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PLANT REGENERATION IN MESOCOTYL CALLUS OF *BRASSICA JUNCEA* VAR. POORBIRAYA

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Abstract

Mesocotyl segments of *Brassica juncea* var. *poorbiraya* from *in vitro* grown seedlings were tested for their callogenic and thence morphogenic potential with various treatments of plant growth regulators. Calli grown at 1.0 mg/l of BAP gave a maximum frequencey (2.25 buds/culture) of shoot regeneration. Regenerated buds were induced to root when cultured on half strength MS medium containing IAA @ 0.5 mg/l. Embryogenesis was induced in mesocotyl callus in MS medium containing 2,4-D (0.022 mg/l) + KIN (0.215 mg/l). Phenotype of regenerated plants looked similar to *in vitro* grown *Brassica juncea* plants.

Introduction

Plant tissue culture techniques are being used in a variety of ways in oil crop improvement. The development of efficient methods of plant cell culture and, in particular, high frequency reliable plant regeneration are essential prerequisites for the aplication of biotechnology to plant breeding (Jones, 1988). Extensive tissue culture research has been done on *Brassica napus, B. campestris* and *B. oleracea* (Iizuka *et al.,* 1978: Dunwell, 1981; Lichter, 1982; Xu *et al.,* 1982; Lazzeri, 1984 a,b; Singh, 1984) but *Brassica juncea* has been relatively less explored.

Shoot formation directly on cotyledon and hypocotyls of *B. juncea* were reported by Hui & Zea (1978) and George & Rao (1980). A direct and via callus regeneration by using internodal and hypocotyl segments of *B. juncea* have been reported (Ahmad *et al.,* 1990, Shahzadi *et al.,* 1991). The present study describes the procedures developed to regenerate *Brassica juncea* through mesocotyl derived calli.

Material and Methods

a) ***Plant Material:*** Seeds of *Brassica juncea* var. *Poorbiraya* were sterilized with ethanol for 1-2 min., followed by $HgCl_2$ (0.1%) for 15 minutes and rinsed repeatedly with sterile distilled water were then transferred in culture tubes, 1-2 seeds per tube. Hypocotyl and epicotyl explants were aseptically removed from 3-4 day old seedlings and remaining mesocotyl portions were left behind for callus induction in the same culture tube.

b) ***Media and Culture Conditions:*** Murashige & Skoog (1962) mineral salt and vitamins, including 30 g/l sucrose and 7 g/l agar was used as culture medium. Growth regulators were added before sterilization of media. Calli were subcultured every 3-4 weeks. pH of media was adjusted at 5.7 with NaOH prior to the addition of agar. Twenty ml of medium in cul ture tubes/flasks was sterilized at 121°C for 15 minutes. All cultures were incubated at 26±1°C under a 16 h photoperiod at 3 Klux of cool white fluorescent light.

Table 1. Role of KIN* and BAP* in callus induction in mesocotyl explants of *Brassica juncea* var. *Poorbiraya*.

Hormones (mg/l)	Callus (Quantity)** %			Proliferation +/-
	poor	Good	Copious	
KIN				
0.1	75	25	0	-
0.3	71	28	0	-
0.5	75	25	0	-
0.75	66	33	0	-
1.0	70	20	10	+
2.5	77	22	0	1
2.0	80	10	10	1
2.5	60	20	20	1
BAP				
0.01	83	16	0	-
0.03	85	0	14	-
0.05	57	42	0	+
0.1	50	50	0	+
0.2	40	50	10	+
1.0	14	28	57	+
2.0	0	12.5	87.5	+
3.0	55	44	0	+
5.0	80	20	0	-

* Average callus induction for KIN in 10 and BAP in 14 days.
** No. of replicates = 9.

Results and Discussions

a) ***Initiation, Growth and Maintenance of Callus*:** Choice of optimal salt mixture was not experimented upon as there were enough reports (Hui & Zee 1978; George & Rao 1980; Kao *et al.*, 1987; Ahmad *et al.*, 1990; Shahzadi *et al.*, 1991) recommending MS(1962) medium for Tissue culture studies of *Brassica juncea*. Callus induction from mesocotyl explants on MS medium supplemented with KIN (0.1- 2.5 mg/l) usually occurred in 9-10 days (Table 1). Very little or no callus growth was observed in treatments containing lower (0.75 mg/l) concentration of KIN. Calli formed were usually pale white to brown, and upropagatable due to root differentiation. Calli induced at 1 mg/l of KIN were better and could be grown by subculturing on-to fresh medium. Calli formed at higher than 1.5 mg/l levels became brown and died after 3 weeks.

Very little callus growth was observed in treatments having 0.01-0.02 mg/l of BAP. Calli could not be maintained due to root formation which intiated at a very early stage. BAP 1-2 mg/l was the optimal concentration range for callus induction. Calli were green in colour, soft in texture and retained their vigour throughout subculturing. Quantity of callus mass decreased at higher BAP concentration. In treatments containing 3 and 5 mg/l of BAP 88% of cultures exhibited poor callus growth. Where 0.03-0.05 mg/l each

Fig.1. *Brassica juncea* var. *Poorbiraya* mesocotyl callus induced and maintained on medium containing 2 mg/l BAP.

of 2,4-D and BAP were used 60-65% of cultures exhibited little or moderate callus growth. Callus quality improved at higher concentrations of the growth regulators. Upto 70 to 75% cultures exhibited good callus growth in treatments containing 0.1-2.0 mg/l each of 2,4-D and BAP. Calli formed were green in colour, nodular and compact in texture (Fig.1).

