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IN VITRO REGENERATION POTENTIALITY OF *BRASSICA* GENOTYPES IN DIFFERENTIAL GROWTH REGULATORS

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Abstract

Petiole of six genotypes of oilseed *Brassica* viz. Tori-7, Sampad, Kallyania, BARI Sarisha-7, BARI Sarisha-8, and MM 20-3 were cultured in MS medium with different concentrations of BAP, NAA, and $AgNO_3$ for callus induction and subsequent plant regeneration. The highest percentage of callus induction (91.43%) was observed in Tori-7 in the media supplemented with 2 mg/L BAP, 0.1 mg/L NAA and 2.0 mg/L $AgNO_3$. Calli were maintained in order to get sufficient number of regenerants. With the increased concentration of BAP, the highest percentage (57.14) of regenerants were found in Tori-7 followed by Sampad (33.13%) and BARI Sarisha-8 (31.42%) in MS media supplemented with 2.5 mg/L BAP, 0.1 mg/L NAA and 2.0 mg/L $AgNO_3$. Root formation from the regenerants was found best in half MS medium supplemented with 0.5 mg/L NAA in genotype Tori-7. Regenerated plantlets of four genotypes (Tori-7, BARI Sarisha-8, Kallyania, BARI Sarisha-7) were successfully established in the field.

Keywords : $AgNO_3$, BAP, *Brassica*, NAA, regeneration.

Introduction

Rapeseed, *Brassica rapa* and *Brassica napus* are important oil-yielding crops in Bangladesh. Approximately 70% of the total cultivated mustard in Bangladesh is covered by oilseed *Brassica.* The average yield of local varieties and high yielding varieties are 600-1000 kg/ha and 1400-2000 kg/ha, respectively, which contributes 71.3% of the total oilseed production of Bangladesh (BBS, 2005). Current oilseed production of Bangladesh is about 0.254 million tons, which is 40% of the country's need (FAO, 2001). Conventional breeding approaches can be done to improve the new trait within the species. But conventional breeding programmes alone were not successful enough in *Brassica* due to high degree of segregation upon cross-pollination and unavailability of suitable wild germplasm. Enrichment of genetic variability through mutation, somaclonal variation, and protoplast fusion contributed only a little in the production of disease and pest resistant plants to overcome incompatibility barriers as well as plants with better

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agronomic characters in *Brassica* spp. In this regard, *in vitro* regeneration and transformation have prospects to fulfill breeding needs.

Many efforts have been undertaken to establish a suitable *in vitro* regeneration protocol for *Brassica.* It has consistently proved to be one of the most recalcitrant members of the *Brassicaceae* in tissue culture (Hachey *et al.,* 1991). Due to the recalcitrant nature of *Brassica* tissue *in vitro,* it eluded any notable progress in this regard for a long time. Fortunately, constant efforts with more diverse cultural procedures have overcome many breeding obstacles (Bhojwani *et al.,* 1988). 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. It may be mentioned here that several attempts have been taken to establish *in vitro* regeneration protocol for *Brassica.* A high frequency of shoot regeneration was obtained from some oilseed cultivars (Hachey *et al.,* 1991) and a few leafy vegetable cultivars (Takasaki *et al.,* 1996) of *Brassica* spp. During these attempts, a wide variety of explants were used with the application of several growth regulators to regenerate plantlets with or without intervention of callus. But wide variations were observed among the species in their regeneration potentiality. Narasimhulu and Chopra (1987, 1988) reported that *Brassica rapa* has the lowest frequency of regeneration from cotyledons among the three basic diploid species, *Brassica rapa, Brassica oleracea,* and *Brassica nigra* and their amphidiploids, *Brassica juncea, Brassica napus,* and *Brassica carinata.*

Therefore, present study was undertaken to establish a suitable and reproducible protocol for *in vitro* regeneration of *Brassica rapa* and *Brassica napus* varieties.

