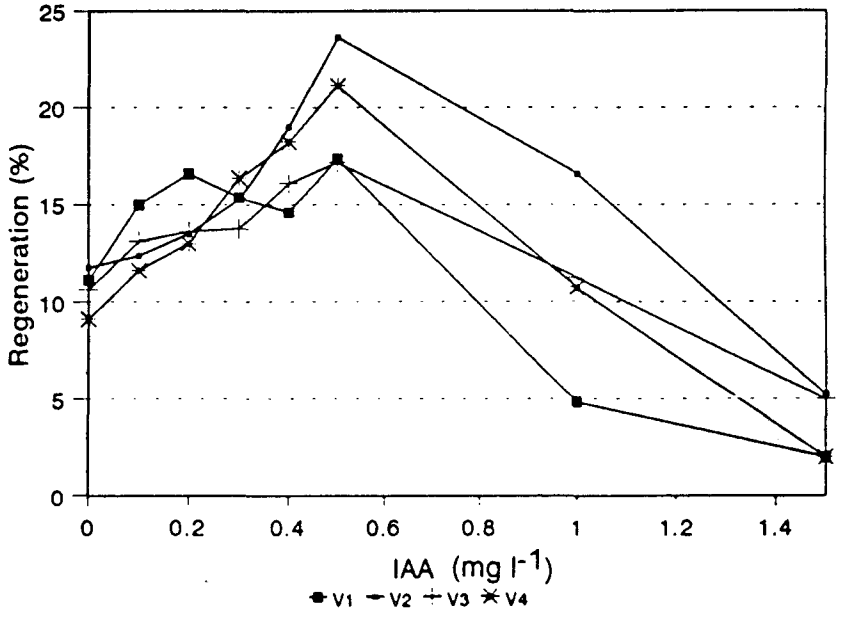

V1-PT 3095 V2-ICMS 7703 V3-732 B V4-81B

Fig. B. Effect of IAA on regeneration of plantlets in immature embryo calli of different genotypes

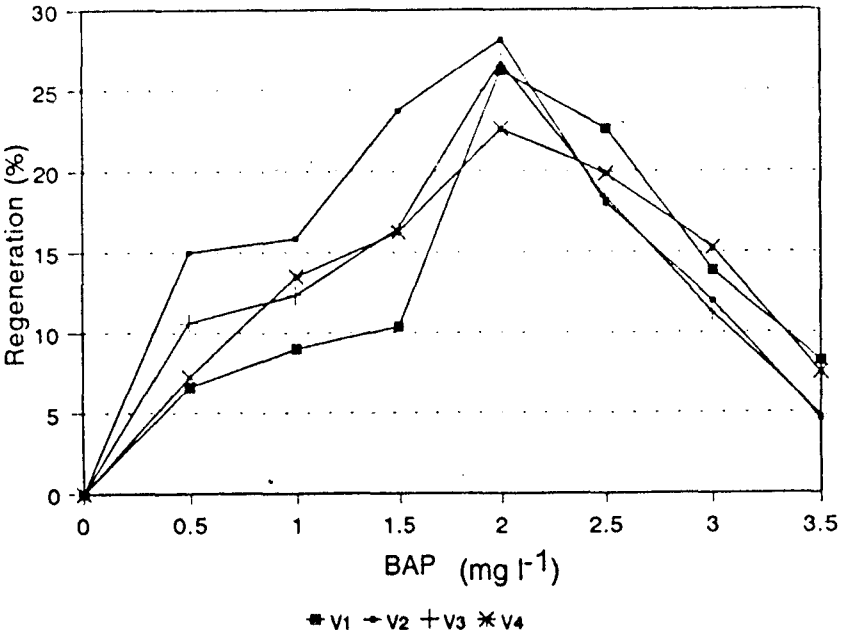

Fig. C. Effect of BAP on regeneration of plantlets in immature embryo calli of different genotypes

The details of the regenerants derived from different explants are furnished in Table 2. Among the four genotypes ICMS 7703 has recorded the highest callus induction frequency (80 %) followed by PT 3095, 732B and 81B. The regeneration efficiency of ICMS 7703 was 54%. The differences in *in vitro* response is due to genotypes of pearlmillet [6]. Such genotypic differences were reported in other cereals also [7, 8]. It was found that the order of suitability of media for callus induction in pearlmillet was N6 (80 %) followed by MS (73 %), whites (51 %) and LS (35%). This difference in callus induction in different media is due to the difference in major nutrients present in the four basal media. The optimum concentration of 2, 4-D for callus induction was observed to vary with different explants but in general ranged from 2.0 to 5.0 mg l^{-1}. The kinetin level that induced good callus ranged from 0.3 to 0.5 mg l^{-1}. Cytokinins in combination with an auxin appears essential for the onset of growth and induction of embryogenesis in cereals [9].

It was concluded from present study that the ideal explant for *in vitro* culture of pearl millet is immature inflorescence and the morphogenetic potential was high in ICMS 7703. This study indicates the possibility for inducing genetic variability through tissue culture for pearlmillet improvement.

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