J. Sher-e-Bangla Agric. Univ., 8(1): 1-6, January 2014 ISSN 1997-6038

IN VITRO ORGANOGENESIS AND PLANT REGENERATION IN *BRASSICA* SPECIES

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ABSTRACT

High frequency regeneration of plants from *in vitro* cultured tissues is perquisite to use genetic transformation to enrich oleiferous *Brassica spp.* Emphasis was given in this study on callus induction aptitude and subsequent plant regeneration from cotyledon and stem segment of three of *Brassica spp.* viz BARI sarisa-8 (*Brassica napus*), Daulat (*Brassica juncea*) and Sonali (*Brassica campestris*). Two milligram per litre of Kn, BAP (1.0, 2.0, 3.0 mg/l) and constant concentration of NAA (0.5 mg/l) were used in MS medium. The result showed that stem segment produced maximum percentage of callus and subsequent shoot regeneration in all the four treatments BARI sarisa -8 Showed best performance in callus induction and it take minimum (6-7 days) for callus initiation. Shoot initiation potentiality also highest in the same variety under studied. The variety sonali showed poor performance for all the parameter under studied. Rooting occurred simultaneously from regenerated shoot on half strength MS medium supplemented with 0.5 mg/l IBA. Regenerated plantlets were successfully transferred to pots containing a mixer of soil and vermiculite.

Keywords: callus, regeneration, *Brassica spp.*, growth hormone

INTRODUCTION

Mustard and rapeseed is one of the important edible oil source crops of Bangladesh. The geographical and agro climatic condition are favorable for cultivation of mustard in this country. The yield potentiality of available released mustard varieties are very low in Bangladesh as compared to other mustard producing countries like India and Canada. Poor crop husbandry, low yielding varieties, inadequate manuring, severe disease and pest infestation fail to avail high yield as well as oil content. From the above circumstances, creation of novel germplasm through techniques of tissue culture and gene transfer holds great potential for improving the quality and agronomic traits of mustard and rapeseed. The generation of transgenic plants is an integrated process, which involves many different factors such as plant regeneration, the choice of regenerable explants, culture condition and transformation techniques. Therefore, selection of genotypes with higher regeneration rates will help improve the efficiency of genetic transformation. Moreover, explant types and growth regulators potentiality on the regeneration need to assess in cell culture technology. Hence, the present study was undertaken to investigate the *in vitro* organogenesis and plant regeneration potentiality of three *Brassica spp.*

Materials and Methods

The experiment was conducted in the tissue culture laboratory of the Biotechnology Division, Bangladesh Agricultural Research Institute (BARI), Joydevpur, Gazipur-1701, Bangladesh.

Preparation of explants

Seeds of three different genotypes of *Brassica spp.* (Table.1) were obtained from Oil Seed Division of Bangladesh Agricultural Research Institute (BARI). Mature seeds of described materials were

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surface sterilized with 0.1 % $HgCl_2$ for 5 minutes, washed with sterile distilled water 3 times and placed for germination in half strength MS (Murashige and Skoog, 1962) medium in incubation room. Cotyledon (1-2 mm) and stem segment (2-3 mm) from seven days old seedlings were used as source of explants.

Preparation of media for callus induction, shoot regeneration and root initiation

MS medium supplemented with auxin (0.5 mg/l NAA), cytokinin (2.0 mg/l, Kn) and BAP (1.0 mg/l, 2.0 mg/l, and 3.0 mg/l) were used for callus induction and shoot differentiation. Thus four treatments were used for callus induction and shoot differentiation. They are as follows

T1: MS medium supplemented with 2.0 mg/l Kn and 0.5 mg/l NAA
T2: MS medium supplemented with 1.0 mg/l BAP and 0.5 mg/l NAA
T3: MS medium supplemented with 2.0 mg/l BAP and 0.5 mg/l NAA
T4: MS medium supplemented with 3.0 mg/l BAP and 0.5 mg/l NAA

Half strength MS medium supplemented with 0.5 mg/l IBA and 0.1 mg/l IAA separately were used for root induction. Callus induced and direction shoot regenerated materials were used for the above mentioned treatments. Medium was solidified with 0.7% agarose (Difco-brand Bacto Agar) maintaining P^H 5.8. The medium were autoclaved at 121^0 C with 1.15 kg cm^{-2} pressure for 25 minute. The culture was incubated at 25^0 C ± 1^0 C under white light intensity (2500-3000 lux).

