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IN VITRO REGENERATION POTENTIALITY OF OIL SEED *BRASSICA* GENOTYPES WITH DIFFERENTIAL BAP CONCENTRATION

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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 with the media supplemented with 2 mgL^{-1}BAP, 0.1 mgL^{-1} NAA and 2.0 mgL^{-1} $AgNO_3$. 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 medium supplemented with 2.5 mgL^{-1} BAP, 0.1mgL^{-1} NAA and 2.0mgL^{-1} $AgNO_3$. Root formation from the regenerants was found best in half MS medium supplemented with 0.5mgL^{-1} NAA in genotype Tori-7. Regenerated plantlets of 4 genotypes (Tori-7, BARI Sarisha-8, Kallyania, BARI Sarisha-7) were successfully established in the field.

Introduction

Rapeseed, *Brassica rapa* and *Brassica napus* are important oil-yielding crops in Bangladesh. Approximately 70% of the total cultivated mustard in Bangladesh is the variety of either *Brassica rapa* or *Brassica napus*. The average yield of local varieties and high yielding varieties are 600-1000 kg/ha and 1400-2000 kg/ha respectively which contributes to 52% of the total production and 61.2% of the oilseed production of Bangladesh (Anon., 2007). Current oilseed production of Bangladesh is about 0.254 million ton which is 40% of the country need (Anon., 2005).

Conventional breeding approaches can be adopted to improve the new trait within the species. But conventional breeding program alone was 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 agronomic characters in *Brassica.* But *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*. Though, *Brassica spp*., has consistently proved to be one of the most recalcitrant members of the *Brassicaceae* in tissue culture (Hachey *et al*., 1991).

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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). Hachey *et al*., 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. 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 & 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*.

So present study was undertaken to establish a suitable and reproducible protocol for *In vitro* regeneration of *Brassica rapa* and *Brassica napus* varieties where a different explant, petiole was taken to observe the regeneration potentiality.

Materials and Methods

The experiment was conducted at the Tissue Culture Laboratory of the Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh. Six oilseed *Brassica* genotypes viz., Tori-7, Sampad, Kallyania of *Brassica rapa* and BARI Sarisha-7, BARI Sarisha-8 and MM 20-3 of *Brassica napus* were used for this experiment. Seed of Sampad was collected from Bangladesh Agricultural University and those of other five genotypes were collected from Bangladesh Agricultural Research Institute. Half strength MS (Murashige & Skoog, 1962) medium supplemented with 20 mg/L sucrose was used for seed germination. For callus induction and shoot regeneration 5 different combinations each containing MS + 0.1mg/L NAA+ 2mg/L $AgNO_3$ with different concentrations of BAP (T_1-0 ppm, T_2-1 ppm, T_3- 1.5 ppm, T_4- 2 ppm and T_5-2.5 ppm) were used. For root initiation three treatments each containing half-strength MS medium supplemented with three different concentration 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; for plantlet regeneration data were recorded on days to shoot initiation, number of callus showing shoots, total number shoot per Petri dish 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 6 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 Tables 1 and 2. To examine the effect of phytohormones, the mean values of 5 different combinations of phytohormones were found statistically significant for the parameters of days required for callus initiation, number of

callus/Petri dish, nature of callus. T_4 (MS+2 mgL-1BAP+0.1 mgL-1NAA+2 mgL-1$AgNO_3$) was found the best for the number of callus per Petridish whereas; in consideration of the nature of callus T_3 (MS+1.5 mgL-1BAP+0.1 mgL-1NAA+2 mgL-1$AgNO_3$) was the best performer (Fig. 1A & 1B). Maximum number of callus/Petri dish (4.13) was found in T_4 (Table 3) followed by T_5 (MS+2.5 mgL-1BAP+0.1 mgL-1NAA+2 mgL-1$AgNO_3$) and T_3 (MS+1.5 mgL-1BAP+0.1 mgL-1NAA+2 mgL-1$AgNO_3$). Days required for callus initiation was minimum (6.19days) in T_4. Abundance of compact to friable natured calli was found in T_3, T_4, while the rest showed moderate in abundance (Table 3).

