International Journal of Scientific Engineering and Applied Science (IJSEAS) – Volume-2, Issue-3, March 2016
ISSN: 2395-3470
www.ijseas.com

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

Dipti[1], Veena Chawla[1], Neelam R. Yadav[2], R. C. Yadav[2] and Priyanka[1]

[1]Deptartment of Genetics and Plant Breeding, CCS HAU, Hisar.

[2]Department of Molecular Biology, Biotechnology and Bioinformatics, CCS HAU, Hisar.

ABSTRACT:

Anther culture is a biotechnological technique that can be used for production of pure lines in many species. In order to access the anther culture response in *Brassica juncea,* four different genotypes (RH 406, RH 555, RH 749 and RH 832) and their F_1's with Ogura Restorer (OR) as the background were evaluated using two basal media viz., MS and B5 with different concentrations of phytohormones. Anthers were cultured at uninucleate stage and subsequent plant regeneration was studied on MS media with different concentrations and combinations of phytohormone. Maximum callusing (%) response of anthers was observed in F_1, OR x RH 749 (20.37±1.44) and maximum percent embryogenic calli obtained were 20.35±2.68 in same genotype on medium supplemented with B5 as basal medium with 100 mg/l sucrose, 30 mg/l glutathione, 100 mg/l serine, 0.05 mg/l BAP, 0.5 mg/l NAA and 10 mg/l silver nitrate. Rooting and hardening of plantlet was done on MS medium supplemented with different concentration of NAA and IBA. Out of sixteen anther culture media combinations tried, the best media were found to be ACM15 and ACM16 (containing B5 as basal medium with 100 mg/l sucrose, 30 mg/l glutathione, 100 mg/l serine, 0.05 mg/l BAP, 0.5 mg/l NAA and 10 mg/l silver nitrate in ACM15 and 20 mg/l silver nitrate in ACM 16) for all parents and F_1's.

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

INTRODUCTION

Indian mustard is an important oilseed crop of the *Brassicaceae* family. Due to its wide usage as oil, forage, condiments and for medicinal purposes, the crop holds a great economic importance. *Brassica species* range from noxious weeds to leaf and root vegetables to oilseed and condiment crops. The family *Brassicaceae* (= *Cruciferae*) contains over 338 genera and 3709 species (Al-Shehbaz *et al*., 2006; Warwick *et al*., 2006). Through interspecific hybridization in all possible combinations, three basic diploid plant species *B. rapa* (A genome, n=10), *B. nigra* (B genome, n=8) and *B. oleracea* (C genome, n=9) gave rise to three amphidiploid species *B. juncea* (AB genome, n=18), *B. napus* (AC genome, n=19), and *B. carinata* (BC genome, n=17) usually illustrated by 'U' triangle (U, 1935). Among different species *B. napus*, *B. rapa* and *B. juncea* have greatest importance in the world as sources of edible oil, *B. oleracea* as a source of vegetable crops and *B. nigra* as sources of condiment mustard. *Brassica* oilseed crops annually occupy over 26 million hectares of the world's agricultural land. Due to their ability to survive and grow at relatively low temperatures, makes them well adapted to cultivation at high elevations and in the subtropics.

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 contribution of rapeseed-mustard to the total oilseed acreage and production in India was 23.7% and 26%

respectively during the year 2012-13. In India, it is predominantly cultivated in Rajasthan, U.P., M.P., Haryana, Gujarat, West Bengal, Assam and Bihar states. The yield of rapeseed-mustard was 1176 kg/ha as compared to 955 kg/ha of total oilseeds (Singh, 2014). Indian mustard [*Brassica juncea* (L.) Czern & Coss.] accounts for about 80 % of the 6.3 m ha area under rapeseed-mustard crops in the country. *Per capita* consumption of edible oil is likely to reach 23-43 kg by 2030 from the present level of 13.4 kg (Singh, 2014).

In recent years, the country produced lesser than that of our national requirement. This gap is increasing day by day as the breeding approaches for Indian mustard improvement remained confined to selection and recombination followed by selection (Mekonnen *et al*. 2014). To address this challenge, it has become apparent that new breeding strategies are imperative to keep pace with emerging needs of *Brassica* oils. For a better breeding program, it should have a broad genetic base. Among CMS types of *Brassicaceae*, the CMS discovered by Ogura (1968), which is now popularly referred to by his name, has been studied most extensively and is used worldwide in F_1 breeding of *B. napus*, *B. juncea*, *B. oleracea* and *R. sativus* (Yamagishi and Bhat, 2014). The existing genetic base, is not enough to meet the challenge. Thus, for creation of variability and its utilization, it is necessary to go for hybridization and selection of desirable types in the succeeding generation to ensure homozygosity of a material during breeding programme. In order to improve the nutritional profiles of the *Brassica* oil and its meal, conventional breeding in combination with modern biotechnology tools such as production of double haploid has led to various agronomical improvements of qualitative and quantitative traits in rapeseed (Gupta, 2009). With the advent of biotechnology tools, plant tissue culture techniques have been refined and protocols have been developed to supplement the conventional crop improvement approaches. Anther culture is an efficient way of producing doubled haploid plants in Brassica species Prem *et al.,* 2008. 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).

