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Optimization of callus induction and plantlet regeneration through filament culture of five oilseed *Brassica* species

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Abstract: Filaments of five *Brassica* species namely BARI sariaha-7, Tori-7, Agrani, Daulat and Safal were cultured *in vitro* to observe their regeneration potentiality. Different concentrations and combinations of growth regulators were supplemented on MS medium. The range of callus induction was 12.50-93.75 %. Maximum callus induction 90.63 % was observed on MS + 4 mgL^{-1}2, 4-D + 1.0 mgL^{-1} BAP. Among the genotypes, BARI sarisha-7 showed the highest percentage of callus induction (81.25%). Among the treatments the highest percentage of shoot regeneration (93.75 %) was observed on MS + 4 mgL^{-1} BAP + 1.0 mgL^{-1} NAA. BARI Sarisha-7 also showed the highest rate of plant regeneration (79.17 %). Root induction was highest (87.50) on half strength MS medium supplemented with 1.0 mgL^{-1} IBA and 0.5mgL^{-1} NAA. The plantlets with sufficient roots thus obtained were transferred successfully to plastic pots and subsequently to the field. BARI Sarisha-7 and Tori-7 survived easily in the pots as well as in the field but Safal was very poor in survivability both in the pots and in the field.
Keywords: Oilseed *Brassica* species*;* filament culture; 2, 4-D, BAP and NAA treatment; *in vitro* regeneration

Introduction

Bangladesh is principally an agricultural country and produces good member of oilseed crops like mustard, sesame, groundnut, linseed, niger, safflower, sunflower, soybean, castor etc. The first three of these are considered as the major oil crops. The oleiferous *Brassica* (rapeseed and mustard) which is commonly termed as "mustard" plants and play an important role in vegetable oil production of the world. It is the third most important edible oil source in the world after soybean and palm (FAO 2003; Piazza and Foslia, 2001; Walker and Booth, 2001). This crop supplies about 13.2% of the world's edible oil (Downey and Robbelen, 1989). It is now realized that regeneration of plants from tissue culture is an important and essential component of plant breeding and biotechnological research. The totipotency of the cell or tissue open up several new contingencies in plant breeding progress that provided gene manipulation and selection of desirable character. High frequency regeneration of plants from *in vitro* culture tissue is a pre-requisite for successful application of tissue culture technique for crop improvement. During last decades, considerable efforts have been made to develop *in vitro* technique for regeneration of *Brassica* spp. During these attempts a wide variety of explants have been used such as leaves (Radke *et al*., 1988); roots (Xu *et al*., 1982); anther (Robin, 2005b; Zhang Enhui *et al*., 2006); filament (Bhuyan M. A. A., 2006); cotyledon (Ono *et al*., 1994); hypocotyls (Suri *et al*., 2005) and protoplasts (Kik and Zaal, 1993).

Therefore, to harvest the multifarious merits of filament culture the present research work had been planned and carried out. Main objectives were- establishment of a suitable and reproducible protocol for *in vitro* regeneration of *Brassica* plantlets through filament culture, optimization of the suitable combination and concentration of hormones on selected media for regeneration of *Brassica* genotypes, and finally observation of the genotypic variation for callus induction and plantlet regeneration.

Materials and Methods

Experimental materials: Filaments of five *Brassica* species (BARI sarisha-7, Tori-7, Agrani, Daulat and Safal) were used as experimental material for this experiment.

Media: MS medium supplemented with 1.0, 2.0 and 4.0 mgL^{-1} 2,4-D along with the constant addition of 1.0 mgL^{-1} BAP were used for callus induction. Likewise, MS medium supplemented with 2.0, 3.0 and 4.0 mgL^{-1} BAP along with the constant addition of 1.0 mgL^{-1} NAA were used for shoot initiation while half strength MS (Murashige and Skoog, 1962) medium with supplementation of 1.0, 2.0 & 3.0 mgL^{-1} IBA with 0.5mg/L NAA in each treatment were used for root initiation.

