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Effect of various growth regulators on direct regeneration of Brassica spp. using stem disc as explants

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ABSTRACT: Culture conditions for shoot regeneration, proliferation and rooting of *Brassica* spp. were optimized by a judicious selection of explants and manipulation of hormonal combinations in the culture medium. High frequency bud break (100%) and direct shoot regeneration were induced from stem disc explants and cultured on MS medium with 3 mg/L BAP. The shoots are proliferated from stem disc within 14 days of inoculation on MS medium containing 2.0-4.0 mg/L BAP alone without intervening callus. Regenerated shoots produce good quality roots on MS 1/2 medium with IBA 0.5 mg/L was used for quality root formation. Regenerated plants get established in pots containing sterile soil followed by their transfer to natural soil under full sun.

Keywords: *Brassica* spp., micropropagation, culture media, explants, growth regulators.

INTRODUCTION

The application of gene transfer techniques for crop improvement requires an efficient method through which plants can be regenerated from single, cell, tissues or organs via organogenesis or somatic embryogenesis *in vitro*. Plant propagation via organogenesis is one of the techniques used in plant tissue culture to obtain large number of plants irrespective of season and with conservation of space and time.

The genus *Brassica* compresses many commercially important vegetable and oilseed crops. Oilseed *Brassica* is one of the most important sources of edible vegetable oil, industrial oil and protein rich product in the world. Oilseed *Brassica* ranks third after soybean and palm oil in global production (Canola Council of Canada, 1006).

The average yield of local varieties and high yielding varieties are 600-1000 kg/ha and 1400-2000 kg/ha which contributes only 71.3% of total oilseed production of Bangladesh (BBS, 2005). Conventional breeding programme can be used to improve new traits within species. But these programmes alone were not successful enough in *Brassica* due to high degree of segregation upon cross pollination and unavailability of suitable wild germplasm. Many efforts have been undertaken to establish a suitable *in vitro* regeneration protocol. In 1991, Hachey and co-workers obtained a high frequency of shoot regeneration from some oilseed cultivars and Takasaki *et al.* (1996) got shoot from a few leafy vegetable cultivars of *Brassica* spp. Several attempts have been taken to establish *in vitro* regeneration protocol for brassica.

Various tissues have been used in organogenesis of brassica crops like hypocotyls (Cardoza and Stewart, 2003; Munir *et al.*, 2008), cotyledon and leaves (Kennedy *et al.*, 2005; Javed *et al.*, 2012), shoot tip (Widiyanto and Erytrina, 2001; Abbas *et al.*, 2012; Asim, 2012), roots (Kranthi *et al.*, 2005) and protoplast (Kaur *et al.*, 2006). Direct organogenesis has been achieved in a variety of *Brassica* spp. such as from the stem sections of *Brassica juncea* (Barfied and Pra, 1991), petioles of *Brassica napus* (Ghanya *et al.*, 2007), hypocotyls of *Brassica napus* (Phogat *et al.*, 2000) and cotyledonary explant of *Brassica compestris* spp. Pekinesis (Zhang *et al.*, 1998).

In vitro regeneration is influenced by many factors such as culture environment, culture medium composition, explant source and genotype (Zhang *et al.*, 1998; Rajicie *et al.*, 2001; Bano *et al.*, 2010). This paper presents the findings of an experiment to work out a suitable protocol for efficient regeneration in *Brassica compestris* using stem disc as explant and role of growth regulators in regeneration.

MATERIAL AND METHODS

Stem disc explants from well grown up *Brassica* plants were used. Vegetatively propagated plants raised under moist conditions were carefully brought to laboratory and washed 3-4 times under running tap water and then different explants were treated and soaked with detergents like teepol or tween-20 + systematic fungicide Bavistin (0.5%) + 0.2% streptomycin sulfate. Further surface sterilization of these explants was done with smercuric chloride ($HgCl_2$) at concentration of 1% for 5 minutes duration and initially these explants washed with double sterilized water for 3-4 times before inoculation. The borosilicate glasswares used for experiment were soaked in chromic acid (5%) solution for six hours followed by thorough washing with running tap water.

