International Journal of Research Studies in Biosciences (IJRSB)
Volume 3, Issue 4, April 2015, PP 33-44
ISSN 2349-0357 (Print) & ISSN 2349-0365 (Online)
www.arcjournals.org

Comparative Response of Different Genotypes of *Brassica* to Anther Culture

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Abstract: *Anther culture is a biotechnology technique that can be used for production of pure lines in many species. The aim of this experiment is to study comparative response of anther culture on different genotypes of Brassica. In order to access the effect of anther culture response in different genotypes of Brassica, three varieties of B. napus [GSL1, DGS1 and RSPN25) and three varieties of Brassica juncea (RSPR01, Varuna and Kranti) were evaluated. The best response of callus induction was observed in MS medium supplemented with 2, 4-D (1.0 mg l^{-1}) and NAA (0.5 mg l^{-1}). Enhancement of regeneration of plantlets was achieved by sub culturing embryogenenic callus on media containing different concentrations of cytokinins [BAP and Kinetin] and the best response of embryogeniccalli was got with kinetin [2.0 mg l^{-1}]. The best rooting was achieved with 0.4 mg/l IBA. After 5 days of rooting root samples were taken for confirmation of haploid nature of the plants. Haploid frequency was more than 50 %. Rooting and hardening of plantlet was done on MS medium supplemented with NAA (0.5mgl^{-1}). Among the genotypes used in present study, Brassica napus (DGS1) showed highest response.*

Keywords: *Anther culture, Haploids, Brassica napus, Brassica juncea, Embryogenesis.*

1. INTRODUCTION

Brassica is the most economically important genus in the Brassicaceae family (syn. Cruciferae). *Brassica* occupy a prominent place in world's agrarian economy as vegetables, oilseeds, feed and fodder, green manure and condiments. They also contain a large number of novel photochemical, some of which are anti-carcinogenic (Steinmetz and Potter, 1996).Oilseed brassica is the third leading source of vegetable oil in the world after soybean and palm oil and contributes significantly to the economy of many countries (Gupta and Pratap, 2007). In India oilseeds are the second largest agricultural commodity after cereals sharing 13 percent of countries gross cropped area, and contributing nearly 5 percent to gross national product and 10 percent to the value of all agricultural products (Hedge, 2000).At global level, rapeseed-mustard crops are grown in 53 countries spreading over the 6 continents, covering an area of 22.33 million hectare with on average production of 33.17 million tonnes and an average yield of 1468 kg/hectare ranging from 333 kg/hectare (Tazikistan) to 6667 kg/hectare (Algeria) (Pandey., et al 2013).The state of Jammu and Kashmir accounts for just about 2.1 percent of total rapeseed-mustard in India. The state occupies an area of 7.92 thousands ha with a production of 6.10 thousand million tonnes and productivity of 6.28 qtls per hectare (Anonymous 2007). Anther culture is an efficient way of producing doubled haploid plants in Brassica species. Compared with the traditional production of genetically stable homozygous lines, microspore culture dramatically speeds up breeding process and facilitates the selection of recessive traits (Henderson and Pauls, 1992). Microspore culture gives less response in B. rapa (Guo and Pulli, 1996) compared with B. napus (Zhou et al., 2002; Gu et al. 2004 and Leroux et al., 2009).

In the present paper, we used different genotypes of *Brassica* to evaluate the effect of anther culture response for haploid production.

2. MATERIAL AND METHODS

Donor plants: Experimental material comprised of anthers (explant) got from three varieties of *B. juncea* (RSPR01, Varuna and Kranti) and three varieties of *B. napus* (GSL1, DGS1 and RSPN 25). All the genotypes were collected from the Division of Plant Breeding and Genetics at

SKUAST-J, Chatha, Jammu. Closed flower buds of 2-2.5 mm size of all selected genotypes, were collected at appropriate stage and time. Disease free flower buds, mostly from uninucleate to binucleate stage were collected during morning hours between 8:00 AM to 10:00 AM and evening hours between 4:00 PM to 6:00 PM. It is this time that the development stage of microspores would just before the nuclear mitosis stage.

Callus induction media: The B5 medium (Gamborg et al, 1968) and MS medium (Murashige and Skoog, 1962) procured in readymade form without sugar and agar from Hi- Media laboratories, were used for callus induction. Sucrose was used as carbon source in the callus induction media. 24.17 g/l of B5 medium in powder form and sucrose 30 g ml^{-1} were added in distilled water. The medium was fortified with different concentrations of phytohormones i.e. 2, 4-D (1.0- 3 mg/l), NAA (0.5-3.0 mg/l), BAP (1.0-5.0 mg/l) and codes were assigned to them for different combination of concentration which are shown in table 1. The pH of medium was adjusted at 5.8 using 0.1N NaOH or1N HCl and volume made up to 1 litre. Semisolid medium prepared by adding 8 g/l agar was autoclaved at 120^0C temperature and 15 psi pressure for 20 minutes in test tubes and flasks containing 10-20 ml of medium, respectively. The medium was incubated in dark at 25 ± 2^0C for one week before being used for inoculations of anthers. Similarly 4.41 g/l of MS medium in powder form and sucrose 30 g ml^{-1} were added in distilled water. The medium was fortified with different concentrations of phytohormone i.e. 2, 4-D (1.0- 3 mg/l l), NAA (0.5-3.0 mg/l), BA (1.0-5.0 mg/l) and codes were assigned to them for different combination of concentration which are shown in table 1. The pH of medium was adjusted at 5.8 using 0.1N NaOH or 1N HCl and volume made up to 1 litre. Semisolid medium prepared by adding 8 g/l agar was autoclaved at 120^0C temperature and 15 psi pressure for 20 minutes in test tubes and flasks containing 10-20 ml of medium, respectively. The medium was incubated in dark at 25 ± 2^0C for one week before being used for inoculations of anthers. Observations with regard to callus induction frequency were made within 4 ± 1 weeks of inoculation.