Culture method: Before culture surface sterilization of flower buds was carried out in the Laminar Air Flow Cabinet. They were rinsed in 0.1% $HgCl_2$ for one minutes, and then thoroughly washed with sterilized distilled water for three times. Then the following culture methods were employed in the present study:

i) Explant culture

Filament culture: Filaments were aseptically removed from flower buds using fine tweezers and incubated into 8 cm petridishes each containing 10 ml MS medium with different hormone concentrations. Four filaments were incubated in each culture vessel. The culture vessel containing explants were placed under fluorescent light in a room with controlled temperature (25 ± 1^0C) and light (16 hrs). The culture vessels were checked daily to record the response and development of contamination.

ii) Sub-culture or transfer of the callus

a. For shoot regeneration: Two weeks after inoculation, the regenerated calli attained convenient size. The calli were cut into few pieces and again placed them into the small vials with shoot regeneration media. Sub-culture was done in the MS media containing different concentration of BAP and NAA.

b. For root initiation: The sub-cultured calli contained to proliferate and differentiated into shoots. When these shoots grew 2-3 cm in length, they were

rescued aseptically from the cultured vials and were separated from each other and again cultured on vials with freshly prepared root induction medium to induce root. The vials containing plantlet incubated under continuous light.

Results and Discussion

Callus Induction

Callusing performance from the genotype BARI sarisha-7 showed excellent result in all the combinations, of which MS + 4 mgL^{-1} 2,4-D + 1.0 mgL^{-1} BAP (Plate-b) showed 93.75% callusing and MS+2mgL^{-1}2,4-D + 1.0mg L^{-1} BAP showed 81.25% callusing. Callusing performance was lowest (68.75%) in MS+1mgL^{-1}2,4-D + 1.0mg L^{-1} BAP. The genotype Tri-7 also showed the best performance (87.50%) with MS + 4mgL^{-1}2,4-D + 1.0mg L^{-1} BAP (Plate-a). Callus induction was the lowest in Safal (37.50%) (Table1). It was observed that callus induction ability of all the genotypes showed better in all the combinations of phytohormones (Table 2). It was also observed that the genotype Safal and the combination MS+1mgL^{-1}2,4-D+1.0mgL^{-1}BAP showed the lowest performance. This finding showed similarity with those of Javed and Hassan (1992), Anuradha and Chopra (1989) and Ratan *et al.* (2001). They reported that BAP and kientin promote callus induction from hypocotyl.

Table 1. Response of cultivars on callus induction

Cultivars	Days to callus induction	Percent callus induction	Weight of callus (g)	Color of callus	Nature of callus	Abundance of callus
BARI sarisha-7	6.47c	81.25a	0.142a	2.833a	Compact	2.958a
Tori-7	6.95bc	58.33ab	0.115b	2.771ab	Compact	2.917ab
Agrani	7.15b	41.67b	0.103ab	2.708b	Compact	2.875b
Daulat	7.95ab	31.25bc	0.078bc	2.521bc	Friable	2.792bc
Safal	8.67a	22.92c	0.052c	2.292c	Loose	2.729c

Note: Mean values having common letter in the column are statistically identical and those having different letters are statistically different

Table 2. Effects of different concentration and combinations of phytohormone for callus induction

Phytohormone combinations	Genotypes	No. of explants showing callus induction	% callus induction	Days req. for callus induction
MS+1mgL^{-1} 2,4-D + 1.0 mgL^{-1} BAP	BARI Sarisha-7	11	68.75c	6.688de
	Tori-7	06	37.50e	7.015cd
	Agrani	04	25.00fg	7.375d
	Daulat	02	12.50g	7.850c
	Safal	02	12.50g	7.850c
MS+2mgL^{-1} 2,4 -D + 1.0 mgL^{-1} BAP	BARI sarisha-7	13	81.25ab	6.480de
	Tori-7	08	50.00cd	7.202cd
	Agrani	05	31.25de	7.651cd
	Daulat	04	25.00ef	8.850b
	Safal	03	18.75f	9.000a
MS+ 4mgL^{-1} 2,4-D + 1.0 mgL^{-1} BAP	BARI sarisha-7	15	93.75a	6.250e
	Tori-7	14	87.00b	7.168cd
	Agrani	11	68.75c	8.115ab
	Daulat	09	56.25d	8.125ab
	Safal	06	37.50e	8.405ab

Organogenesis via calli

Different concentrations of BAP with constant concentration of NAA on MS media were used to observe the regeneration capacity of the calli. Among the genotypes the regeneration performances were found better in BARI sarisha-7 (79.17%) (Plate-c) followed by Tori-7 (72.92%) and Agrani (64.58%) (Table-3). The MS medium supplemented with 4.0 mgL^{-1} BAP+ 1.0 mgL^{-1} NAA showed the highest percentage (72.25%) of shoot regeneration (Table -4).