Rapidly proliferating, soft textured and green calli were formed when NAA and BAP were used in the medium (Table 2). NAA (0.4 mg/l) + BAP (0.2 mg/l) was found the optimum concentration as 72.7% of cultures produced fast growing calli. These calli were maintained by subculturing to the same treatment after 4-5 weeks. Higher than 1.0 mg/l) concentrations of NAA and BAP were not suitable for callogenesis on mesocotyl explants.

Rapidly proliferating whitish-green embryogenic calli were obtained with 2,4-D (0.02-0.5) + KIN (0.2-1.0 mg/l) from mesocotyl explants. Calli were maintained on subculturing to fresh medium containing same concentration of PGRs after a regular interval of 4 weeks. Media containing 2,4-D (1.0 mg/l) and KIN (2.0-3.0 mg/l) were not found suitable for callus growth.

b) ***Regeneration through Mesocotyl Callus:*** The established callus cultures were taken to auxinless media. Wherever shoot regeneration was intended, the concentration of cytokinin was either retained or increased. Calli grown at 1 mg/l of KIN, when sub-cultured on the same or shifted to higher (2,3 or 4 mg/l) KIN media, did not show any morphogenic response. Calli induced at 1 and 2 mg/l of BAP regenerated shoot buds during third passage on maintenance medium containing same concentration of BAP (Table.3). Regeneration frequency was 2.25 and 2 buds/culture respectively. Cytokinin containing media, facilitates subsequent shoot regeneration. *In vitro* organogenesis in media containing only one cytokinin has been reported (Reilly & Washer, 1977; Winton & Verhagen 1977; Von Arnold & Eriksson, 1978; Shahzadi *et al.,* 1991). *Brassica juncea* shoots regenerated roots when transferred to media containing IAA (0.5 mg/l) and half strength MS salts (Fig.2 & 3).

Table 2. Effect of 2,4-D, NAA, BAP and KIN, in various combinations on callusing from mesocotyl explants of *Brassica juncea* var. *Poorbiraya*.

Hormones (mg/l)		Callus (Quantity) %			Proliferation +/-
		Poor	Good	Copious	
2,4-D	**BAP**				
0.03	0.03	66	33	0	-
0.1	0.1	0	18	81	+
0.5	0.5	0	16	83	+
0.75	0.75	20	26	53	+
1	1	20	20	60	+
2	2	0	25	75	+
	KIN				
0.02	0.2	0	12.5	87.5	+
0.05	0.4	0	0	100	+
0.1	0.8	8	8	83	+
0.2	1.6	0	11	88	+
0.4	3.2	37.5	25	37.5	+
0.5	1.0	36	45.5	18	+
1	2	25	66.5	8	-
1	3	40	40	20	-
NAA	**BAP**				
0.1	0.5	30	40	30	+
0.2	0.1	33	25	41	+
0.4	0.2	0	27	72	+
0.6	0.3	0	40	60	+
0.8	0.4	15	53	30	+
1	0.5	50	42	7	+
1	1	66	33	0	-
2	2	87.5	12.5	0	-
3	3	85	14	0	-

1. Average callus induction in 11 days with NAA + BAP, 16 days with 2,4-D + KIN & 24 days with 2,4-D + BAP. 2. No. of replicates = 10

Calli induced under various concentrations of 2,4-D (0.03-0.8 mg/l) + BAP (0.03-1.0 mg/l) when subcultured to media containing only BAP (1 mg/l) did not show any regeneration except one callus line induced at 1.0 mg/l each of 2,4-D and BAP. Regeneration frequency was 1.3 buds/culture and was achieved in 15-16 days after transferring to auxin less medium. Regenerated shoots developed roots when cultured on MS (half strength) medium and either no PGR or with IAA at 0.5 mg/l (Fig.4).

Calli initiated and maintained at NAA (0.6 mg/l) + BAP (0.3 mg/l) (Table.3) regenerated a single shoot bud in 50- 54 days after media containing BAP (1.0 mg/l). No shoot formation was observed in any of the other calli grown at various concentrations of NAA and BAP. Failure of calli induced at higher concentrations of NAA could probably be attributed to the fact that PGR, modify membrane permeability (Glinka & Reinhold, 1971), alter the uptake of other hormones (Durlay *et al.*, 1976) and thus affect