Materials and Method

The experiment was conducted at the tissue culture laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh during the period from September 2004 to July 2005. Six oilseed *Brassica* genotypes were used for this experiment. The genotypes were Tori-7, Sampad, Kallyania of *Brassica rapa* and BARI Sarisha-7, BARI Sarisha-8, and MM 20-3 of *Brassica napus.* Seeds of Sampad were collected from Bangladesh Agricultural University (BAU) and those of other five genotypes were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur. Half strength MS (Murashige and Skoog, 1962) medium supplemented with 20 mg/L sucrose was used for seed germination. For callus induction and shoot regeneration, five different combinations each containing MS + 0.l mg/L NAA+ 2 mg/L $AgNO_3$

with different concentrations of BAP viz., 0 ppm, 1 ppm,1.5 ppm, 2 ppm, and 2.5 ppm were used. Again, for root initiation, three treatments each containing half-strength MS medium supplemented with three different concentrations of IBA (0 ppm, 0.1 ppm, and 0.5 ppm) were used. The regenerated plantlets were transplanted from culture vessel to plastic pots containing 25% garden soil + 50% Sand + 25% Cowdung in growth chamber. For callus induction, data were recorded on days to callus initiation, percent callus induction and nature of callus (loose, friable, and compact texture of callus were scored one, two, and three, respectively); for plantlet regeneration data were recorded on days to shoot initiation, number of callus showing shoots, total number of shoots per petridish and for root initiation, data were recorded on percent shoot showing root. The collected data were analyzed with proper statistical methods.

Results and Discussion

Investigations of *in vitro* regeneration potentiality of these six genotypes were accomplished with callus induction, maintenance of calli, organogenesis, and finally plantlet regeneration and their establishment in field conditions.

Callus induction: Callus induction performances of the genotypes under each treatment were evaluated and the results are presented in Table 1 and 2. To examine the effect of phytohormones, the mean values of five different combinations of phytohormones were found statistically significant for the parameters of days required for callus initiation, number of callus/petridish, nature of callus. T_4 (MS+2mg/L BAP+0. 1 mg/L NAA+2 mg/L $AgNO_3$) was found the best for the number of callus per petridish whereas, in consideration of the nature of callus T_3 (MS+1.5 mg/L BAP+0.1 mg/L NAA+2 mg/L $AgNO_3$) was the best performer (Fig lA & 1B). Maximum number of calli/petridish (4.13) was found in T_4 (MS+2mg/L BAP+0.l mg/L NAA+2 mg/L $AgNO_3$) (Table 2) followed by T_5 (MS+2.5 mg/L BAP+0.1 mg/L NAA+2 mg/L $AgNO_3$) and T_3 (MS+1.5 mg/L BAP + 0.1 mg/L NAA+2 mg/L $AgNO_3$). Days required for callus initiation was minimum (6.l9 days) in T_4 (MS+2 mg/L ^{BAP} +0.1 mg/L NAA+2 mg/L $AgNO_3$). Abundance of compact to friable natured calli was found in T_3 (MS+1.5 mg/L BAP+0.1 mg/L NAA+2 mg/L $AgNO_3$), T_4 (MS+2mg/L BAP+0.1. mg/L NAA+2 mg/L $AgNO_3$), while the rest showed moderate in abundance (Table 2).

Table 1. Effects of different combinations of phytohormone for callus induction from petiole segment of six *Brassica* genotypes.