Table1. *Different concentration of media with growth hormone for callus induction*

S.No	Medium code	MS media supplemented with	B_5 media supplemented with
1	M-1	BAP 1.5 mg/l	BAP 1.5 mg/l
2	M-2	BAP(1.5mg/l) + NAA(0.5mg/l)	BAP(1.5mg/l) + NAA(0.5mg/l)
3	M-3	NAA(0.5mg/l)	NAA(0.5mg/l)
4	M-4	BAP(1.5mg/l) + 2, 4 D(1.0mg/l)	BAP(1.5mg/l) + 2, 4 D(1.0mg/l)
5	M-5	2,4D(1.0mg/l) + NAA (0.5mg/l)	2,4D(1.0mg/l) + NAA (0.5mg/l
6	M-6	2, 4 D (1.0mg/l)	2, 4 D (1.0mg/l)

Regeneration medium: MS medium (Murashige and skoog, 1962) in readymade form, without sugar and agar, was procured from Hi- media Laboratories. For every litre of medium, 4.41 g MS medium and 30 g sucrose were added to distilled water. Different concentration of phytohormone i.e. BAP 0.5-5.0 mg/l and Kinetin 0.5-3.0 mg/l were used to prepare different media combinations. The pH of medium adjusted at 5.8 using 0.1 N NaOH or 1N HCl and then the total volume made upto one litre. Agar (8 g/l) was used as gelling agent to prepare semisolid medium. The medium was autoclaved at 120^0C temperature and 15 psi pressure for 20 minutes. The codes were assigned to the regeneration media as depicted in table 2. Observations with regard to number of plants regenerated were made within $5\pm$ 1 weeks of inoculation at different concentration of BAP and Kinetin.

Table2. *Combination of growth hormone for regeneration*

S.No	Medium code	MS medium supplemented with
1	RM1	BAP 1.0 mg/l
2	RM2	BAP 2.0 mg/l
3	RM3	BAP 3.0 mg/l
4	RM4	kinetin 1.0 mg/l
5	RM5	kinetin 2.0 mg/l
6	RM6	kinetin 3.0 mg/l

Rooting media: The regenerated shoots were subculture in rooting medium (within 5 ± 1 of culture initiation) of MS medium supplemented with IBA at the concentration 0.1-0.6 mg/l. The codes were assigned to the regeneration media as depicted in table 3.

Table3. *Different concentration of growth hormone with MS medium for in vitro rooting*

S.No	Medium code	Medium detail
1	R1	MS
2	R2	MS +IBA (0.2 mg/l)
3	R3	MS +IBA (0.4 mg/l)
4	R4	MS +IBA (0.6 mg/l)

3. IN VITRO CULTURING

Anther plating: Twenty to thirty flower buds, for each genotype were cut on a sterile Petri plate provided with lining of sterilized filter paper inside, under laminar airflow bench. Individual flower buds were cut at the base with sharp surgical sterilized scissors to free anther from the filament. With the help of sterilized pointed forceps the floret were picked at the apex and tapped on the rim of flask/test tubes containing callus induction media such that anthers fell on the surface of the medium. About 20-25 anthers per test tube were inoculated under aseptic conditions in laminar airflow cabinet. Tubes and flasks after inoculation were plugged with cotton plug and sealed with parafilm and incubated in dark at 25 ± 2^0C. Cultures were periodically observed for response of anthers culture to callus induction at different media combinations. Observations with respect to response of genotype for callus induction were recorded 4 ± 1 week of inoculation.

Regeneration: The calli induced were sub-cultured for further proliferation on the same media in which callus induction had taken place. Large compact pieces of one month old calli were cut into small pieces (2-3 mm diameter) with sterilized blade inside laminar air flow cabinet. With the help of pointed forceps, they were picked and placed inside the test tubes containing semisolid regeneration media. The culture tube were sealed with parafilm and incubated under cool white florescent light (2500 lux) in dark /light conditions for 16/8 h, respectively at 25 ± 2^0C. Observations with regard to number of plant regenerated were made within 5 ± 1 weeks of inoculations.

Rooting of regenerated shoots: The fully formed plantlets were taken out from culture vessels. The agar was removed from the roots and plantlets shifted to MS basal medium supplanted with various concentration of auxin in large size test tube for proper development of roots and shoot and incubated for 15 days under light/dark period of 16/8 h, respectively, at 25 ± 2^0C.

Observations recorded: For each genotype, anthers in aseptic cultures were counted and observations with respect to response of anthers to callusing were recorded. Data with respect to callus induction frequency (%), regeneration frequency (%) and haploid induction frequency (%) were worked out following Otani *et al* (2005).