In the present paper, we used different genotypes of *B. Juncea* and their crosses with Ogura restorer as background to evaluate the effect of anther culture response for haploid production.

MATERIAL AND METHODS

Donor plants: Experimental material comprised of anthers (explant) from four varieties of *B. juncea* (RH 406, RH 555, RH 749 and RH 832). In addition, crosses were attempted to get F_1 using Ogura restorer line as a female parent with the four genotypes**.** All the genotypes were collected from the area of oilseed section of Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar. Closed flower buds of 2-2.5 mm size of all selected genotypes, were collected at uninucleate to binucleate stage during morning hours.

Culture media: During the present investigation, 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. MS medium was used with 30 g/l sucrose, 0.1 g/l inositol and 8.0 g/l agar and B5 (Gamborg's) medium was used with 100 g/l sucrose and 3.0 g/l gelrite. Growth additives like serine, glutamine, glutathione and silver nitrate were used to enhance embryogenesis. Growth regulators, BAP and NAA were used alone or in various combinations with basal medium which are shown in table 1. All the components of the medium were mixed and the pH was adjusted to 5.8 with the help of 1N NaOH/ 1N HCl,

then the final volume was made by adding double distilled water followed by addition of agar or gelrite. The conical flask containing media and sealed with a cork and aluminium foil was sterilized in an autoclave at 121°C, 15psi for 20 mins. The sterilized medium was poured into petri plates/250 ml Jam bottles for inoculation / culturing under aseptic conditions in laminar air flow. The petri plates/Jam bottles were stored at 25±2° C and used within 3-4 days of its preparation. Similarly rooting media with MS basal supplemented with different concentrations of IBA and NAA were prepared.

Table 1: Different media used for anther culture of Indian mustard genotypes and their F_1.

Sr. No.	Media code	Medium and the supplements used
1	ACM1	B5+ 30 g/l sucrose
2	ACM2	MS+ 30 g/l sucrose
3	ACM3	B5+ 30 g/l sucrose +0.05 mg/l BAP + 0.5 mg/l NAA
4	ACM4	MS+ 30 g/l sucrose +0.06 mg/l BAP + 0.6 mg/l NAA
5	ACM5	B5+ 80 g/l sucrose +0.05 mg/l BAP + 0.5 mg/l NAA
6	ACM6	MS + 100 g/l sucrose + 0.05 mg/l BAP + 0.5 mg/l NAA
7	ACM7	MS + 100 g/l sucrose + 30 mg/l glutathione + 100 mg/l serine + 0.05 mg/l BAP + 0.5 mg/l NAA
8	ACM8	B5 + 100 g/l sucrose + 30 mg/l glutathione + 100 mg/l serine + 800 mg/l glutamine 0.05 mg/l BAP + 0.5 mg/l NAA
9	ACM9	B5 + 130 g/l sucrose + 30 mg/l glutathione + 100 mg/l serine + 800 mg/l glutamine + 0.05 mg/l BAP + 0.5 mg/l NAA
10	ACM10	B5 + 80 g/l sucrose + 100 mg/l serine + 0.08 mg/l BAP + 0.2 mg/l NAA
11	ACM11	B5 + 100 g/l sucrose + 30 mg/l glutathione + 0.05 mg/l BAP + 0.2 mg/l NAA+ 10 mg/l Silver nitrate
12	ACM12	B5 + 80 g/l sucrose + 30 mg/l glutathione + 100 mg/l serine + 0.05 mg/l BAP + 0.5 mg/l NAA + 20 mg/l silver nitrate
13	ACM13	B5 + 100 g/l sucrose + 100 mg/l serine + 0.02 mg/l BAP + 0.2 mg/l NAA + 20 mg/l silver nitrate
14	ACM14	B5 + 110 g/l sucrose + 30 mg/l glutathione +100 mg/l serine + 0.01 mg/l BAP + 0.1 mg/l NAA + 10 mg/l silver nitrate
15	ACM15	B5 + 100 g/l sucrose + 30 mg/l glutathione + 100 mg/l serine + 0.05 mg/l BAP + 0.5 mg/l NAA + 10 mg/l silver nitrate
16	ACM16	B5 + 100 g/l sucrose + 30 mg/l glutathione + 100 mg/l serine + 0.05 mg/l BAP + 0.5 mg/l NAA + 20 mg/l silver nitrate