Root initiation

Half strength MS medium with supplementation of 1.0, 2.0 & 3.0 mgL^{-1} IBA with 0.5mg/L NAA in each treatment were used to observe rooting response of regenerated shoots of *Brassica* species (Plate d). The highest percentage of root induction (87.50%) was observed in BARI sarisha-7 and lowest (50.00%) in Safal respectively (Table 5). Among the three treatments half MS medium supplemented with 1.0mgL^{-1} IBA + 0.5mgL^{-1} NAA was found to be the best (85.50%) for root initiation.

Table 3. Response of cultivars on shoot regeneration

Cultivars	Number of Shoot initiation	Days to shoot initiation	Percent shoot initiation
BARI Sarisha-7	3.167a	21.23c	79.17a
Tori-7	2.917ab	22.47bc	72.92ab
Agrani	2.583ab	26.47b	64.58ab
Daulat	2.333b	27.35ab	58.33b
Safal	1.417c	28.93a	35.42c

Table 4. Effects of media with hormone supplements on shoot regeneration

Media with different hormone supplements	Days to shoot initiation	Percent shoot initiation
MS+ 2.0 mg/L BAP +1.0mg/L IAA	25.95a	50.00b
MS+ 3.0 mg/L BAP +1.0mg/L IAA	25.10ab	60.00ab
MS+ 4.0 mg/L BAP +1.0mg/L IAA	24.55b	72.25a

Establishment of plantlets

After sufficient development of root system the small plantlets were taken out from the culture vessels without damaging roots. Excess agar around the roots was washed off by running tap water to prevent microbial infection and transplanted in small plastic pots having soil: sand: cowdung (1:2:1). The pots were then transferred into the growth chamber for proper hardening of the plantlets and then these were planted in earthen pots (Plate e & f) having soil: sand: cowdung (1:2:1).

Table 5. Effects of different combinations of phytohoromone in half strength MS medium on root initiation

Phytohormone combinations	Genotypes	% root initiation
½ MS+ 0.5mgL^{-1} NAA + 1.0mgL $^{-1}$ IBA	BARI sarisha-7	87.50a
	Tori-7	87.50a
	Agrani	75.00ab
	Daulat	75.00ab
	Safal	75.00ab
½ MS+ 0.5mgL^{-1} NAA + 2.0mgL $^{-1}$ IBA	BARI sarisha-7	87.50a
	Tori-7	68.75bc
	Agrani	62.50c
	Daulat	56.25cd
	Safal	50.00d
½ MS+ 0.5mgL^{-1} NAA + 3.0mgL $^{-1}$ IBA	BARI sarisha-7	75.00ab
	Tori-7	62.50c
	Agrani	56.25cd
	Daulat	50.00d
	Safal	50.00d

The survival rate of plantlet from filament in pot as well as in soil was the highest in the genotype BARI Sarisha-7 (80.00% and 66.67%, respectively) followed by Tori-7 (71.43% and 54.54%, respectively). The survival rate of plantlet from filament in pot was the lowest in the genotypes Daulat and Safal (33.33%) (Table 6).

In this experiment, *in vitro* regeneration potentiality of five *Brassica* genotypes has been observed and an efficient as well as reproducible protocol for regeneration of the genotypes has been developed using filament as explants. Since genetic engineering of crop plants relies on the development of efficient methods for the regeneration of viable shoots (using filament) from cultured tissues, this protocol can be followed for genetic manipulation for improvement of *Brassica* species.