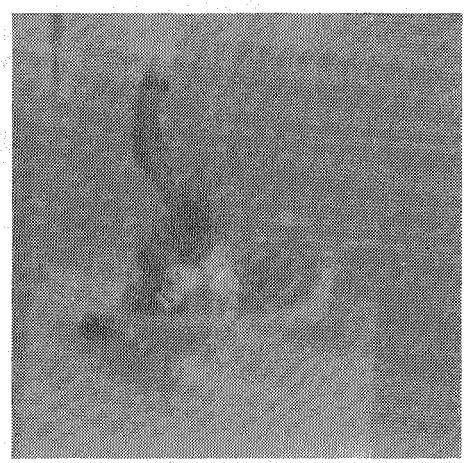

Fig.2. Bud regeneration in mesocotyl callus of *B. juncea* var. *Poorbiraya* with BAP (1.0 mg/l).

the system adversely at the level of absorption. NAA and 2,4-D at lower levels did not interfere with the cytokinin (BAP) activity, whereas residual effects of higher levels probably suppressed the process of shoot regeneration from callus. Comparison could be made in terms of regeneration frequency and time taken for regeneration through various routes (Table.3). Best regeneration in mesocotyl callus was obtained on medium containing BAP (1-2 mg/l) which showed better regeneration frequency (2-2.25 buds/culture, 15-16 days) and NAA + BAP (1 bud/culture, 50-54 days) (Fig. 2 & 3).

c) ***Embryogenesis:*** Minute embryoid like structures with dark green tips appeared on callus surfaces against the back ground of slightly pale white callus mass on medium containing 2,4-D (0.022 mg/l) + KIN (0.215 mg/l). Such differentiating structures were not observed in any of the other combination of 2,4-D and KIN. Bhattacharya (1980) was also able to obtain somatic embryogenesis through leaf petiole callus of *Brassica*

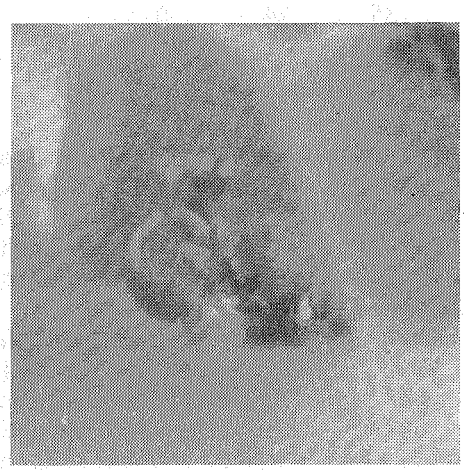

Fig.3. Bud regeneration in mesocotyl callus of *B. juncea* var. *Poorbiraya* with BAP (2.0 mg/l).

Table 3. Regeneration through mesocotyl callus of *Brassica juncea* var. *Poorbiraya*.

Growth regulators used for callus induction (mg/l)		Growth regulators used for callus proliferation (mg/l)		Growth regulators used for shoot differentation (mg/l)		Average time for shoot regeneration (days)	Regeneration frequency (buds/culture)	Rooting (media)
Auxin	BAP	Auxin	BAP	Auxin	BAP			
NAA (0.6)	0.3	NAA (0.6)	0.3	-	1.0	54	1	MS(half strength) + IAA (0.5 mg/l)
NAA	0.3	NAA (0.6)	0.3	-	2.0	42	1	"
-	1.0	-	1.0	-	1.0	14	2.25	"
-	2.0	-	2.0	-	2.0	14	2	"
2,4-D	1.0	2,4-D (1.01)	1.0	-	1.0	16	1.3	MS(half strength) and no PGR.

"Time for regeneration" was calculated from time of shifting of calli from maintenance medium to regeneration medium and regeneration observed.

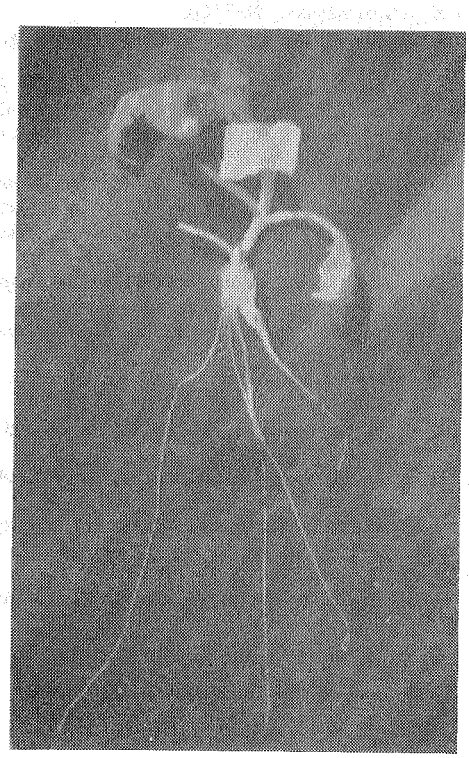

Fig.4. Root formation on regenerated *Brassica* buds by IAA at 0.5 mg/l.

campestris at only one treatment containing 2,4-D and KIN at 0.022 and 0.215 mg/l respectivity. By the end of fourth week, embryoids became quite large in size. Dark green whorl type structures appeared on the callus surfaces. Callus masses at this stage when transferred to hormoneless media showed initiation of healthy shoots in 6-8 days. Regeneration frequency was very low and root system was also not fully developed. Formation of somatic embryos in calli derived from mesocotyl explants was also confirmed by histological study.

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