Regeneration medium: MS medium in readymade form containing 4.41 g medium powder and 30 g sucrose were added to distilled water. Further, 1.0 mg/l BAP and 0.3 mg/l 2, 4-D were used to prepare media for callus maintenance. Observations with regard to number of plants regenerated were made within 5+ 1 weeks of inoculation. The calli from anthers with size more than 7.0 mm were transferred to shoot induction medium with

different concentration of phytohormones (Table 2) and incubated at 25±2°C in light (5000 lux) with 16 hrs. /8 hrs; light/dark regime. Regenerated shoots were then put on shoot multiplication medium (MS + BAP ;2.0 mg/l) to increase the copy number of anther culture derived plants.

Table2. Combination of growth hormone for plant regeneration in Indian mustard hybrids.

Sr. No.	Media code	Purpose	Media used
1	CM	Callus maintenance	MS + 30 g/l sucrose + 1.0 mg/l BAP + 0.3 mg/l 2, 4-D
2	SI1	Shoot induction	MS + 30 g/l sucrose + 2.5 mg/l Kin + 0.2 mg/l NAA
	SI2	Shoot induction	MS + 30 g/l sucrose + 1.0 mg/lBAP

Rooting media: The regenerated shoots were subcultured in rooting medium containing MS medium supplemented with IBA and NAA The codes were assigned to the regeneration media as depicted in table 3.

Table 3: Different media used for root formation in Indian mustard

Sr. No.	Medium code	Rooting media
1	RM1	MS basal
2	RM2	Half strength MS basal
3	RM3	MS + IBA (0.2mg/l)
4	RM4	MS + IBA (0.5 mg/l)
5	RM5	MS + NAA (0.2mg/l)

Anther plating: The immature floral buds collected from the field (Plate A & B) were kept in refrigerator at 4 °C for 2 days and then surface sterilized with distilled water containing 2-3 drops of tween 20, 3-4 washings were given to remove the detergent completely. In laminar air flow, these buds were then surface sterilized with freshly prepared 70% ethanol for less than 1 min. followed by washing thrice with autoclaved distilled water to remove traces of ethanol. These sterilized buds, were opened by making a cut with the help of forceps and the anthers aseptically and carefully removed were placed in petriplates (Plate C) containing anther culture media **(**Table 1). The anthers were given heat shock treatment by keeping the inoculated petriplates in incubator at 32°C for 2 days and then cultures were shifted to the culture room at 25±1°C in the dark.

Transfer of embryos raised from anther culture: After 2-3 weeks when the anthers became brown and swollen, the percent response of anthers was recorded. After 6-7 weeks when the anthers developed into embryos-(Plate D & E), these were transferred on to MS medium containing 1.0 mg/l BAP and 0.3 mg/l 2, 4-D for callus maintenance and the calli from anthers with size more than 7.0 mm were transferred to shoot induction medium (Table 2**).** Only those calli were selected which were produced after burst opening of anther lobes, any callus formation from the sides of anthers or anther wall and the filament was rejected in order to avoid any diploid/somatic embryogenesis. The emerging embryos (globular and heart shaped) were transferred to growth regulator free B5 medium for germination.

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 supplemented with various concentration of auxin in large sized jam bottles for proper development of roots and incubated for 15 days under light/dark period of 16/8 h, respectively, at 25+20C.

Observations recorded: For each genotype, anthers in aseptic cultures were counted and observations with respect to response of anthers to callusing were recorded.

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.

RESULTS AND DISCUSSIONS

Since the first report of in vitro production of doubled haploid plants in the 1960s, there has been much progress in protocol development, efficiency and application of the double haploid method. There are several endogenous and exogenous factors which play important role in *in vitro* haploid production. In the present investigations experiments were conducted to know about the factors affecting haploid production and their regeneration, required for haploid formation and results so obtained are being discussed below.

Effect of microspore development stage: The stage of microspores development at the time of inoculation is a complex and one of the most critical factors that strongly affects the induction of androgenesis i.e. success of anther culture (Mishra and Goswami, 2014). *Brassica* microspores are responsive to culture only at specific stages of their development. In the present investigation anther culture was attempted in four hybrids of *B. juncea*. Buds were collected from field in morning hours. Microspore stage and size of the bud were standardized by acetocarmine staining. Buds of size (2 - 2.5 mm) containing anthers with pollen grains at mid to late uninucleate stage were cultured. Kott *et al.* (1988a) reported that the highest frequency of embryogenesis in *B. napus* occurred when microspores were cultured at late uninucleate stage. Similarly, microspores of *B. napus,* 'Topas', were most responsive at late uninucleate to early binucleate stage (Pechan *et al.,* 1991). The stage of microspores could be correlated with the length of bud (Baillie *et al.,* 1992; Pechan *et al.,* 1988).