- Callus induction frequency (CIF)

$$\frac{\text{No. of calli induced}}{\text{No. of anthers cultured}} \times 100$$

- Callus regeneration frequency (CRF)

$$\frac{\text{No. of calli showing regeneration}}{\text{No. of calli cultured}} \times 100$$

- Haploid induction frequency (HF)

$$\frac{\text{No. of haploids induced}}{\text{No. of anthers cultured}} \times 100$$

Statistical analysis: The data obtained during the experiment were analyzed statistically following the Completely Randomized Design (Panse and Sukhathme, 1985). The analysis of variance (ANOVA), along with SE (Mean), Critical difference (CD) and coefficient of variance (CV) values were worked out as per standard formula. The analyzed results were tabulated for interpretation.

4. RESULTS

Effect of growth regulators on anthers forming Callus: Six media combinations (designated as M1, M, M3, M4, M5, and M6) tested for callus induction during the presented study was based on MS and B5 basal medium. Different media used had different combinations and concentration of inorganic and organic salts and plant growth regulators. These were tested for callus initiation in different varieties of *Brassica spp*.

From the table 4 and figure 1it was observed that highest mean percentage of callus induction (36.71) in DGS1 was possible when MS medium was supplemented with 2, 4 D (1.0 mg/l) + NAA (0.5 mg/l) followed by GSL1, where the mean percentage of callus induction was (36.69).B_5 medium showed relatively lower percentage (13.34) of callus induction as compared to MS medium. It was evident that 1.0 mg/l 2, 4 D + NAA (0.5 mg/l) proved to be excellent growth regulator in terms of callus induction followed by 1.5 mg/l BA+ 0.5 mg/l NAA.

In case of *B. juncea*, the maximum mean percentage of anthers forming callus was shown in Varuna(18.97) in MS medium supplemented with1.0 mg/l 2, 4 D + 0.5 mg/l NAA (table 5 figure 2). B_5 medium showed relatively lower percentage (9.78) of callus induction as compared to MS medium. It was evident that 1.0 mg/l 2, 4 D + NAA (0.5 mg/l) proved to be excellent growth regulator in terms of callus induction followed by 1.5 mg/l BA+ 0.5 mg/l NAA. Other growth regulators used, showed significantly lower values for callus induction.

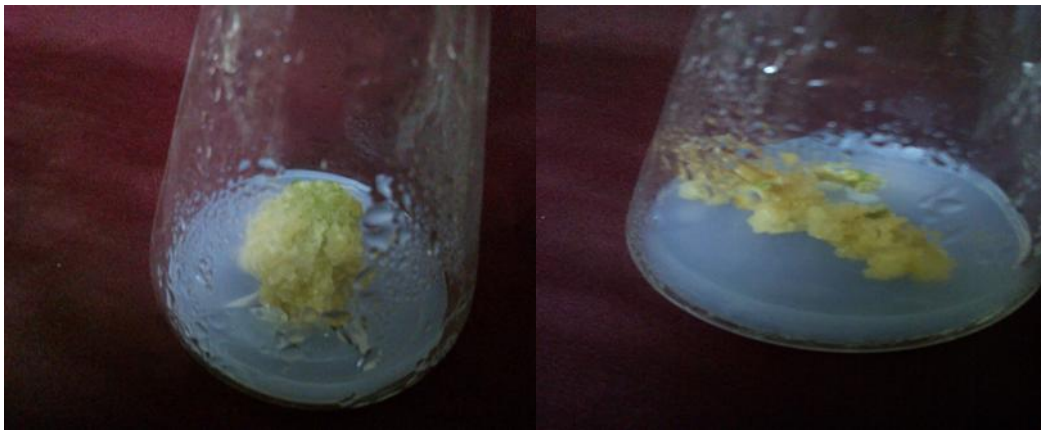

Fig1. *Callogenesis into Brassica napus* **Fig2.** *Callogenesis into Brassica juncea*

Table4. *Effect of growth regulators on Callus Induction Frequency (CIF) in Brassica napus*

Genotype	Media components	MS Medium			B_5 Medium		
		Anthers cultured	Calli obtained	CIF (%)	Anthers cultured	Calli obtained	CIF (%)
GSL1	M_1	205	80	39.02 (38.67)	205	117	57.07 (49.07)
	M_2	256	0	0 (0)	256	0	0 (0)
	M_3	300	117	39 (38.63)	300	62	20.67 (27.04)
	M_4	142	0	0 (0)	142	0	0 (0)
	M_5	256	233	91.01 (72.54)	256	0	0 (0)
	M_6	205	104	50.98 (45.41)	205	0	0 (0)
	Means			**36.69 (32.54)**			**12.96 (12.69)**
	CD(0.05)			**0.48**			**0.26**
	SE(m) ±			**0.16**			**0.09**
	M_1	244	98	40.16 (39.40)	244	144	59.01 (50.19)
	M_2	177	0	0 (0)	177	0	0 (0)
	M_3	313	110	35.14 (36.344)	313	67	21.04 (27.54)