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 mgL^{-1} BAP+0.1 mgL^{-1} NAA+2 mgL^{-1} $AgNO_3$ (T_1)	Kallyania	35	6	17.14	8.08
	Sampad	35	7	20.00	8.14
	Tori-7	35	14	40.00	8.06
	BARI Sarisha-8	35	11	31.43	7.06
	BARI Sarisha-7	35	9	25.71	7.36
	MM 20-3	35	6	17.14	7.82
MS+1 mgL^{-1} BAP+0.1 mgL^{-1} NAA+2 mgL^{-1} $AgNO_3$ (T_2)	Kallyania	35	9	25.71	7.04
	Sampad	35	13	37.14	7.22
	Tori-7	35	23	65.71	6.80
	BARI Sarisha-8	35	21	60.00	6.76
	BARI Sarisha-7	35	16	45.71	6.38
	MM 20-3	35	12	34.29	7.08
MS+1.5 mgL^{-1} BAP+0.1 mgL^{-1} NAA+2 mgL^{-1} $AgNO_3$ (T_3)	Kallyania	35	12	34.29	7.26
	Sampad	35	14	40.00	7.10
	Tori-7	35	27	77.14	6.42
	BARI Sarisha-8	35	25	71.43	6.30
	BARI Sarisha-7	35	17	48.57	6.04
	MM 20-3	35	12	34.29	6.84
MS+2 mgL^{-1} BAP+0.1 mgL^{-1} NAA+2 mgL^{-1} $AgNO_3$ (T_4)	Kallyania	35	16	45.71	6.02
	Sampad	35	18	51.43	6.12
	Tori-7	35	32	91.43	6.30
	BARI Sarisha-8	35	27	77.14	6.46
	BARI Sarisha-7	35	18	51.43	6.06
	MM 20-3	35	13	37.14	6.2
MS+2.5 mgL^{-1} BAP+0.1 mgL^{-1} NAA+2 mgL^{-1} $AgNO_3$ (T_5)	Kallyania	35	14	40.00	6.3
	Sampad	35	15	42.86	6.26
	Tori-7	35	31	88.57	6.74
	BARI Sarisha-8	35	27	77.14	6.48
	BARI Sarisha-7	35	17	48.57	6.50
	MM 20-3	35	15	42.86	6.3

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

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

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 AgNO3. (B) Callus initiation of BARI Sarisa-8on MS+ 2 mg/L BAP+ 0.1 mg/L NAA+ 2mg/L AgNO3. (C) Shoot initiation of Tori-7 on MS+ 2 mg/L BAP+ 0.1 mg/L NAA+ 2 mg/L AgNO3. (D) Shoot initiation of Tori-7 on MS+ 2 mg/L BAP + 0.1 mg/L NAA + 2 mg/L AgNO3. (E) Initiation of root from regenerated shoot of BARI Sarisa-8 (left) Tori-7(right) on ½MS+ 0.5 mg/L NAA. (F) Acclimatized plantlet of Tori-7 in pot. (G) Established plantlet of Tori-7. ((Magnification, A, B, C =2X; D, E, F, G =1X).

To find out the effect genotypes, the mean values of genotypes for the parameters days to callus initiation, number of callus per Petri dish, 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 (6.61days), BARI sarisha-8 (6.84 days), Tori-7 (6.86 days) where as both Sampad and Kallyania were delayed (7 days) in callus initiation. Tori-7 showed highest (5.08) number of callus/Petri dish followed by BARI Sarisha-8 (4.44), BARI Sarisha-7, Sampad (2.68) and MM 20-3(2.32). Number of callus per Petri dish 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) (Table 2). Based on above findings, it can be said that Tori-7 was better in callus growth than other genotypes.

All parameters were found highly significant for hormone × genotype interactions. Early callusing was found in the interactions of T_4 with Tori-7. (Data not shown). Compact callus was found on the interaction of T_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 was the best performer with the interaction of Tori-7, BARI Sarisha-7, BARI Sarisha-8 and MM-20-3. T_5 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 (MS+2.5 mgL-1BAP+0.1 mgL-1NAA+2 mgL-1$AgNO_3$) showed the highest shoot regeneration (43.80%) followed by T_4 (41.42%), T_3 (37.61 %) and T_2 (27.61%).

Table 3. Effect of different concentration of phytohormone on shoot regeneration of *Brassica* genotypes.

Supplements	Genotypes	Number of explants incubated	Number of explant showing shoot	Shoot regeneration (%)
MS+0 mgL^{-1}	Kallyania	35	2	5.71
BAP+0.1 mgL^{-1}	Sampad	35	3	8.57
NAA+2 mgL^{-1}	Tori-7	35	5	14.29
$AgNO_3$ (T_1)	BARI Sarisha-8	35	4	11.43
	BARI Sarisha-7	35	1	2.86
	MM 20-3	35	4	11.43
MS+1 mgL^{-1}	Kallyania	35	6	17.14
BAP+0.1 mgL^{-1}	Sampad	35	9	25.71
NAA+2 mgL^{-1}	Tori-7	35	16	45.71
$AgNO_3$ (T_2)	BARI Sarisha-8	35	9	25.71
	BARI Sarisha-7	35	11	31.43
	MM 20-3	35	7	20.00
MS+1.5 mgL^{-1}	Kallyania	35	13	37.14
BAP+0.1 mgL^{-1}	Sampad	35	14	40.00
NAA+2 mgL^{-1}	Tori-7	35	17	48.57
$AgNO_3$ (T_3)	BARI Sarisha-8	35	13	37.14
	BARI Sarisha-7	35	13	37.14
	MM 20-3	35	9	25.71
MS+2 mgL^{-1}	Kallyania	35	14	40.00
BAP+0.1 mgL^{-1}	Sampad	35	16	45.71
NAA+2 mgL^{-1}	Tori-7	35	19	54.29
$AgNO_3$ (T_4)	BARI Sarisha-8	35	14	40.00
	BARI Sarisha-7	35	13	37.14
	MM 20-3	35	11	31.43
MS+2.5 mgL^{-1}	Kallyania	35	15	42.86
BAP+0.1 mgL^{-1}	Sampad	35	16	45.71
NAA+2 mgL^{-1}	Tori-7	35	20	57.14
$AgNO_3$ (T_5)	BARI Sarisha-8	35	14	40.00
	BARI Sarisha-7	35	14	40.00
	MM 20-3	35	12	34.29

