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Regeneration and *In Vitro* Flowering in *Brassica Campestris* (L.) Var. *Bhavani*

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Abstract

Multiple shoot formation and *in vitro* flowering was found in *Brassica campestris* (L.) var. Bhavani. Maximum numbers of shoots were produced in both cotyledonary node and shoot apex explants on MS-media supplemented with BA (2.5 mg/l) + IAA (1.0 mg/l) + Kn (0.5 mg/l). Maximum flowering (50%) was noted at IBA (1.5 mg/l) + IAA (1.0 mg/l) + Kn (0.5 mg/l) in the shoots from cotyledonary nodes. *In vitro* flowering may contribute in many ways to Brassica Improvement Programs. The shoots rooted well in the half and full strength media each with IBA (1.0 mg/l) and NAA (1.0 mg/l) and the plantlets have been maintained.

Keywords: *Brassica campestris*, *In vitro* flowering, Regeneration.

Introduction

Brassica campestris is an important source of vegetable oil. *In vitro* techniques have been applied in Brassicas from different point of views and organogenesis, somatic embryogenesis and regeneration were achieved (Antonio *et al*., 1987; Jain *et al*., 1988; Ono *et al*., 1994; Koh and Loh, 2000; and Khan *et al*., 2002). *Brassica campestris*, in contrast to other species of Brassica, has consistently been proved more difficult to regenerate *in vitro* (Dunwell, 1981; Dietert *et al*., 1982; Schenck and Röbbelen, 1982; Glimelius, 1984; and Lazzeri and Dunwell, 1984a, b). Nevertheless, *in vitro* flowering has been reported as a rare process of importance of high genetic purity (Stephen and Jayabalan, 1998). The *in vitro* flowering has been found in *B. oleracea* and *B. napus* (Vandana *et al*., 1995; and Koh and Loh, 2000) and in other crops like coriander (Stephen and Jayabalan, 1998) and maize (Mandal *et al*., 2000).This paper presents the findings of an experiment to work out a suitable protocol for the efficient regeneration in *B. campestris* and the role of phytohormones on *in vitro* flowering in this species.

Materials and methods

The seeds of *B. campestris* (L.) were washed in running tap water for 30 min and treated with 2% bavastin solution and few drop of Tween-80 for 20 min. Then after, thoroughly washed seeds were surface sterilized with 90% alcohol for 1 min and immersed in 0.1% $HgCl_2$ solution for 5-7 min and rinsed thoroughly with autoclaved distilled water. The sterilized seeds were germinated on MS basal medium. Cotyledonary nodes and shoot apices were excised from seven days old aseptically grown seedlings and cultured on MS media containing 3% sucrose and 0.7% agar with various concentrations/combination of auxins (IAA, IBA and NAA) and cytokinins (BA, Kn). The pH of the medium was adjusted to 5.8 prior to autoclaving at 121˚C

for 30 min. All cultures were maintained at 25±2˚C under 16h/8h photoperiod.

Results and discussion

Shoot induction was observed, in both, the cotyledonary nodes and shoot apex explants within 8-10 days of culture at all the hormonal combinations (Figures A, B; Table 1). The number of shoots ranged form 4 to 9 in cotyledonary nodes and from 3 to 8 in shoot apex explants. The maximum shoots were observed at BA (2.0-2.5 mg/l) + IAA (0.5-1.0 mg/l) + Kn (0.5 mg/l) combinations. The BA concentration 2.0-2.5 mg/l appeared as optimum for shooting. George and Rao (1980) observed maximum regeneration from cotyledon explant in *B. juncea* with BA and NAA rather than BA alone. Hachey *et al*. (1991) have also reported efficient regeneration in *B. campestris* with BA in combination with NAA.