DGS1	M_4	244	0	0 (0)	244	0	0 (0)
	M_5	177	165	93.22 (74.84)	177	0	0 (0)
	M_6	313	162	51..76 (45.986)	313	0	0 (0)
	Means			**36.71 (32.72)**			**13.34 (12.96)**
	CD(0.05)			**0.53**			**0.26**
	SE(m) +			**0.179**			**0.09**
RSPN25	M_1	244	51	20.90 (27.18)	244	0	0 (0)
	M_2	177	79	44.63 (41.88)	177	0	0 (0)
	M_3	313	34	10.86 (19.23)	313	0	0 (0)
	M_4	224	0	0 (0)	224	0	0 (0)
	M_5	177	115	64.98 (53.66)	171	68	38.42 (38.28)
	M_6	313	0	0 (0)	313	0	0 (0)
	Means			**23.56 (23.65)**			**6.40 (6.38)**
	CD(0.05)			**0.52**			**0.10**
	SE(m) +			**0.17**			**0.04**

Figures in parenthesis represent square root transformed values

Table5. *Effect of growth regulators on Callus Induction Frequency (CIF) in Brassica juncea*

Genotype	**Media components**	**MS Medium**			**B_5 Medium**		
		Anthers cultured	**Calli obtained**	**CIF (%)**	**Anthers cultured**	**Calli obtained**	**CIF (%)**
Kranti	M_1	244	54	22.13 (28.06)	244	0	0 (0)
	M_2	177	33	18.64 (25.56)	177	35	19.77 (26.75)
	M_3	313	0	0 (0)	313	72	23.00 (26.99)
	M_4	177	37	20.90 (27.20)	244	26	10.66 (21.44)
	M_5	244	102	41.80 (40.28)	177	0	0 (0)
	M_6	313	0	0 (0)	313	0	0 (0)
	Means			**17.24 (20.18)**			**8.91 (12.53)**
	CD(0.05)			**0.44**			**0.43**
	SE(m) +			**0.15**			**0.14**
Varuna	M_1	205	48	23.41 (28.94)	205	0	0 (0)
	M_2	256	52	20.31 (26.77)	256	52	20.31 (26.75)
	M_3	300	0	0 (0)	300	75	25.00 (26.99)
	M_4	256	64	25 (30.04)	142	19	13.38 (21.44)
	M_5	142	64	45.07 (42.17)	256	0	0 (0)
	M_6	205	0	0 (0)	205	0	0 (0)
	Means			**18.97 (21.32)**			**9.78 (12.53)**
	CD(0.05)			**0.64**			**0.44**
	SE(m) +			**0.21**			**0.19**
	M_1	177	0	0 (0)	244	46	18.85 (25.73)

RSPR01	M_2	177	0	0 (0)	177	22	12.43 (20.64)
	M_3	313	0	0 (0)	313	0	0 (0)
	M_4	244	27	11.06 (19.42)	244	0	0 (0)
	M_5	244	50	20.42 (26.91)	177	122	68.93 (56.19)
	M_6	313	31	9.90 (18.96)	313	0	0 (0)
	Means			**6.90 (10.88)**			**16.70 (17.09)**
	CD(0.05)			**0.72**			**0.36**
	SE(m) ±			**0.24**			**0.12**

Figures in parenthesis represent square root transformed values

Standardization of media for Callus regeneration through influence of Plants Growth Regulators: Calli induced were transferred to regeneration media containing various concentrations of growth regulators (designated as RM1, RM2, RM3, RM4, RM5 and RM6). Results depicted in table 6 and figure 3 showed that the cultivars differed in their number of regenerated plants. Maximum of 75 and 72.34 percent plants were obtained in DGS1 and GSL1 of *Brassica napus* on the media combination RM5 (MS+ Kinetin (2.0 mg/l). However, minimum regeneration frequency of 28.12 in DGS1 and 26.92 in GSL1 was recorded when MS supplemented with Kinetin (3.0 mg/l).

The data represented in table 7 and figure 4 revealed that Varuna showed the maximum percentage (73.76) of shoot regeneration in case of *Brassica juncea* when MS media was supplemented with 2.0 mg/l Kinetin.