Effect of culture composition: A pivotal role in the induction of androgenesis is played by the culture medium composition. The diverse genotypes show very different basal medium requirements to induce pollen-derived plant formation. The nutritional requirements of the excised anthers are much simpler than those of isolated microspores (Reinert and Bajaj, 1977). The most commonly used basal media for anther culture are N6 medium (Chu, 1978), modified MS medium (Murashige and Skoog, 1962; Nitsch and Nitsch, 1969) medium, B5 medium (Gamborg *et al.,* 1968) and many others. In present study, modified MS and B5 media with different concentrations and combinations of various growth regulators were used and response in terms of (%) swollen brown anthers was recorded to be maximum on modified B5 basal media, ACM9, ACM12, ACM13, ACM15 and ACM16 (Table 1).

***Effect of carbohydrate source*:** A carbohydrate source is essential for embryo production in anther culture because of its osmotic and nutritional effects. Sucrose is the most common carbohydrate used in plant tissue and it is the major translocation carbon source used in anther culture. Sucrose concentration in induction medium has a major effect on osmosis and the development of embryos is apparently influenced by osmosis (Mishra and Goswami, 2014). Along with it sucrose also determines the pollen viability and embryo induction in the anthers. It not only inhibits growth of anther wall, but specifically induces divisions in pollen grains.

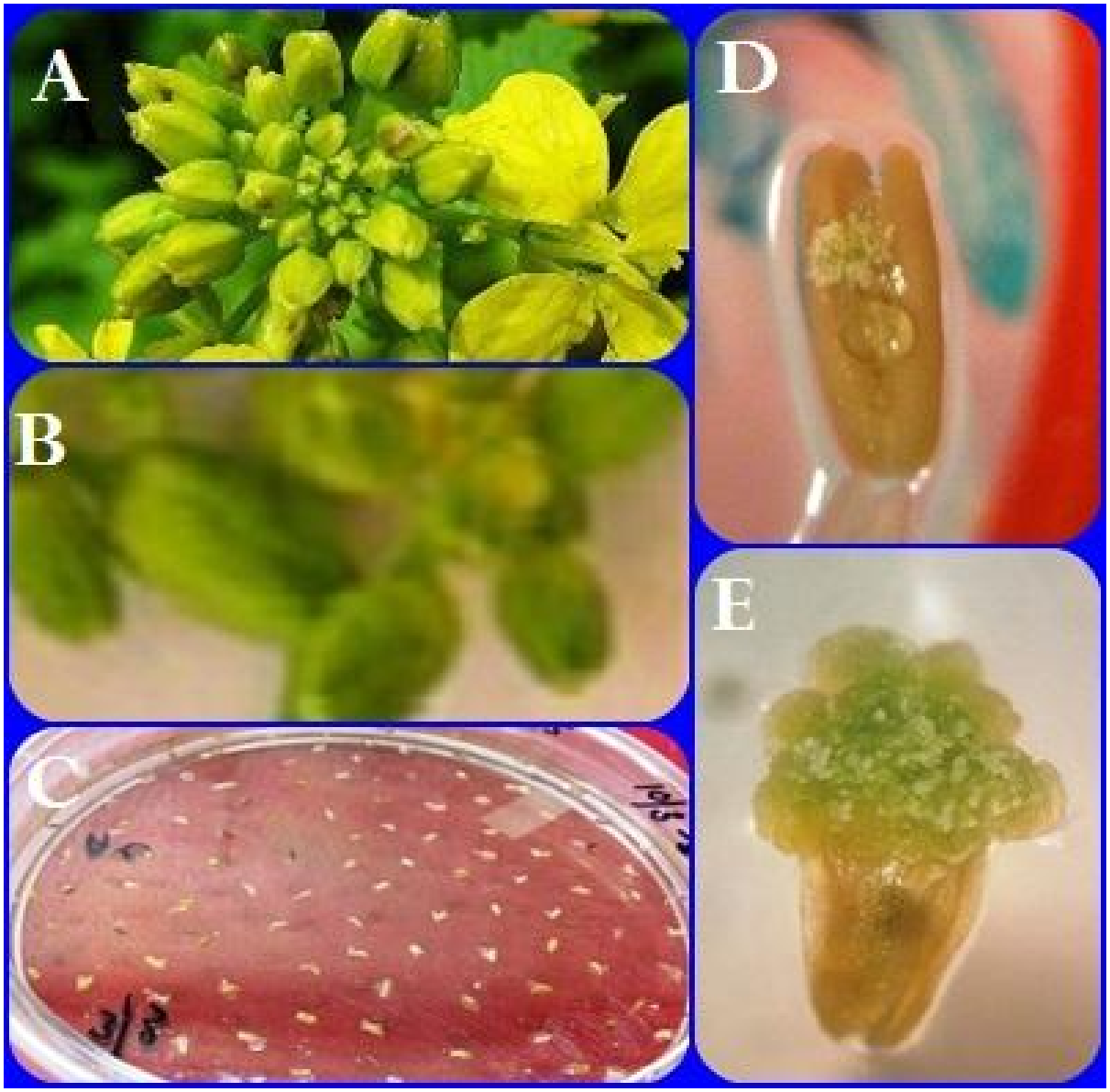
In our present investigation different concentrations (8 % - 13 %) of sucrose were used in culture medium, but the good and consistent response was observed in all the four genotypes on media (ACM15 and ACM16) comprising 10 % sucrose. Similar response of all the F_1's was recorded on medium with 13 % sucrose (ACM9) except in F_1 OR x RH 749 (with no response). No regeneration was recorded on the media that contained 8 % or 11 % of sucrose (Table 1).