Supplements	Genotypes	Number of explants incubated	Number of explants producing callus	Callus induction (%)	Days required for callus initiation
MS+0 mg/L	Kallyania	35	6	17.14	8.08
BAP+0.1	Sampad	*35*	7	20.00	8.14
mg/L	Tori-7	35	14	40.00	8.06
NAA+2 mg/L	BARI Sarisha-8	35	11	31.43	7.06
$AgNO_3$ (T_1)	BARI Sarisha-7	35	9	25.7!	7.36
	MM 20-3	35	6	17.14	7.82
MS+1 mg/L	Kallyania	35	9	25.71	7.04
BAP+0.l	Sampad	35	13	37.14	7.22
mg/L	Tori-7	*35*	23	65.71	6.80
NAA+2 mg/L	BARI Sarisha-8	35	2l	60.00	6.76
$AgNO_3$ (T_2)	BARI Sarisha-7	35	16	45.71	6.38
	MM 20-3	35	12	34.29	7.08
MS+l.5 mg/L	Kallyania	35	12	34.29	7.26
BAP+0.1	Sampad	35	14	40.00	7.10
mg/L	Tori-7	35	27	77.14	6.42
NAA+2 mg/L	BARI Sarisha-8	35	25	71.43	6.30
$AgNO_3$ (T_3)	BARI Sarisha-7	35	17	48.57	6.04
	MM 20-3	35	12	34.29	6.84
MS+2 mg/L	Kallyania	35	16	45.7!	6.02
BAP+0.1	Sampad	35	18	51.43	6.12
mg/L	Tori-7	*35*	32	91.43	6.30
NAA+2 mg/L	BARI Sarisha-8	35	27	77.14	6.46
$AgNO_3$ (T_4)	BARI Sarisha-7	*35*	18	51.43	6.06
	MM 20-3	*35*	13	37.14	6.2
MS+2.5 mg/L	Kallyania	*35*	14	40.00	6.3
BAP+0.1	Sampad	*35*	15	42.86	6.26
mg/L	Tori-7	35	31	88.57	6.74
NAA+2 mg/L	BARI Sarisha-8	35	27	77.14	6.48
$AgNO_3$ (T_5)	BARI Sarisha-7	35	17	48.57	6.50
	MM 20-3	35	15	42.86	6.3

To find out the effect of genotypes, the mean values of genotypes for the parameters days to callus initiation, number of calli per petridish, nature of callus were found statistically significant. MM 20-3 started callus initiation early (6.46 days) compared to other genotypes, such as BARI Sarisha-7 (6.61 days), BARI Sarisha-8 (6.84 days), Tori-7 (6.86 days) where both Sampad and Kallyania were delayed (7 days) in callus initiation. Tori-7 showed highest (5.08) number of calli/petridish followed by BARI Sarisha-8 (4.44), BARI Sarisha-7, Sampad (2.68), and MM 20-3 (2.32). Number of calli/petridish was lowest (2.28) in Kallyania. Tori-7 showed compact natured callus (2.77) followed by Sampad (2.48) and Kallyania (2.38). Friable callus was found in MM 20-3 (1.869) (Data not shown). Based on above findings, it can be said that Tori-7 was better in callus growth than other genotypes.

Table 2. Performance of different combinations of phytohormone on callus induction of *Brassica* genotypes.

Phytohormone combinations	Characteristics of callus		
	Days to callusing	Number of calli/petridish	Texture of callus
MS+0mg/L BAP+0.1 mg/L 1NAA+2 mg/L $AgNO_3$ (T_1)	7.753 A	1.767D	1.577D
MS+1 mg/L BAP+0.1 mg/L INAA+2 mg/L $AgNO_3$ (T_2)	6.880 B	3.133C	2.387C
MS+1.5 mg/L BAP+0.1 mg/L INAA+2 mg/L $AgNO_3$ (T_3)	6.660 C	3.567B	2.962A
MS+2 mg/L BAP+0.1 mg/L INAA+2 mg/L $AgNO_3$ (T_4)	6.193 E	4.133A	2.533BC
MS+2.5 mg/L BAP+0.1 mg/L INAA+2 mg/L $AgNO_3$ (T_5)	6.430 D	3.967A	2.646AB

All parameters were found highly significant for hormone × genotype interactions. Early callusing was found in Tori-7 with T_4 (MS+2mg/L BAP+0.1 mg/L NAA+2 mg/L $AgNO_3$). (Data not shown). Compact callus was found on the interaction of T_3 (MS+1.5 mg/L BAP+0.l mg/L NAA+2 mg/L $AgNO_3$) with Tori-7 followed by Sampad, Kallyania, BARI sarisha-8, and BARI Sarisha-7. MS medium without BAP showed friable callus with MM 20-3. In all combinations, MM 20-3 produced mostly friable calli. It can be concluded from the present finding that T_4 (MS+2mg/L BAP+0.1 mg/L NAA+2 mg/L $AgNO_3$) was the best performer with the interaction of Tori-7, BARI Sarisha-7, BARI Sarisha-8, and MM-20-3. T_5 (MS+2.5 mg/L BAP+0. 1 mg/L NAA+2 mg/L $AgNO_3$) also promoted satisfactory result in this experiment.