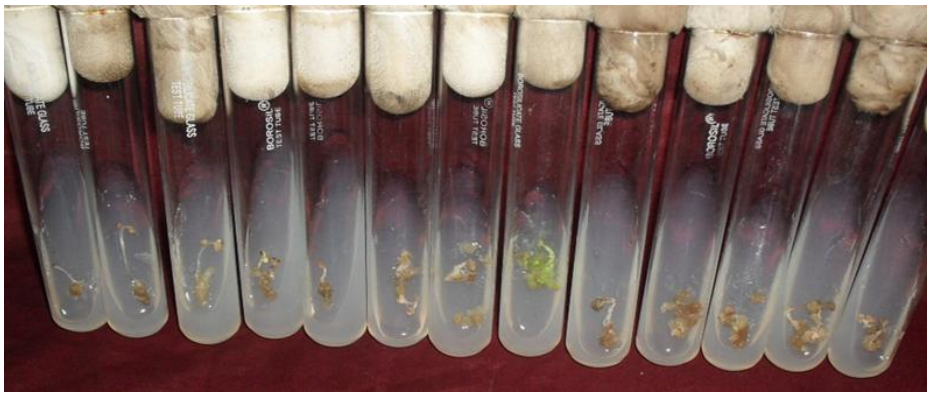

Fig3. *Regeneration in Brassica napus*

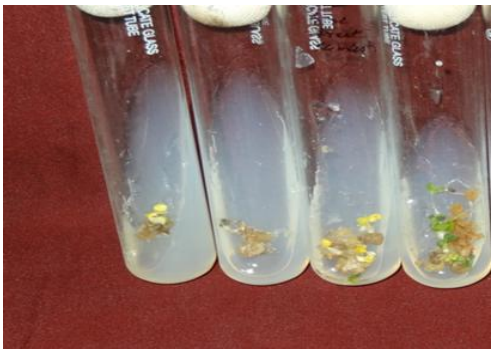

Fig4. *Regeneration in Brassica juncea*

Table6. *Effect of growth regulators on callus regeneration in Brassica napus*

S. No	Regeneration media	GSL1			DGS1			RSPN25		
		Calli cultured	Calli regenerated	CRF (%)	Calli cultured	Calli regenerated	CRF (%)	Calli cultured	Calli regenerated	CRF (%)
1	RM_1	104	54	51.92	64	34	53.12	100	42	42
2	RM_2	141	89	63.12	64	43	67.19	100	60	60
3	RM_3	104	42	40.38	64	28	43.75	100	29	29
4	RM_4	104	35	33.65	64	21	32.81	100	36	36

5	RM_5	141	102	72.34	64	48	75	100	62	62
6	RM_6	104	*28*	26.92	64	18	28.12	100	38	38
	Mean			**48.06**			**49.98**			**44.5**
	CD (0.05)			**1.43**			**3.02**			**0.56**
	SE(m) ±			**0.48**			**1.09**			**0.52**

Table7. *Effect of growth regulators on callus regeneration in Brassica juncea*

S. No	Regeneration media	**Kranti**			**Varuna**			**RSPR01**		
		Calli cultured	Calli regenerated	CRF (%)	Calli cultured	Calli regenerated	CRF (%)	Calli cultured	Calli regenerated	CRF (%)
1	RM_1	64	23	35.93	100	20	20	63	10	15.87
2	RM_2	64	34	53.12	141	81	56.74	62	30	48.38
3	RM_3	64	21	32.81	100	36	36	63	20	31.74
4	RM_4	63	20	31.75	100	32	32	64	16	25
5	RM_5	62	37	59.68	141	104	73.76	64	38	59.37
6	RM_6	63	21	33.33	100	20	32	64	16	25
	Mean			**35.64**			**41.75**			**34.23**
	CD (0.05)			**2.85**			**1.83**			**2.73**
	SE(m) ±			**0.95**			**0.61**			**0.91**

Standardization of medium for root formation (Rhizogenesis) through influence of Growth regulators: Micro shoots were aseptically excised devoid of any callus and transferred to rooting medium containing MS medium as basal medium and different auxins. Each treatment comprised of thirty micro shoots which were replicated four times. The results were recorded after 4 ±1 weeks of subculture. The data presented in table 8and figure 5 indicates the main effects of genotypes.

DGS1 shows the maximum percentage of rooting of micro shoots (33.78) followed by GSL1 (29.03), Varuna, (25.83), RSPN 25 (23.95), Kranti (20.77) and RSPR01 (17.79). The result also revealed the main effects of IBA influenced the rooting of micro shoots. Maximum percentage of rooting was observed with 0.4 mg/l IBA (30.72) followed by 0.2 mg/l IBA (26.26) all other combinations show lower percentage of rooting the best combination where 42.47 percent of rooting could be achieved was in DGS1 when MS medium was supplemented with IBA (0.4 mg/l).

Table8. *Influence of growth regulators on in vitro rooting of shoots (%)*

S. No	**Genotype**		**R1**	**R2**	**R3**	**R4**	**Mean**
1		GSL1	19.28	25.89	40.09	30.89	29.03
2		DGS1	22.37	35.15	42.47	35.15	33.78
3	*B. napus*	RSPN25	17.71	22.91	32.29	22.92	23.95
4		Kranti	16.90	23.25	23.25	19.68	20.77
5		Varuna	19.95	30.56	26.40	26.40	25.83
6	*B. juncea*	RSPR01	11.70	19.82	19.82	19.82	17.79
		Mean	**14.66**	**26.26**	**30.72**	**25.81**	
		CD(0.05)	**NS**	**NS**	**6.23**	**7.73**	
		SE(M)±	**3.01**	**3.44**	**2.08**	**2.58**	

N.S: Non Significant

Fig5. *In vitro* rooting

Influence of different combinations and media formation on Haploid frequency: The data represented in Table 9 and figure 6revealed that among three genotypes of *B.napus* maximum mean percentage of haploids was obtained in DGS1 (50.78) followed by GSL1 (48.58) and RSPN25 (47.44) and the data represented in Table 10and figure 7 revealed that among three genotypes of *B.juncea* maximum mean percentage of haploids was obtained in Varuna (44.53) followed by (42.94) Kranti and RSPR01 (42.33). Among all the six genotypes maximum frequency of haploids was obtained in DGS1 (34.42).