***Effect of growth regulators*:** Sixteen media combinations (designated as ACM1-16) tested for callus induction during the presented study was based on MS and B5 basal media. Different media used had different combinations and concentration of inorganic and organic salts and plant growth regulators. Among various media (Table 1) tried for anther culture, ACM1-5, ACM 8, ACM 10, ACM 11 and ACM 14 did not respond at all. Pre-culture on ACM1 and ACM2 did not show any difference in anther culture response. Thereafter the anthers were directly cultured on selected media. For genotype RH 406 and RH 555, maximum response (27.17±1.40 and 21.51±1.29 respectively) was recorded on ACM 15. RH 749 and RH 832 showed maximum response on medium ACM16 (i.e. 25.09±1.12 and 28.20±0.21respectively). Other responsive media were ACM 6, ACM 7, ACM 9, ACM 12, ACM 13, ACM 15 and ACM 16.

Out of 16 media combinations tried, highest percent responsive anthers (2.80 ± 0.34) were observed on medium ACM16 in F_1 (OR x RH 832) and lowest percent responsive anthers were recorded on medium ACM9 (6.30±3.20) in OR x RH 406. Highest callusing anthers (Plate F) were observed in OR x RH 749 (20.37±1.44) and highest per cent embryogenic calli (Plate G) (20.35±2.68) were obtained in OR x RH 749 on medium ACM15 while lowest no. of anthers (%) showing callusing were observed on medium ACM6 (4.58 ± 2.29) in OR x RH 832 (Table 4). Anthers were unresponsive to media ACM1, 2, 3, 4, 5, 8, 10, 11 and 14 and showed no callusing or embryogenesis (Table 4 and 5) on these media.

Minimum no. of days (25.01 ± 1.73) to response was recorded in hybrid OR x RH 406 on medium ACM15 medium while maximum days (33.80 ± 1.28) to response were observed on medium ACM9 in OR x RH 406 (Table 3).

In present investigation, highest no. of shoots per explant (Plate j) in OR x RH 406 was recorded on medium SI1 medium (MS + 30 g/l sucrose + 2.5 mg/l Kn + 0.2 mg/l NAA). In OR x RH 555 maximum response in terms of no. of shoots per explant (7.54±0.81) was observed on SI2 medium (MS + 30 g/l sucrose + 1.0 mg/l BAP). Maximum no. of shoots per explant in OR x RH 749 (10.50±0.81) and OR x RH 832 (9.49±0.83) were recorded on SI1 medium (Table 6).



A) Buds in field, (B) Collected buds (C)Anthers placed on media (D) Cultured anther after two weeks. (E) Cultured anther after 3 weeks

ISSN: 2395-3470
www.ijseas.com

Table 8: Response of anthers (%) in four hybrids of *B. juncea* cultured on different media combinations