Table 1. Identifying characters of three *Brassica* genotypes.

Characters	Name of genotypes		
	Sonali	**Daulat**	**BARI sarisa-8**
Species name	*Brassica campestris*	*Brassica juncea*	*Brassica napus*
Variety developed by	BARI	BARI	BARI
Year of release	1979	1988	1994
Maturity period (days)	90-98	95-100	105-110
Fruit type	2-Chambered	2-Chambered	2-Chambered
Seed color	Yellow	Brown	Brown
Seed yield Kg/ha	1800-2000	1200-1400	1000-1200
Oil content (%)	43-44	35-36	38-40

Source: (Rahman, 2002)

Transfer of plantlet to soil

Medium attached to the roots was gently washed from well developed plantlet and the plantlet in length of 5-8 cm were transferred to plastic pot containing sterile soil:sand:cowdung (1:2:1) mixture. Plantlets were then covered with a moist polythine bag to maintain 90±0.05 RH for 7-15 days and then this cover gradually perforated for adaptation of plantlets to dry condition and finally after 15-20 days plantlets were transferred to soil.

Data collection and statistical analysis

The effect of different treatments and response of different genotypes of *Brassica* spp. for callus induction and plant regeneration data were collected during the experiment period. Date were recorded on the days to callus initiation, percentage of callus induction, size of callus (in cm), color of callus (grading as 1 for yellow, 2 for creamy and 3 for green), nature of callus (grading as 1 for loose, 2 for friable and 3 for textured), abundance of callus (grading as 1 for poor, 2 for moderate and 3 for plenty),

weight of callus (g), days to shoot initiation, percentage of shoot regeneration and percentage of regenerated plantlets were recorded. The data for the parameters under present study were statistically analyzed wherever applicable. The experiments were conducted in tissue culture laboratory and arranged in completely randomized design (CRD). The analysis of variance for different parameters were performed and the mean values were compared by Duncan's Multiple Range Test (DMRT).

RESULT AND DISCUSSION

Callus induction

Callus induction potentiality and days to callus induction from stem segment and cotyledon were presented in Table 2. It was observed that stem segment showed higher potentiality than cotyledon as explants in most of the varieties under study. The variety Sonali showed lowest percent (72%) of callus induction rate in all the treatment. Cent percent (100%) callus induction was noticed in most of the treatment when stem segment used as explants. Duration of callus induction is very closed in most of the treatment and in all the varieties. However, minimum 5-6 days were required for callus induction in the treatment MS + 2 mg/l Kn+ 0.5 mg/l NAA on the variety BARI Sarisa-8 and it was maximum (11-12 days) on MS media supplemented with 3.0 mg/l BAP and 0.5 mg/l NAA. The variety BARI Sarisa-8 showed best performance in respect of callus induction and time duration in all the treatment under investigation. Callus induction in *Brassica* spp. was reported by Du *et al.* (2000) in the treatment MS media supplemented with 2.0 mg/l Kn and 0.5 mg/l NAA.