Different concentrations of BAP showed significant variations for number of callus with shoot/Petri dish and number of shoot/callus (Table 3). Number of callus with shoot/Petri dish was highest (3.03) in T_5 (Fig. 1C) followed by T_4 (2.90) and T_3 (2.63). Number of shoot/callus was highest (3.01) in T_5 and lowest (1.26) in T_1. So, it is clear that BAP at 2.5 mgL^{-1} concentrations along with 0.1 mgL^{-1} NAA and 2 mgL^{-1} $AgNO_3$ was the best for number of callus with shoot/Petri dish and number of shoot/callus and total number of shoots/Petri dish. 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 callus with shoot/Petri dish and number of shoot/callus.Number of callus 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. (Data not shown).

Results related to hormone × genotype interaction for the characters of shoot regeneration such as number of callus with shoot per Petri dish and number of shoot 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 shoot per Petri dish 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 with the interaction of Tori-7 was the best performer for shoot regeneration.

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

Supplements	Genotypes	Number of callus with shoots/Petri dish	Total number of shoots/callus
MS+0 mgL^{-1}	Kallyania	0.40KL	0.80JK
BAP+0.1 mgL^{-1}	Sampad	0.60J-L	1.20IJ
NAA+2 mgL^{-1}	Tori-7	1.00I-K	2.00GH
$AgNO_3$ (T_1)	BARI Sarisha-8	0.80I-L	1.60HI
	BARI Sarisha-7	0.20L	0.40K
	MM 20-3	0.80I-L	1.60HI
MS+1 mgL^{-1}	Kallyania	1.2H-J	2.10F-H
BAP+0.1 mgL^{-1}	Sampad	1.8GH	2.40D-G
NAA+2 mgL^{-1}	Tori-7	3.2B-D	3.10A-C
$AgNO_3$ (T_2)	BARI Sarisha-8	1.80GH	2.40D-G
	BARI Sarisha-7	2.20FG	2.60C-G
	MM 20-3	1.40HI	2.20E-H
MS+1.5 mgL^{-1}	Kallyania	2.6D-F	2.80B-E
BAP+0.1 mgL^{-1}	Sampad	2.8C-F	2.90A-D
NAA+2 mgL^{-1}	Tori-7	3.4A-C	3.20A-C
$AgNO_3$ (T_3)	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 mgL^{-1}	Kallyania	2.8C-F	2.90A-D
BAP+0.1 mgL^{-1}	Sampad	3.2B-D	3.10A-C
NAA+2 mgL^{-1}	Tori-7	3.8A-B	3.40AB
$AgNO_3$ (T_4)	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 mgL^{-1}	Kallyania	3.00C-E	3.00A-D
BAP+0.1 mgL^{-1}	Sampad	3.2B-D	3.10A-C
NAA+2 mgL^{-1}	Tori-7	4.00A	3.50A
$AgNO_3$ (T_5)	BARI Sarisha-8	2.80C-F	2.90AB
	BARI Sarisha-7	2.80C-F	2.90A-D
	MM 20-3	2.40E-G	2.70C-F

Regeneration of root: Induction of root from regenerated shoots showed wide variations according to genotypes and different concentrations of NAA in the medium (Data not shown). Tori-7 had the highest percentage (55.56) of rooted shoots. It was also highest in ½ MS + 0.5 mgL^{-1} NAA (40.67) (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 maximum number of shoots with root (2.22) was found in ½ MS + 0.5 mgL^{-1} NAA followed by ½ MS + 1 mgL^{-1} NAA (1.5) and ½ MS (1.11).

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 (Data not shown). The effects of phytohormone × genotype interactions in producing root were found statistically significant. Maximum number of shoots with root was found in Tori-7 on ½ MS + 0.5 mgL^{-1} NAA (Data not shown).

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).

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

Supplements	Variety	Number of shoot incubated	Number of shoot 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^{-1}	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^{-1}	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

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