The flowering was observed in these shoots after 35-40 days of inoculation at BA (2.0-2.5 mg/l) + IAA (1.0 mg/l) + Kn (0.5 mg/l) and at IBA (1.0-1.5 mg/l) + IAA (1.0 mg/l) + Kn (0.5 mg/l) combinations (Figs. C, D). This exogenous hormonal supply might have been added up to the endogenous contents, raising the hormonal level required for triggering the flowering. A maximum of 12 flower buds was recorded from the shoots of an explant. Almost 50% shoots had flowers and maximum flowering was noted at IBA (1.5 mg/l) + IAA (1.0 mg/l) + Kn (0.5 mg/l) and minimum at BA (2.0 mg/l) + IAA (1.0 mg/l) + Kn (0.5 mg/l) combinations. Also, the shoots from cotyledonary node explant showed better flowering response as compared to that of shoot apex explants. But, these *in vitro* flowers were smaller than *in vivo* ones. 1.5 mg/l IBA seemed optimum for flowering in the shoots from cotyledonary node explants. Vandana *et al*. (1995) have reported *in vitro* flowering and pod formation in cauliflower with IAA and Kn. Stephen and Jayabalan (1998) opined that flowering was considered as a complex process regulated by both internal and external factors and its induction under *in vitro* culture is extensively rare. While Zimmerman *et al*. (1985) were of the opinion that the interaction of carbohydrate and other nutritional factors with endogenous growth regulators can influence some biological parameters, which are altered when plant changes from juvenile to mature phase. Sheeja and Mandal (2003) have also reported *in vitro* flowering and fruit formation in tomato at high level of endogenous auxins. Jabeen *et al*. (2005) have reported that auxins support *in vitro* flowering in *Solanum nigrum*.

The shoots transferred to half and full strength media each supplemented with IBA (1.0 mg/l) and NAA (1.0 mg/l) produced roots. The plantlets have been maintained.

It is evident from these results that maximum regeneration and *in vitro* flowering can be obtained at BA (2.5 mg/l) + IAA (1.0 mg/l) + Kn (0.5 mg/l) and at IBA (1.5 mg/l) + IAA (1.0 mg/l) + Kn (0.5 mg/l) respectively. These protocols may be utilized for maximum regeneration and *in vitro* flowering in *B. campestris* genotypes. *In vitro* flowering can be of much value to circumvent the flowering time and also to accentuate the pod formation to facilitate the *Brassica* Improvement Programs.

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Figure (A-D). Shoots and plantlets with flowering in *B. campestris*. (A) Multiple shoots from cotyledonary node (B) A plantlet (C) Shoot with flower buds (D) Plantlets with flower buds and flowers

Table 1. Phytohormonal concentrations influencing *in vitro* response in *Brassica campestris* var. *bhavani*

Phytohormonal concentration	Cotyledonary node			Shoot apex			Flowering %
	No. of shoots /explant	No of flower buds /explant	No of flowers/ explant	No of shoots /explant	No of flower buds/explant	No of flowers /explant	
BA(1.0mg/l)+IAA(0.5mg/l)+Kn(0.5mg/l)	2.70±0.23	--	--	3.21±0.28	--	--	--
BA(2.0mg/l)+IAA(1.0mg/l)+Kn(0.5mg/l)	4.96±0.34	5.28±0.77	4.14±0.72	4.68±0.34	--	--	25%
BA(2.5mg/l)+IAA(1.0mg/l)+Kn(0.5mg/l)	5.13±0.34	5.57±0.67	4.85±0.47	4.81±0.41	6.60±0.81	4.60±0.74	28%
BA(3.0mg/l)+IAA(1.0mg/l)+Kn(0.5mg/l)	2.76±0.25	--	--	2.50±0.30	--	--	--
IBA(1.0mg/l)+IAA(1.0mg/l)+Kn(0.5mg/l)	4.07±0.37	6.42±0.75	4.85±0.76	3.36±0.35	5.28±0.42	3.85±0.59	48%
IBA(1.5mg/l)+IAA(1.0mg/l)+Kn(0.5mg/l)	3.18±0.37	8.41±0.77	6.25±0.95	2.90±0.37	5.37±0.41	4.87±0.39	50%

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