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In vitro response of explant size and age on multiple shoot induction from shoot tips of castor (*Ricinus communis* L.)

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Abstract : The response of shoot tips to initiate multiple shoots was studied in two genotypes of castor GCH 4 and Kranthi. *In vitro* shoot bud induction response varied with the explant size and age. Five mm shoot tips or shoot tips obtained from nine day old seedlings initiated a maximum of 4.6 and 5.6 shoot buds per explant on MS + BAP 5.0 mg/1 respectively. Elongated shoots were induced for rooting on MS +IBA 0.1 mg/1. Complete plants were hardened and later transferred to pots and grown up to maturity of primary spikes.

Key words: Multiple shoot bud induction, Ricimus communis

Introduction

Castor *(Ricinus communis* L.) is an important non edible oilseed crop of India. Andhra Pradesh is the second largest producer of caster with a production of 1.3 lakh tonnes. The productivity in the state as compared to national average of 833 kg / ha is however low at 333 kg / ha (Damodaram and Hegde, 2002). To increase the yield potential, new biotechnological techniques that include production of novel tissue culture induced variations appear promising (Sujatha and Reddy, 1998). Not much headway has been made in generating plant types that tolerate abiotic stresses by conventional breeding methods. As such, plants regenerated *in vitro* could be screened for drought stress by growing them in nutrient solutions that decrease the osmotic potential (Bhojwani and Razdan, 1983). Seedling parameters like seed germination percent, seedling vigour index and nitrate reductase activity / proline proved useful in selecting drought stress tolerant genotypes (Manjula *el al.,* 2003). Poly ethylene glycol (PEG) induces water stress in the medium by acting as a non penetrating agent and lowers the water potential of the medium (Srivastava, 1998). Using PEG, screening procedures have been developed to obtain drought stress tolerant callus in castor (Manula *et al*., 2001) and drought tolerant plants in groundnut (Venkateshwarlu *et al.,* 1998). The present study was therefore taken up to standardize *in vitro* protocol for multiple shoot bud induction and their transfer to pot culture as a first step.

Materials and Methods

Seeds of two castor genotypes GCH 4 and Kranthi were surface sterilized with $HgCl_2$ (0.1%) for ten minutes, washed with sterile water and sown in plastic pots containing quartz sand and grown *in vivo* in *rabi*, 2003. Three to eleven day old seedlings were used for the study. Five cm long shoot tips cut from the seedlings were surface sterilized with $HgCl_2$ 0.1% for ten minutes, washed with sterile

Table 1. Response of shoot tips to explant size and explant age on shoot bud differentiation at 21 DAI on MS + BAP 5.0 mg/l.

Shoot buds formed				Shoot buds formed			
Explant size (mm)	Genotypes		Mean	Explant age (days)	Genotypes		Mean
	GCH 4	Kranthi			GCH 4	Kranthi	
1	1	1	1	3	1	1	1
2	1.7	1.7	1.7	5	3.2	1.3	2.2
3	2.2	2.8	2.5	7	4.2	3.2	3.7
4	4.2	3.8	4	9	4.6	5.6	5.1
5	4.3	5.2	4.7	11	4	3.3	3.6
6	3.8	4.2	4				
Mean	3.1	3.4		Mean	3.4	2.9	

	S.Em+	CD (p=0.05)		S.Em+	CD (p=0.05)
Size (S)	0.04	0.08	Age (A)	0.08	0.17
Genotype (G)	0.03	0.06	Genotype (G)	0.05	0.11
S x G	0.06	0.12	A x G	0.11	0.24

distilled water and used for culture *in vitro*. Five mm sized shoot tips were cultured on MS medium (Murashige and Skoog, 1962). The medium was fortified with BAP 2-6 mg/1. Shoot tips were inoculated on the media and standardized initially for multiple shoot bud induction. Explant size ranging from 1 to 6 mm and explants from 3 to 11 day old seedlings were cultured on MS +BAP 5.0 mg/l to improve the shoot bud induction response. Six replications with a minimum of ten cultures per replication were inoculated. Cultures were maintained at 25$\pm$2^{o}C and data recorded three weeks of culture on the multiple shoot bud initiation response. The data was analyzed in two factor randomized block design following Panse and Sukhatme (1985). Shoots elongated in the culture tubes and measuring 1.5 cm were cut and transferred to MS + IBA 0.1 mg/l for rooting with 80% success. Rooted plants were hardened for three months following the standard procedures outlined by Bhojwani and Razdan (1983). A total of twelve plants were raised in pots till maturity of primary spikes.

Results and Discussion

Manipulation in the size of the shoot tip cultures altered the shoot bud induction response on MS containing BAP 5.0 mg/1 (Table 1). Culture of 1.0 to 6.0 mm sized shoot tips of the two genotypes resulted in 1.0 to 4.7 multiple shoot buds. Genotypes X explant size interaction revealed five mm sized shoot tips to give optimum response. A maximum of 4.3 and 5.2 shoot buds initiated in GCH 4 and Kranthi respectively. Sujatha and Reddy (1998) reported in aruna genotype that 3.0 mm sized shoot tips gave the maximum response. However, in the present study increase

or decrease in the size of shoot tips decreased the morphogenetic response, thus indicating that the size of the explant used for culture decides the response. In other studies, size and quality of seed material inoculated influenced the tissue culture response (Kumar, 2002).

Seedling age manipulation similar to change in size of the explant used, altered the multiple shoot bud response (Table 1). Shoot tips from 3-11 day old seedlings initiated 1.0 to 5.1 shoot buds per shoot tip. Optimum response was obtained when shoot tips were obtained from nine day old seedlings and cultured on MS + BAP 5.0 mg/1. Genotypic variation was also evident in cultures. A maximum of 4.6 and 5.6 shoot buds were initiated from GCH 4 and Kranthi respectively. Use of explants from older seedlings decreased the organ genetic response. Kumar (2002) also reported that explant factors like physiological age influenced shoot induction response. Shoots measuring 1.5 cm were cut and transferred to MS + IBA 0.1 mg/1 for rooting with 80% success. Rooted plants were hardened for three months. A total of twelve plants were raised in pots till maturity of primary spikes.

The use of 5.0 mm sized shoot tips obtained from nine day seedlings and cultured on MS medium incorporated with BAP 5.0 mg/1 gave the maximum shoot bud induction response. Shoots rooted on MS + IBA 0.1 mg/1 were further hardened and raised until maturity of primary spikes.

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