Organogenesis via callus: Percent of shoot regeneration increased with the increasing concentration of BAP. Shoot regeneration was found highest in Tori-7 (43.99%) followed by Sampad (33.13%) and BARI Sarisha-8 (31.42). Among the phytohormone combinations, T_5 showed the highest shoot regeneration (43.80%) followed by T_4 (41.42%), T_3 (37.61 %), and T_2 (27.61%) (Table 3).

Different concentrations of BAP showed significant variations for number of callus with shoot/petridish and number of shoots/callus (Table 4). Number of calli with shoots/petridish was highest (3.03) in T_5 (Fig.1 C) followed by T_4 (2.90) and T_3 (2.63). Number of shoots/callus was highest (3.01) in T_5 and lowest (1.26) in T_1. So, it is clear that BAP at 2.5 mg/L concentrations along with 0.1 mg/L NAA and 2 mg/L $AgNO_3$ was the best for number of callus with shoots/petridish and number of shoots/callus and total number of shoots/petridish. Similar result was reported by Ohara *et al.* (2000).

Mean values of six genotypes were found statistically significant for all the characters of shoot regeneration like number of calli with shoots/petridish and number of shoots/callus. Number of calli with shoot was highest in Tori-7 and lowest in MM 20-3. It was observed that among the genotypes tested, Tori-7 showed maximum number of shoots/callus followed by Sampad, BARI Sarisha-8, and Kallyania which is shown in Fig. 1D.

Results related to hormone × genotype interaction for the characters of shoot regeneration, such as number of callus with shoot per petridish and number of shoots per callus are presented in the Table 4. These parameters were found statistically significant, indicating significant differences among the interactions for those characters. Number of callus with shoots per petridish was highest on T_5×Tori-7 (4.0) and T_4 × Tori-7 (3.8) whereas, lowest was found in T_1 with BARI Sarisha-7(0.20). Number of shoots/callus was highest (3.5) on T_5 × Tori-7 and lowest (0.40) in T_1 × BARI Sarisha-7. From the above discussion, it may be concluded that T_4 performed best with the interaction of Tori-7 for shoot regeneration.

Table 3. Effect of different concentrations of phytohormone on shoot regeneration percent in *Brassica* genotypes.

Supplements	Genotypes	Number of explants incubated	Number of explants producing short	Days required for callus initiation
MS+0 mg/L BAP+0.11 mg/L NAA+2 mg/L $AgNO_3$ (T_1)	Kallyania	35	2	5.71
	Sampad	35	3	8.57
	Tori-7	35	5	14.29
	BARI Sarisha-8	35	4	11.43
	BARI Sarisha-7	35	1	2.86
	MM2O-3	35	4	11.43
MS+1 mg/L BAP+0.1 mg/L NAA+2 mg/L $AgNO_3$ (T_2)	Kallyania	35	6	17.14
	Sampad	35	9	25.71
	Tori-7	35	16	45.71
	BARI Sarisha-8	35	9	25.71
	BARI Sarisha-7	35	11	31.43
	MM 20-3	35	7	20.00
MS+1.5 mg/L BAP+0.1 mg/L NAA+2 mg/L $AgNO_3$ (T_3)	Kallyania	35	13	37.14
	Sampad	35	14	40.00
	Tori-7	35	17	48.57
	BARI Sarisha-8	35	13	37.14
	BARI Sarisha-7	35	13	37.14
	MM 20-3	35	9	25.71
MS+2 mg/L BAP+0.1 mg/L NAA+2 rngL^{-1} $AgNO_3$ (T_4)	Kallyania	35	14	40.00
	Sampad	35	16	45.71
	Tori-7	35	19	54.29
	BARI Sarisha-8	35	14	40.00
	BARI Sarisha-7	35	13	37.14
	MM2O-3	35	11	31.43
MS+2.5 mg/L BAP+0.1 mg/L NAA+2 mg/L $AgNO_3$ (T_5)	Kallyania	35	15	42.86
	Sampad	35	16	45.71
	Tori-7	35	20	57.14
	BARI Sarisha-8	35	14	40.00
	BARI Sarisha-7	35	14	40.00
	MM 20-3	35	12	34.29