Table 6. Survival rate of regenerants from filament of five genotypes of *Brassica* spp. after transfer in soil

	Genotypes	Regenerants	
		No. of plants survived	Survival rate (%)
In plastic pots (with soil)	BARI sarisha-7	12	80.00a
	Tori-7	10	71.43ab
	Agrani	8	61.54b
	Daulat	6	50.00bc
	Safal	3	42.86c
In earthen pots (with soil)	BARI sarisha-7	8	66.67a
	Tori-7	6	60.00ab
	Agrani	4	50.00b
	Daulat	2	33.33c
	Safal	1	33.33c

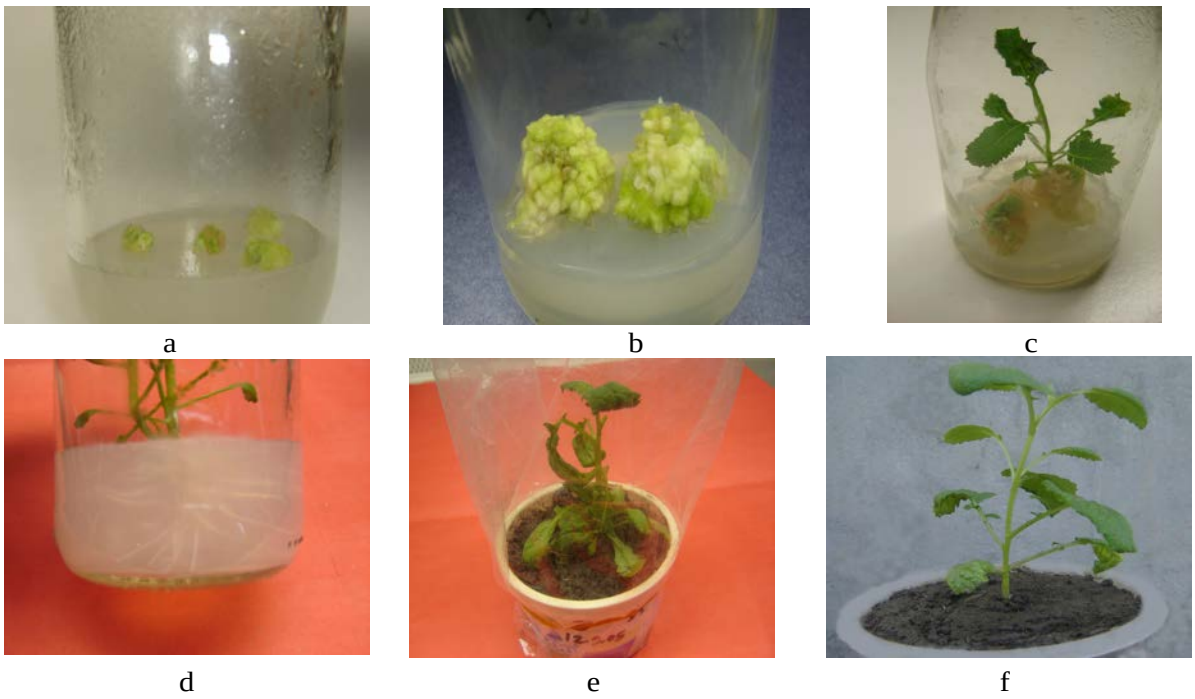

Plate a) Callus initiation from filament of the genotype Tori -7 in MS +4mgL^{-1} 2,4-D +1.0mgL^{-1} BAP after 15 days of inoculation, **b)** Proliferated callus from filament of the genotype BARI Sarisha-7 in MS + 4 mgL^{-1}2,4-D + 1.0 mgL^{-1} BAP after 25 days of inoculation **c)** Shoot regeneration from filament derived calli of the genotype BARI Sarisha-7 in MS + 4 mgL^{-1}BAP + 1.0 mgL^{-1}NAA **d)** Initiation of roots from regenerated shoots of the genotype Tori-7 in half MS + 1.0 mgL^{-1} IBA + 0.5mgL^{-1}NAA **e)** Regenerated plant of BARI Sarisha-7 from filament in plastic pot covered with polythene bag kept in net house for hardening **f)** Regenerated plant from filament of the genotype BARI Sarisha-7 in earthen pot.

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