Culture media and conditions: MS medium (Murashig and Skoog, 1962) supplemented with 30g/L (w/v) sucrose, 100 mg/L myoinositol, 0.5 mg/L nicotinic acid, 0.5 mg/L pyridoxine HCl, 2.0 mg/L glycine, 0.1 mg/L thiamine along with auxins i.e. (NAA) naphthalene acetic acid, Indole-3 butyric acid (IBA), Indol-3-acetic acid (IAA) and 2,4-dichloro-phenoxy acetic acid (2, 4-D) in the concentration ranging from 0.1-1 mg/L and cytokinines-Benzyl amino purine (1-4 mg/L) and kinetin (kin) (1-2 mg/L) were used either individually or in combination for initial and establishment of culture. The pH of MS medium was adjusted to 5.8 by 1N NaOH. The culture vials are autoclaved at 121^{o}C at 106 KPa for 20 minutes.

Shoot induction and multiplication: Explants were placed in Borosil glasswares containing 100ml culture medium in flask and sealed with non-absorbant cotton. Cultures were maintained at 25 $\pm$ 2^{o}C under a 16 hrs photoperiod with a light intensity of 50 μE mol $m^{-2}s^{-2}$ supplied through cool white florescent tubes (Philips, India). After shoot induction, shoots were excised and sub-cultured twice at every third week interval. Shoot formed in clusters on establishment medium were excised after 6-weeks and cultured on shoot multiplication media. MS medium containing the same organic compounds as used in establishment medium in combination with either 1 or 2 mg/L BAP or kinetin.

Rooting: Shoots were isolated and cultured on basal MS 1/2 liquid medium with various concentrations of auxins for rooting. After 4 weeks on rooting media, the percentage of rooted shoots, number of roots and number of fasciculated roots formed on each shoot were recorded.

Ex vitro growth and acclimatization: For *ex vitro* growth, plantlets developed after rooting were taken out of medium were washed thoroughly with double distilled water to remove traces of medium. These were then transferred to small polythene bags containing 25% garden soil + 50% sand + 25% cowdung in growth chamber. Potted bags were suitably watered from time to time. After about 2 weeks, potted bags were taken out from culture room and polythene bags were removed for 3 hrs. daily to expose the plants to conditions of natural humidity. After 6 weeks, the plants were transferred to field containing cowdung manure.

RESULTS AND DISCUSSION

The regeneration of pathogen free plants is one of the best achievements of plant tissue culture. There was no sign of growth when MS media without growth regulators was used. In the present study, high concentration of cytokinin induces average number of shoots and auxin induces roots, MS media was found to support a greater response for shoot regeneration than other media.

Shoot proliferation: Bud breakage and development of shoots from stem disc was a function of cytokinein activity. There was no sign of bud breakage even after 4 weeks on MS medium devoid of growth regulators. If two cytokinins tested BAP was more effective than kinetin in inducing bud breakage

as well as multiple shoot formation. There was a direct correlation between increase in concentration of BAP upto optimum level (4.0 mg/l) and percentage of bud break. At different concentration of cytokinein BAP (1.0, 2.0, 3.0, 4.0 mg/L) and auxin IAA, IBA, NAA alone failed to response for callus induction. But direct regeneration of shoot takes place by using cytokinins alone. In the present study, stimulatory effect of singular supplement of BAP on bud breakage and multiple shoot proliferation in *Brassica* spp. was similar to that earlier reported in various plants including Occimum species (Pattnaik and Chand, 1996), *T. indica* (Rani *et al.*, 2010). MS medium supplemented with BAP 2.0 and 3.0 mg/L showed 100 per cent bud break after 10-14 days of inoculation. But BAP (1.0 mg/L) in combination with NAA (1.0 mg/L) supplemented to MS medium showed 100% bud break alongwith moderate callusing and 8 number of shoots/explant after 14 days of inoculation. However, with increase in concentration of BAP i.e. 2.0 mg/L with NAA 0.5 mg/L showed only callusing no bud break. Our observations on sprouting at higher concentration of BAP were in accordance with those of Pattnaik and Chand (1996) in O. amranum and O. sanctum and *T. indica* (Rani *et al.*, 2010). The average number of shoots/explant are 11.56 on 3.0 mg/L BAP and length of shoots is 0.5-2.3 cm while at 2.0 mg/L BAP, the average of shoots is 10.4 with average length is 0.5-2.5 cm.