Table 4. Effects of Hormone x Genotype interactions on shoot regeneration parameters of *Brassica* genotypes.

Supplements	Genotypes	Number of calli with shoots/petridish	Total number of shoots/callus
MS+0 mg/L BAP+0.1 mg/L NAA+2 mg/L $AgNO_3$ (T_1)	Kallyania	0.4OKL	0.80JK
	Sampad	0.60J-L	1.201J
	Tori-7	1 .001-K	2 00GH
	BARI Sarisha-8	0.80l-L	I .60H1
	BARI Sarisha-7	0.20L	0.40K
	MM 20-3	0.801-L	.60Hl
MS+1 mg/L BAP+0.1 mg/L NAA+2 mg/L $AgNO_3$ (T_2)	Kallyania	1.2H-J	210F-H
	Sampad	1.8GH	2.40D-G
	Tori-7	3.2B-D	3.10A-C
	BARI Sarisha-8	1.80GH	2.40D-G
	BARI Sarislia-7	2.20FG	2.60C-G
	MM 20-3	1.40H1	2.20E-H
MS+1.5 mg/L BAP+0.1 mg/L NAA+2 mg/L $AgNO_3$ (T_3)	Kallyania	2.6D-F	2.80B-E
	Sampad	2.8C-F	2.90A-D
	Tori-7	3.4A-C	3.20A-C
	BARI Sarisha-8	2.60D-F	2.80B-E
	BARI Sarisha-7	2.60D-F	2.80B-E
	MM 20-3	1.80GH	2.40D-G
MS+2 mg/L BAP+0.1 mg/L NAA+2 mg/L $AgNO_3$ (T_4)	Kallyania	2.8C-F	2.90A-D
	Sampad	3.2B-D	3.10A-C
	Tori-7	3.8A-B	3.40AB
	BARI Sarisha-8	2.80C-F	2.90AB
	BARI Sarisha-7	2.60D-F	2.80B-E
	MM 20-3	2.20FG	2.60C-G
MS+2.5 mg/L BAP+0.1 mg/L NAA+2 mg/L $AgNO_3$ (T_5)	Kallyania	3.00C-E	3.00A-D
	Sampad	3.2B-D	3.10A-C
	Tori-7	4.00A	3.50A
	BARI Sarisha-8	2.80C-F	2.9OAB
	BARI Sarisha-7	2.80C-F	2.90A-D
	MM 20-3	2.40E-G	2.70C-F

Table 5. Effects of different combinations of phytohormone in half strength MS medium on root initiation of *Brassica* genotypes.

Supplements	Variety	No. of incubated	No. of shoots with root	Root formation (%)
½ MS	Kallyania	15	5	33.00
	Sampad	15	0	0.00
	Tori-7	15	6	40.00
	BARI Sarisha-8	15	4	26.67
	BARI Sarisha-7	15	5	33.33
	MM 20-3	15	0	00.00
½ MS + 0.5 mg/L NAA	Kallyania	15	9	60.00
	Sampad	15	0	0.00
	Tori-7	15	11	73.33
	BARI Sarisha-8	15	10	66.67
	BARI Sarisha-7	15	6	40.00
	MM 20-3	15	4	26.67
½ MS+ 1 mg/L NAA	Kallyania	15	5	33.33
	Sampad	15	1	6.67
	Tori-7	15	8	53.33
	BARI Sarisha-8	15	7	46.67
	BARI Sarisha-7	15	5	33.33
	MM 20-3	15	1	6.67