S. No.	Media code	Explant response (%)	Days to response	Explant response (%)	Days to response	Explant response (%)	Days to response	Explants response (%)	Days to response
		RH 406		**RH 555**		**RH 749**		**RH 832**	
1	ACM6	0	0	0	0	0	0	10.04 ± 0.42	28.04 ± 0.22
2	ACM7	0	0	10.04 ± 0.42	31.58 ± 0.25	0	0	0	0
3	ACM9	9.30 ±1.08	42.80 ± 1.5	18.42± 1.23	31.79 ± 0.21	0	0	16.23±0.43	32.16 ± 0.11
4	ACM12	05.03± 1.23	23.03± 1.23	0	0	0	0	13.21±1.20	31.13 ± 1.15
5	ACM13	0	0	14.26±1.4	34.12 ± 1.13	4.01± 1.22	33.21± 2.11	0	0
6	ACM15	27.17±1.40	29.04 ± 1.04	21.51±1.29	33.42 ± 2.43	14.45±1.40	33.12 ± 2.14	22.13±0.24	36.70 ± 3.11
7	ACM16	20.27±0.31	36.25 ± 2.37	18.18±1.22	35.40 ± 2.44	25.09±1.12	41.46 ± 2.11	28.20±0.21	36.43 ± 2.30
		OR x RH 406		**OR x RH 555**		**OR x RH 749**		**OR x RH 832**	
1	ACM6	0	0	0	0	0	0	7.94±0.76	25.32 ± 0.97
2	ACM7	0	0	9.05±4.64	25.58 ± 0.65	0	0	0	0
3	ACM9	6.30±3.20	33.80 ± 1.28	11.08±1.07	26.79 ± 0.48	0	0	12.04±0.33	27.26 ± 0.41
4	ACM12	0	0	0	0	0	0	6.91±3.69	26.25 ± 3.37
5	ACM13	0	0	10.66±5.92	25.24 ± 1.93	0	0	0	0
6	ACM15	17.87±1.30	25.01 ± 1.73	16.91±1.89	29.47 ± 3.73	9.37±1.40	29.75 ± 3.34	17.47±0.84	29.00 ± 3.93
7	ACM16	14.97±0.62	28.85 ± 3.37	13.18±1.82	32.40 ± 4.74	20.08±1.32	32.76 ± 6.17	20.80±0.34	31.53 ± 5.90

- ACM1-5, 8, 10, 11 and 14 did not respond.

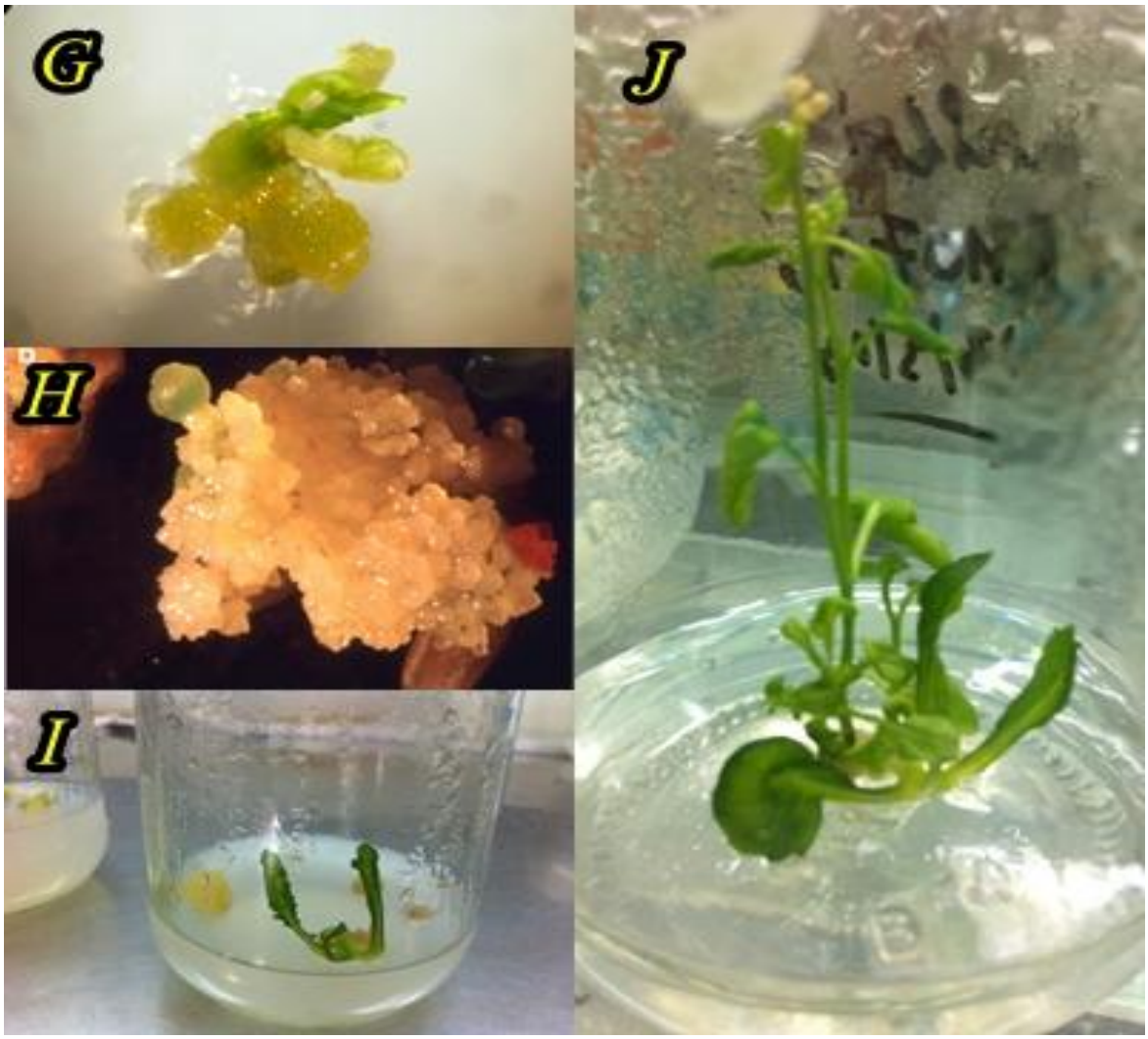
Table 9: Callus response of anthers (%) of four hybrids of *B. juncea* cultured on different media combinations