Fig6. *Regeneration of haploid plants in Brassica napus*

Fig7. *Regeneration of haploid plants in Brassica juncea*

Table9. *Influence of growth regulators on haploid frequency (HF) in Brassica napus*

S. No	Regeneration media	GSL1			DGS1			RSPN25		
		Anthers cultured	Haploid regenerated	HF (%)	Anthers cultured	Haploid regenerated	HF (%)	Anthers cultured	Haploid regenerated	HF (%)
1	RM_1	244	30	12.29	205	27	13.17	297	24	8.08
2	RM_2	177	87	49.15	256	130	50.78	142	69	46.94
3	RM_3	313	110	35.14	297	104	35.01	205	64	31.22
4	RM_4	244	48	19.65	142	28	19.72	256	47	18.21
5	RM_5	177	86	48.58	256	130	50.78	137	65	47.44
6	RM_6	313	114	36.42	205	76	37.07	256	83	32.42
	Mean			33.54			34.42			30.71
	CD(0.05)			2.36			1.98			1.96
	SE(M)+			0.79			0.66			0.65

Table10. *Influence of growth regulators on haploid frequency (HF) in Brassica juncea*

S.No.	Regeneration media	Kranti			Varuna			RSPR01		
		Anthers cultured	Haploid regenerated	HF (%)	Anthers cultured	Haploid regenerated	HF (%)	Anthers cultured	Haploid regenerated	HF (%)
1	RM_1	244	23	9.42	205	25	12.20	297	19	6.40
2	RM_2	177	32	18.08	256	46	17.96	142	25	17.60
3	RM_3	313	84	26.84	297	85	28.62	205	52	25.36
4	RM_4	244	38	15.57	142	26	18.30	258	34	13.17
5	RM_5	177	76	42.94	256	114	44.53	137	58	42.33
6	RM_6	313	87	27.80	205	58	28.29	256	71	27.73
	Mean			**23.44**			**24.98**			**22.09**
	CD(0.05)			**1.51**			**1.56**			**1.39**
	SE(M)+			**0.51**			**0.52**			**0.46**

5. DISCUSSION

Callus induction: Significantly higher number of induced callus was formed in the investigation when the medium was solid. This is in concurrence with the findings ofStipic and Campion (1997) where significantly higher induction frequencies were achieved from cauliflower anthers cultured on solid media. However, studies of Yang *et al.* (1992), on anther culture revealed increased induction frequency on a liquid culture medium as well.

Inclusion of auxins and cytokinins in induction media has been shown to influence embryogenesis in number of species, including tomato (DeBergh and Nitsch, 1973), tobacco (Nitsch, 1969),

wheat and barley (Clapham, 1977). In the present study of*Barssica* spp. Maximum number of induced anthers were obtained when one of the induction media viz. MS medium was supplemented with 2, 4-D (1.0 mg/l) with NAA (0.5 mg/l) followed by BA (1.5 mg/l) and NAA (0.5 mg/l). The study also revealed that maximum frequency of embryogenesis was achieved when MS medium was supplemented with BA (1.0 mg/l) and 2, 4 D (1.0 mg/l). Similar results were reported by Lichter (1981)in *B.napus* where NAA appeared to be essential for the induction of embryogenesis while the simultaneous presence BA increase embryo yield. The existence of complex interaction between explant, absolute concentration of phytohormone and culture media composition for callus induction was evident from the results. Growth conditions of the donor plant had a powerful influence on the frequency of embryogenesis. The observed response in the present study could be attributed to the plants grown under field conditions. Similar results were observed in *Brassica napus* and *B. campestris*by Keller *et al*, (1993) and Keller and Stringham (1978).

Organogenesis: Androgenic embryo development is an indispensible condition for next breeding steps, but not the only one. It is necessary to regenerate the plants from obtained embryo. The regeneration has always been critical as many embryos die (Keller et al, 1982). The choice of proper medium for regeneration is very important and factors like lowering the level of sucrose, elimination of amino acids and also change of hormones are essential mechanisms for obtaining meaningful results (Keller and Armstrong, 1979and Naleezynsks, 1991).

Different media for regeneration of the plants obtained from embryos in the process of androgenesis have been used in the experiments by many workers. Takahota and Keller (1991) used B5 medium without hormones for the regeneration of the plants obtained from the embryos B. oleracea. Several passages induce the formation of shoots. Dore and Boulidard (1988), placed embryos on medium without growth factors transferred in the single complete plants whereas, embryos placed on a medium with 0.1 mg/l of BA formed shoots. Nalecezynska (1991) obtained many shoots from embryos of *B.napus* placed on B5 medium with 20 mg/l of kinetin. Gorecka et al. (2007) obtained highest number of shoots from embryo using B5 medium with 20 mg/l of sucrose along with 20 mg/l of kinetin. Organogenesis depends upon a complex system involving a number of limiting and interacting factors (Narayanswamy, 1977).Regeneration of plants from callus cultures either through organogenesis or embryogenesis is advantageous since enormous number of plants can be produced from callus regeneration. There was significant genotype effect on organogenesis indicated in this study. Previous regeneration studies on Brassica and on other plant species (e.g. barley, soybean) also indicated regeneration/maturation capacity as dependent on genotype and thus under genetic control (Romeijn and Lammeren, 1999; Zamani et al 2003; Klima et al., 2004).

Development of plantlets on MS medium is in conformity with the previous studies concerning Brassica where this medium was effective for regeneration and maturation Rudolf et al., 1999). In this study, callus proliferation, shoot and root development was significantly better on MS medium and organogenesis was induced with different concentration of cytokinins. It is important to note that culture medium is an important factor affecting the plant development (Li et al., 2005, Zhang et al., 2005). A difference in hormonal requirement specific for plant growth, hormonal combinations and their concentration favouring degree of organogenesis was clearly evident during the study. The combination of MS medium supplemented with BA (2.0 mg/l) or Kinetin (2.0 mg/l) was found effective for shoot development. In the present experiment shoots devoid of any callus were taken on rooting media supplemented with different concentrations of auxins (IBA) ranging from 0.2 to 0.6 mg/l. Best rooting was achieved when MS media was supplanted with 0.4 mg/l IBA. Similar reports demonstrating the role of physico-chemical conditions in Brassica include Niv et al., (1999),Momon et al., (2002),Sato et al., (2005) and Zhang et al., (2006). Dore and Boulidard (1988) obtained root formation in the shoots of head cabbage on a medium without hormones. Nalecezynska (1991) used the medium with 5 mg/l of IBA to obtain root formation in the shoots of rapseed.