Regeneration of root: Induction of root from regenerated shoots showed wide variations according to genotypes and different concentrations of NAA in the medium. Tori-7 had the highest percentage (55.56) of rooted shoots irrespective different concentrations of NAA (Fig.1E). Mean values due to different concentrations of NAA for number of shoots with root were highly significant, indicating the presence of variation among the concentrations used for this study. Table *5* indicates that irrespective of genotypes, maximum number of shoots with root (44.44%) was found in $^1/_2$ MS + 0.5 mg/L NAA followed by $^1/_2$ MS + I mg/L NAA (30%), and $^1/_2$ MS (22.16%). Different genotypes showed significant variation in producing root. Tori-7 showed highest number of shoots with root followed by BARI Sarisha-8, Kallyania) and BARI Sarisha-7.

Establishment of plantlets: The regenerated plantlets were transplanted into plastic pots containing sterile soil, sand, and cowdung in a 1: 2: 1 ratio for acclimatization (Fig. 1F). Gradually the plantlets were adapted to the soil (Fig. 1G).

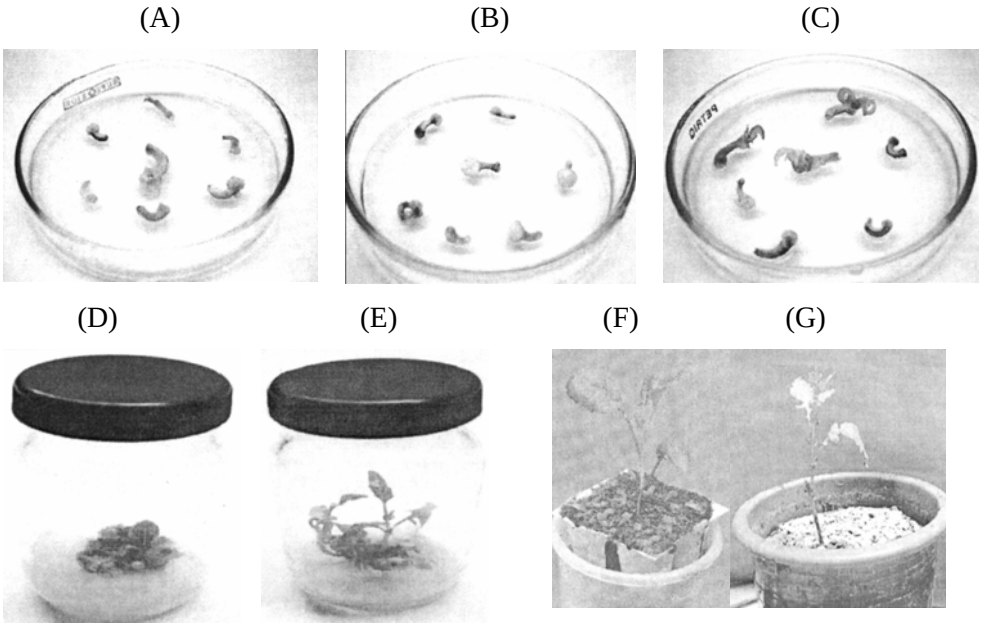

Fig. 1. Different steps of in *vitro* regeneration of *Brassica* genotypes via callus induction.

(A) Callus initiation of Tori-7 on MS+ 2mg/L BAP+ 0.1 mg/L NAA+ 2 mg/L $AgNO_3$. (B) Callus initiation of BARI Sarisa-8 on MS+ 2 mg/L BAP+ 0.1 mg/L NAA+ 2mg/L $AgNO_3$. (C) Shoot initiation of Tori-7 on MS+ 2 mg/L BAP+ 0.1 mg/L NAA+ 2 mg/L $AgNO_3$. (D) Shoot initiation of Tori-7 on MS+ 2 mg/L BAP + 0.1 mg/L NAA + 2 mg/L $AgNO_3$. (E) Initiation of root from regenerated shoot of BARI Sarisa-8 on V2MS+ 0.5 mg/L NAA. (F) Acclimatized plantlet of Tori-7 in pot. (G) Established plantlet of Tori-7.

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