Table 2. Effect of different combination of phytohormone on callus induction in three *Brassica spp.*

Treatments	Brassica varities	Explants			
		Cotyledon		Stem segments	
		% callus induction	Days required for callus induction	% callus induction	Days required for callus induction
MS + 2.0 mg/l Kn + 0.5 mg/l NAA	BARI sarisa-8	100	5-6	100	6-7
	Daulat	92	8-10	100	8-9
	Sonali	92	8-11	100	8-11
MS + 1.0 mg/l BAP + 0.5 mg/l NAA	BARI sarisa-8	80	6-7	96	6-8
	Daulat	76	8-9	92	7-8
	Sonali	72	8-9	80	8-10
MS + 2.0 mg/l BAP + 0.5 mg/l NAA	BARI sarisa-8	96	6-7	100	7-8
	Daulat	100	8-11	100	7-10
	Sonali	96	8-12	96	8-13
MS + 3.0 mg/l BAP + 0.5 mg/l NAA	BARI sarisa-8	100	9-10	100	6-7
	Daulat	100	10-12	100	8-10
	Sonali	96	11-12	100	7-11

Organogenesis

The capacity of cotyledon and stem segments of three *Brassica spp.* to regeneration aptitude was examined. Having used stem segments as the explants, cultivar BARI sarisa-8 (*Brassica napus*) had a higher overall efficiency of regeneration than others. Such genotypic variabilities indicate the genetic control of callus as well as shoot regeneration ability. These findings were in agreement of those obtained by Ben Ghnaya *et al.*, 2008.

Callus morphology and shoot initiation potentiality

The morphological characteristics and shoot initiation capacity of different treatment were presented in Table 3. It showed that explants and hormonal balance had a critical effect on callus morphology and shoot initiation potentiality. MS medium with 3.0 mg/l BAP + 0.5 mg/l NAA was good and effective medium for callus induction as well as shoot formation. In this treatment large size and greenish color callus along with compact to friable in nature obtained in BARI sariasa-8 (*Brassica napus*). Burbulis *et al,* 2008 reported that, the use of BAP in combination with NAA resulted in significant increase (P□0.05) in shoot formation frequency for *Brassica napus.* Abundance of callus was found highest on MS + 2.0 mg/l BAP + 0.5 mg/l NAA in BARI sarisa-8. Weight of callus was found to be highest on treatment MS + 3.0 mg/l BAP + 0.5 mg/l NAA. Finally, it reveal that MS + 3.0 mg/l BAP + 0.5 mg/l NAA Showed best performance in BARI sarisa-8 followed by Sonali. Nasrin (2003) reported similar finding in two genotypes of Brassica when applied on MS+ 2.0mg/L BAP+0.5 mg/L NAA treatment. Shoot induction potentiality was hishest in the variety BARI sarisa-8 in all the treatment and it was lowest in the variety sonali (Table-3).

Table 3. Performance of phytohormone on callus morphology and shoot initiation ability in *Brassica spp.*

Phytohormone combinations	Brassica varieties	Callus morphology					
		Size of callus (cm)	Color of callus	Nature of Callus	Abundance of callus	Weight of callus (g)	Shoot initiation ability (%)
MS + 2.0 mg/l Kn + 0.5 mg/l NAA	BARI sarisa-8	0.49 c d	2.71 b c	2.98 a	2.91 a	2.91 a	40 b
	Daulat	0.39 f	2.50 de	2.74 b	2.54 b c	2.54 b c	29 d
	Sonali	0.42 e f	2.85 a b	2.95 a	2.38 c d	2.38 c d	15 d
MS + 1.0 mg/l BAP + 0.5 mg/l NAA	BARI sarisa-8	0.39 f	2.99 a	2.99 a	2.02 e	2.02 e	48 c
	Daulat	0.20 g	2.45 d e	1.92 e	2.15 d e	2.15 d e	27 d
	Sonali	0.41 e f	2.38 e f	2.93 a	1.99 e	1.99 e	12 d
MS + 2.0 mg/l BAP + 0.5 mg/l NAA	BARI sarisa-8	0.59 b	3.00 a	3.00 a	**2.92 a**	2.92 a	48 a
	Daulat	0.58 b	2.20 g	2.56 c	2.29 c d	2.29 c d	36 b
	Sonali	0.45 d e	2.59 c d	2.95 a	2.65 b	2.65 b	28 c
MS + 3.0 mg/l BAP + 0.5 mg/l NAA	BARI sarisa-8	**0.70 a**	**3.00 a**	**3.00 a**	2.76 a b	2.76 a b	**52 a**
	Daulat	0.51 c	2.23 f g	2.35 d	2.36 c d	2.36 c d	42 a
	Sonali	0.52 c	3.00 a	2.98 a	2.70 a b	2.70 a b	19 d