Haploid frequency: Root tip mitosis chromosome counts were employed to analyse anther derived regenerants for their ploidy level. The haploid frequency in the present study was 50.78 percent in B. napus and 44.53 percent in *B. juncea.* The remaining plants, which were fertile and considered to be diploid, presumably originated as a result of fusion or endoreduplication in the microspores

during anther culture. Similar works have been reported by several workers (Wang et al., 1999). The final ploidy composition of anther derived regenerants has been reported to be species and genotype specific (Wang et al., 1999). It is not yet known why some species and genotypes produce more haploids whereas, others produce majority of diploids. The stage of the pollen development at the time of culture may also affect subsequent ploidy levels of the regenerants for instance culturing pollen at uninucleate stage resulted in the production of more haploids while pollen at bi nucleate stage resulted in more diploids and other higher ploidy levels (sunderland et al., 1974; Keller et al. 1975).

REFERENCES

Anonymous. 2007. Statistical Digest of Jammu and Kashmir. Directorate of Planning and Statistics, Govt of Jammu and Kashmir.

Clapham, D. 1977. Haploid induction in cereals. In applied and fundamental aspects of plant cell. *Tissue and organ culture* Edited by J.Peinert and Y.P.S Bajaj. Springer-Verlag. New York pp. 297-298.

*DeBergh, P. and Nitsch, C. 1973.Primiers results sur la culture de grains de pollen isoles chez le lomote, C.R. Hebd. SeancesAcad Science, 276: 1281-1284. Arjournals. Annual reviews. org/DOI/ABS/10.1146.

*Dore, C. And Boulidard, L. 1988. Production de plantesandrogenetiques de chou a choucroute (*B.oliracea*L.ssp. Capitata) par culture d anthers in vitro. Comportment des lignees haploid doubles (HD) etleurinleretcomme parents d hybrids F_1. Agronomie, 8(10): 851-862. www.inwarz.skierniewice.pl

Gamborg, O.L., Miller, R.A., and Ojma, K. 1968. Nutrient requirements of suspension of soyabean root cells. *Experimental Cell Research*, **50**: 151-158.

Gorecka, K., Krzyzanowska, D., Kowalska, U. 2007. Regeneration and evolution of androgeneic plants of head cabbage (*Brassica oleracea* var. Capitata L.).*Vegetable Crop Research Bulletin*, **67**: 5-15.

Gu, H.H., Zhang, D.Q., Zhou W.J. 2004c. Effect of medium renovation and colchicine treatment on embryogenesis of isolated microspore in *Brassica rapa* ssp. *ChinensisActaAgron. Sin*,**30**: 78-81

Gu, H.H., Zhang, D.Q., Zhou. W.J. 2004b.Embryogenesis and plant regeneration from isolated microspores of winter cauliflower (Brassica oleracea var. botrytis).*Journal of Zhejaiang University* (Agriculture Life Science), **30**: 34-38.

Guo, Y.D., Pulli, S. 1996. High frequency embryogenesis in brassica campestris microspore culture. Plant Cell Tissue Organ Culture, 46: 219-225.

Gupta, S.K and Pratap, A. 2007.History, origin and evolution.In Gupta S.K. (ed.) *Advancesin Botanical Research-Rapeseed Breeding*, **45**: 1-20.

Hedge, D.M. 2000. Oilseed: Technology for high yields. *The Hindu Survey of IndianAgriculture*, pp: 65-71.

Henderson, C.A.P and Pouls, K.P. 1992. The use of haploidy to develop plant that expressseveral recessive traits using light seeded canola (Brassica napus) as an example. *Theoretical andApplied.Genetics*.**83**: 476-479.

Keller W.A. and Stringham, G.R. 1978. Production and utilization of microspore derived haploid plants *Brassica napus* anther culture. In. thrope, T.A (ed) Frontiers of plant tissue culture. 1978, Calgary, Alberta.*The international Association of Plant Tissue Culture*, pp 113-122.

Keller, W.A. and Armstrong, K.C. 1979. Stimulation of embryogenesis and haploid production in *Brassica campestris*anther culture by elevated temperature.*Theoretical Applied Genetics*, **55:** 65-67.

Keller, W.A., Armstrong, K.C. Roche, A.J. de La. 1982.The production and utilization of mocrospopre-derived haploids in *Brassica crops*.In :SkSens; KL Giler (eds) Plant Cell Culture in Crop Improvement. Plenum, Press, New York, pp 169-183.

Keller, W.A., Rajhathy, T., and Lacapra, J. 1975.*In vitro* production of plants from pollen in *Brassica campestris*.*Canadian journal of cytology*, **17**: 655-666.

Klima, M., Vyvadilova, M and Kucera, V. 2004.Production and utilization of double haploids in *Brassica*oleracea vegetables.*Horticulture Scientia* (Prague), **4**: 119- 123.