Table 1: Effect of cytokinins on *in vitro* shoot multiplication and bud breakage of *Brassica* spp., data recorded after 14-days

Medium composition mg/L		% bud break (No. of days for bud break)	Average no. of shoots/explant	Length of shoots
BAP	1.0	89 ± 0.45	6.4 ± 0.19	0.92 ± 0.12
	2.0	100 ± 0.00	10.4 ± 0.16	1.6 ± 0.37
	3.0	100 ± 0.00	11.56 ± 0.17	1.54 ± 0.34
	4.0	85.8 ± 0.66	7.4 ± 0.19	0.68 ± 0.12
Control	2,-4D*	-	-	-
	IAA*	-	-	-
	NAA*	-	-	-
Kin	1.0	0	-	-
	2.0	0	-	-
	3.0	0	-	-

Data represented as Mean value ± Standard error;
** - No response*

Root differentiation: To optimize the rooting response of plantlets raised *in vitro*, different auxins (IAA, IBA and NAA) were tested at various concentrations. In the present study, MS basal medium alongwith different strengths (1/4, 1/2 and full) and different auxins (IAA, IBA and NAA) was used at different concentration. Rooting was observed 100% with all auxins at all concentration including auxin free medium. IBA at low concentration was sufficient for root induction was reported in *Brassica compestris* (Verma and Singh, 2007). In the present study, IBA (0.5 mg/L) was considered to be the best since a maximum number of 30 roots/shoot were obtained.

Table 2: Effect of auxins on formation of roots from excised shoots regenerated *in vitro* from brassica (observation recorded upto 4 weeks after inoculation)

Medium composition mg/L		No. of days for root induction	% rooting	No. of roots/shoot	Root length (cm)
MS (1/4)		7	100	3.6 ± 0.19	2.0 ± 0.35
MS (1/2)		7	100	5.48 ± 0.16	4.5 ± 0.57
MS		6	100	7.2 ± 0.12	3.22 ± 0.35
MS (1/2) + IAA	0.5	10	100	17.24 ± 0.11	2.5 ± 0.00
	1.0	10.	100	14.6 ± 0.19	2.5 ± 0.57
	2.0	12	100	12.4 ± 0.19	3.5 ± 0.57
MS (1/2) + IBA	0.1	6	100	5.4 ± 0.18	3.7 ± 0.36
	0.3	6	100	7.52 ± 0.16	2.38 ± 0.28
	0.5	7	100	30.4 ± 0.19	3.88 ± 0.51
	1.0	7	100	29.7 ± 0.16	1.58 ± 0.18

Data represented as Mean value ± Standard error

Establishment of plants: The regenerated plants were transplanted into plastic bags containing sterile soil, sand and cowdung in a 1:2:1 ratio for acclimatization. Gradually the plantlets were adapted to the soil.

CONCLUSION

In *Brassica* spp., direct shoot formation was maximum (100%) from stem disc (11 shoots/explant) on MS medium with BAP (2.0 – 3.0 mg/L) within 10-14 days required for bud break. MS 1/2 liquid medium with IBA (0.5 mg/L) was sufficient for root formation. The plantlets transferred to sterile soil + sand + cowdung 1:2:1 showed better survival during acclimatization.

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