S. No.	Media code	Callus response (%)			
		OR x RH 406	OR x RH 555	OR x RH 749	OR x RH 832
1	ACM1	0	0	0	0
2	ACM2	0	0	0	0
3	ACM3	0	0	0	0
4	ACM4	0	0	0	0
5	ACM5	0	0	0	0
6	ACM6	0	0	0	4.65±2.33
7	ACM7	0	7.18±3.87	0	0
8	ACM8	0	0	0	0
9	ACM9	0	8.71±0.33	0	7.33±3.69
10	ACM10	0	0	0	0
11	ACM11	0	0	0	0
12	ACM12	0	6.96±3.73	0	6.91±3.69
13	ACM13	0	0	0	0
14	ACM14	0	0	0	0
15	ACM15	13.09±1.07	12.93±2.93	20.37±1.44	13.91±1.43
16	ACM16	11.19±1.29	10.32±1.24	16.00±1.03	15.47±1.24

Table 10: Embryogenic response of anthers (%) of four hybrids of *B. juncea* cultured on different media combinations

S. No.	Media code	Embryogenic response (%)			
		OR x RH 406	OR x RH 555	OR x RH 749	OR x RH 832
1	ACM1	0	0	0	0
2	ACM2	0	0	0	0
3	ACM3	0	0	0	0
4	ACM4	0	0	0	0
5	ACM5	0	0	0	0
6	ACM6	0	0	0	4.58±2.29
7	ACM7	0	5.30±2.66	0	0
8	ACM8	0	0	0	0
9	ACM9	0	5.68±2.85	0	6.36±3.43
10	ACM10	0	0	0	0
11	ACM11	0	0	0	0
12	ACM12	0	7.94±4.53	0	5.67±2.84
13	ACM13	0	0	0	0
14	ACM14	0	0	0	0

15	ACM15	11.52±2.38	10.19±0.67	20.35±2.68	10.19±0.63
16	ACM16	9.66±0.55	4.91±2.67	11.69±1.41	13.34±1.28



G) Androgenesis on cultured anther (H) Embryogenic anther (I) Subcultured explant to fresh media. (j) Regenerated plan

Table 6: Effect of modified MS media on no. of shoots per explant in cultured anthers of *B. juncea* hybrids

S. No.	Media code	OR x RH 406	OR x RH 555	OR x RH 749	OR x RH 832
1.	SI1	5.91 ± 1.68	6.22±0.48	10.50±0.81	9.49±0.83
2.	SI2	4.77±2.06	7.54±0.81	10.13±0.94	8.39± 0.78

Effect of silver nitrate: In present investigation, effect of silver nitrate was studied using B5 media with combinations of growth regulators and silver nitrate (10 mg / l and 20 mg / l). Better response of anther culture was observed on B5 medium supplemented with 20 mg / l silver nitrate as compared to that on B5 medium supplemented with 10 mg / l silver nitrate. Better response of hybrids OR x RH 749 (20.08 ± 1.32) and OR x RH 832 (20.80 ± 0.34) in anther culture was observed on B5 medium supplemented with 20 mg / l (ACM16) silver nitrate as compared to that of hybrids OR x RH 555 (9.37 ± 1.40) and OR x RH 832 (17.47 ± 0.84) on medium supplemented with 10 mg / l silver nitrate (Table 8). These consequences are in close conformity with Malik *et al.* (2001) and Prem *et al.* (2005).

Different media with different combinations of growth regulators were tried for root induction. Since no rooting was observed in shoots developed from anther culture of F_1 hybrids more efforts are needed to critically investigate the rooting conditions with the aim of breaking existing barriers to generate haploid plants and developing successful and better androgenesis.