Leroux, B., Carmoy, N., Giraudet, D., Potin, P., Larher, F and Bodin, M. 2009. Inhibition of ethylene biosynthesis enhances embryogenesis of cultured microspores of *Brassica napus*.*Plant Biotechnology Report*, 3:347-353.

Li, H.Z., Zhou, W.J., Zhang, Z.J., Gu, H.H., Takeuchi, Y. and Yoneyama, K. 2005. Effect of γ Radiation on development yield and quality of microtubers in vitro in *solanumtuberosam* L. *BiologiaPlantarium*, **49:** 625-628.

Lichter, R.1981. Anther culture of Brassica napus in a liquid culture medium. Z. *Physiologiaplantarum*, 103: 229-237.

Momon, G.R., Samantry, S. and Das, P. 2002. In vitro embryogenesis from callus cultures of *Brassica napus*. *ScientiaHorticulturae*, **86**: 61-69.

Naleezynska, A. 1991.Application of doubled haploids in breeding of rape. Part I. Obtaining of homozygous lines. HodRost.Aklim.Nas 35: 3-28 (Polish with English Summary).

Naleezynska, A. 1991.Application of doubled haploids in breeding of rape. Part I. Obtaining of homozygous lines. HodRost. Aklim.Nas 35: 3-28 (Polish with English Summary).

Narayaneswamy, S.1977. Regeneration of plants from tissue cultures.In : Applied and Fundamental Aspects of Plant Cell Tissue Culture (Eds. J.Peinert and Y.P.S Bajaj). Springer, Berlin.179-206.

Nitsch, J.P. 1969. Experimental androgenesis inNicotiana. Phytomorphology, 19: 389-404

Niv, Y.Z., Liu, Y.Z., Wang, L.Z., Yuan, Y.X., Li, S.C. and Fang, Q.J. 1999. A preliminary study on isolated microspores and plant regeneration of resynthesized *Brassica napus* L. *Journal of Sciachuan Agriculture University*, **17**: 167-171.

Otani, M., Wakita, Y and Shimada, T. 2005.Double haploid plant production of transgenic rice (Oryzasativa L.) using anther culture. Plant Biotechnolgy, 22: 141-143.

Pandey, S., Kadbal, M and Tripathi, M.K. 2013.Study of inheritance of erucic acid in Indian mustard (Brassica juncea L).Czern&Coss.Octa Journal of Biosciences, **1**: 77-84.

Romeijn, G. and Lammermen, A.A.M. 1999. Plant regeneration through callus initiation from anthers and ovule of scabiosa columbaria.*Plant Cell Tissue Organ Culture*, **56**: 169-177.

Rudolf, K., Bohanec, B. and Hansen. M. 1999. Microspore culture of white cabbage *Brassica oleracea*var. Capitata L. Genetic improvement of non responding cultivars and effects of genome doubling agents.*Plant Breeding*, **118**: 237-241.

Sato, S., Kotoch, N., Twai, S and Hogimori, M. 2005.Anther culture as a breeding tool in rape 2 progeny analysis of androgenestic lines and induced mutant from haploid cultures.*Breeding Science*, **61**: 26-29.

Steinmetz, K.A and Potter, J.D. 199 6.Vegetable, fruit and cancer prevention. A review *JouranalofAjmer Diet Associoation*. *96:* 1027-1039.

Stipic, M. and Campion, B. 1997.An improved protocol for androgenesis in cauliflowers (Brassica oleracea var. Botrytis).*Plant Breeding*, **116**: 153-157.

Sunderland, N., Collin, G.B and Dunwell, J.M. 1974. The role of nuclear fussion in poleen embryogenesis of *Daturainnoxia*.*Plantaria*. 117: 227-241.

Takahata, Y and Keller, W.A. 1991. High frequency embryogenesis and plant regeneration in isolated microspore culture of *Brassica oleracea*L. *Plant Science*, **74**: 235-242.

Wang, M., Farnham, M.W and Nannes, J.S.P. 1999.ploidy of broccoli regenerated from microspore culture verus anther culture. Plant Breeding, 118(3): 249-252.

Yang, Q., Chauvin, J.E and Herve, Y. 1992.A study of factors affecting anther culture of cauliflower (Brassica oleracea var. botrytis). Plant Cell Organ Culture, 28: 289-296.

Zamani, G., Kovacs, E., Goulo-Vavdinoudi, D.G., Roupakias and Barnabas, B. 2003.regeneration of fertile double haploid plants from colchicines supplemented media in wheat anther culture. *Plant Breeding,***119**: 461-465.

Zhang, G.Q., Zhang, D.Q., Tang, G.X and Zhou, W.J. 2006. Plant development from microspore-derived embryos in oilseed rape as affected by chilling, desiccation and cotyledon excision. *BiologiaPlantarum*, **50**: 180-186.

Zhou, W.J., Hagberg, P and Tang G.X. 2002a .Increasing embryogenesis and doubling efficiency by immediate colchicines treatment of isolated microspores in spring *Brassica napus.Euphytica,***128**: 27-34.

Zhou, W.J., Hagberg, P and Tang G.X. 2002b. Efficient production of doubled haploid plant by immediate colchicine treatment of isolated microspores in winter Brassica napus. *Plant Growth Regulator*, **37**: 185-192.