Note: Color of callus (grading as 1 for yellow, 2 for creamy and 3 for green), nature of callus (grading as 1 for loose, 2 for friable and 3 for textured), Abundance of callus (grading as 1 for poor, 2 for moderate and 3 for plenty).

Root initiation

The shoot induced callus were transferred to rooting medium for root formation (Table-4). It was observed that highest percentage of root initiation was found in BARI Sarisa-8 with combination of ½ MS + 0.5 mg/l IBA. Poor root formation ability was noticed in the variety sonali (10%).

Table 4. Root initiation potentiality of three *Brassica spp.*

Treatment	Brassica varities	Explants	No. of shoots inoculated. explant	No. of shoots showing root initiation	Root initiation ability (%)
½ MS + 0.5 mg/l IBA	BARI sarisa-8	Stem segment	10	6	60
	Daulat	Stem segment	10	4	30
	Sonali	Stem segment	10	1	10
½ MS + 0.1 mg/l IAA	BARI sarisa-8	Stem segment	10	5	50
	Daulat	Stem segment	10	2	20
	Sonali	Stem segment	10	1	10

Establishment of plantlet

Regenerated plantlets with developed root system from induced shoot, transferred to soil: sand: cowdung mixer and placed in a green house. The survival ability of regenerated plantlets studied are presented in Table 5. Highest survival rate (75%) of the plantlets found in BARI Sarisa-8 followed by Daulat (58.33%).

Table 5. Survival rate of regenerated plants of three *Brassica* genotypes after transfer in soil.

Treatment	Materials	No. of plantlets transplanted	No. of plants survived	Survival rate (%)
In Pot	BARI sarisa-8	15	12	75
	Daulat	12	7	58.33
	Sonali	10	10	10

CONCLUSIONS

Plant growth regulators play a vital role in controlling the differentiation process required for regeneration. Significant genotypic variation for shoot regeneration was evident for both explants cotyledon and stem segments. Among the cultured genotypes the stem segment of BARI sarisa-8 (*Brassica napus*) performed highest capacity to regenerate shoots from induced callus. Combination BAP and NAA significantly effect on callus induction and shoot formation frequency.

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Fig. 1. Callus initiatiation from stem segment of the genotype BARI Sarisa-8 (*Brassica napus*) in MS + 3.0 mg/l BAP + 0.5 mg/l NAA

Fig. 2. Shoot from stem segment of the genotype BARI Sarisa-8 (*Brassica napus*)

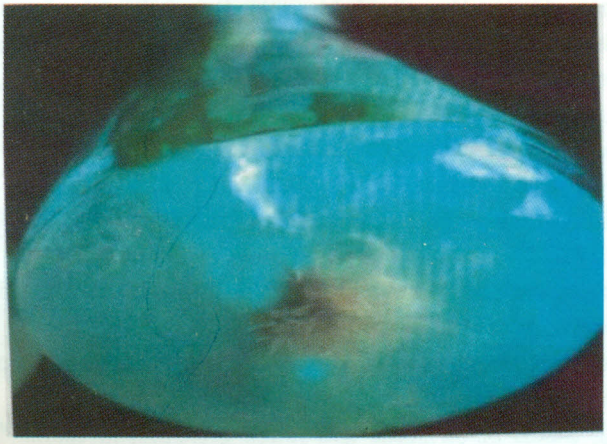

Fig. 3. Initiation of roots from regenerated shoots of the genotype BARI Sarisa-8 (*Brassica napus*) in ½ MS + 0.5 mg/l IBA