REFERENCES

Al-Shehbaz, L.A., Beilstein, M.A. and Kellogg, E. A. (2006) Systematics and phylogeny of the *Brassica*ceae (Cruciferae): an overview. *Pl. Syst. Evol,* **259,** 89-130.

Baillie, A.M.R., Epp, D.J., Hutcheson, D. and Keller, W.A. (1992) In *vitro* culture of isolated microspores and regeneration of plants in *Brassica campestris*. *Plant Cell Report,* **11**, 234-237.

Chu, C.C. (1978) The N6 medium and its applications to anther culture of cereal crops. In*: Proc. Symp. on Plant Tissue Cult,* 25-30.

Gamborg, O.L., Miller, R.A. and Ojima, K (1968) Nutrient requirements of suspension cultures of soybean root cells. *Experimental Cell Research,* **50**, 151-58.

Gupta, S.K. (2009) Microsporogenesis and haploidy breeding. *Biological and breeding of crucifers*, 239-307.

Henderson, C.A.P. and Pauls, K.P. (1992) The use of haploidy to develop plants that express several recessive traits using light seeded Canola (*Brassica napus*) as an example. *Theoretical and Applied Genetics*, **83**, 476-479.

Kott, L.S., Polsoni, L. and Beversdorf, W.I. (1988a) Cytological aspects of isolated microspore culture of *Brassica napus. Canadian Journal of Botany,* **66**, 1658-1664.

Malik, M.R., Rangaswamy, N.S. and Shivanna, K.R. (2001) Induction of microspore embryos in a CMS line of B. *juncea* and formation of the androgenic plantlets. *Euphytica,* **120,** 195-203.

Mekonnen, T. W., Wakjira, A. and Genet, T. (2014) Correlation and path coefficient analysis among yield component traits of Ethiopian mustard (*Brassica carinata* a. brun) at Adet, northwestern, Ethiopia. *J. of Plant Sci,* **2(2),** 89-96.

Mishra, V. K. and Goswami, R. (2014) Haploid Production in Higher Plant. *IJCBS Review Paper* 1(1), [ISSN: 2349–2724]

Murashige, T. and Skoog, F. (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Plant Physiology,* **15**, 473-497.

Nitsch, P. and Nitsch, C. (1969) Haploid plants from pollen grains. *Science,* **163**, 85-87.

Ogura, H. (1968) Studies on the new male sterility in Japanese radish, with special reference to the utilization of this sterility towards the practical raising of hybrid seeds. *Mem Fac Agri Kagoshima Univ*. **6,** 39-78.

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.

Panse, V. G. and Sukhatme, P. V. (1978) Statistical Methods for Agricultural Workers. 3rd edn., ICAR, New Delhi, p.347

Pechan, P.M., and Keller, W.A. (1988) ldentification of potentially embryogenic microspores in *Brassica* napus. *physiologia plantarum,* **74**, 377-384.

Pechan, P.M., Bartels, D., Brown, D.C.W. and Schell, J. (1991) Messenger RNA and protein changes associated milk induction of *Brassica* microspore embryogenesis. *Planta,* **184,** 161-65.

Prem, D., Gupta, K. and Agnihotri, A. (2005) The effect of various exogenous and endogenous factors on microspore embryogenesis in Indian Mustard (*B. juncea*). *In Vitro cellular and development biology-Plant,* **41(3)**, 266- 273.

Reinert, J. and Bajaj, Y.P.S. (1977) Anther culture: haploid production and its significance. In: Reinert J, Bajaj YPS (eds) Applied and fundamental aspects of plant cell, tissue and organ culture. *Springer, pp* 251–267, Berlin.

Singh, D. (2014) Genetic enhancement of mustard for seed yield & its sustainability. *J. of Oilseed Brassica,* **5 (2),** 180-191.

U, N. (1935) Genome analysis in Brassica with special reference to the experimental formation of B. napus and peculiar mode of fertilization. *Jap. J. Bot*, 7, 389–452

Warwick, S.I. (1993) Guide to the wild germplasm of *Brassica* and allied crops: Part I – Taxonomy and genome status in the tribe *Brassicae* (*Cruciferae*). *Agriculture Canada Research Branch Technical Bulletin,* 1993-14E.

Warwick, S.I. Francis, A. and Al-Shehbaz, I.A. (2006) Brassicaceae: species checklist and database on CD-Rom. *Plant Syst. Evol,* **259**, 249-258.

Yamagishi, H. and Bhat, S. R. (2014) Cytoplasmic male sterility in Brassicaceae crops. *Breeding Science,* **64**, 38–47.