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Studies on days to calli appearance in Ethiopian mustard, *Brassica carinata*

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ABSTRACT

The effects of seven genotypes and their cross combinations, two basal media *i.e.*, B5 and MS media, two different sucrose concentrations *i.e.*, 3% and 4% sucrose and three combinations of hormones *viz*. HM_1, HM_2 and HM_3 and their interactions on days to calli appearance in *Brassica carinata* were analyzed by using CPCS software. Analysis of variance revealed that, out of four factors, only genotypes had significant effect on days to calli appearance. Nine out of eleven interactions *viz*., genotypes x hormones, genotypes x media, hormones x media, genotypes x hormones x media, hormones x sucrose, genotypes x hormones x sucrose, genotypes x media x sucrose, hormones x media x sucrose and genotypes x hormones x media x sucrose showed significant effect on days to calli appearance.

KEY WORDS: B_5, DAYS TO CALLI APPEARANCE, HORMONES, MS, SUCROSE

INTRODUCTION

Oilseed crops are the backbone of Indian agricultural economy and occupy an important position in daily diet, being a rich source of fats and vitamins. India is the second largest rapeseed-mustard growing country and accounts for 21.7% area in the world after China. Among oilseeds, rapeseed-mustard is the second most important oilseed crop of the country after groundnut and plays a significant role in Indian oil economy by contributing about 28.6% to the total oilseed production (Shekhawat *et al*., 2014). Rapeseed-mustard is the third important oilseed crop in the world after soybean (*Glycine max*) and palm (*Elaeis guineensis* Jacq.). The crop occupies an area of 33.58 million ha with a total annual production of 67.76 million tonnes and productivity 2018 kg/ha. In production, India ranks third after China (22.9%) and Canada (19.7%). The global production of rapeseed-mustard oil is around 12-14 million tonnes. In India, the crop occupies an area of 6.50 million ha with a total production of 8.02 million tonnes and productivity of 1262 kg/ha (Priyamedha *et al*., 2017).

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Over the last decades, researchers have made great efforts in developing biotechnology methods to facilitate the breeding of *Brassicas*. Research studies indicated that the modern biotechnology will have a major impact in two areas. Firstly, it provides a new range of techniques enabling the efficient selection of favourable variants in plant breeding programmes. Secondly, it provides the opportunity to improve germplasm by increasing its diversity beyond conventional genetic limitations. Due to the relative ease of genetic transformation, *Brassica* oilseed crops have been amongst the first subject to study the full range of modern biotechnology methods (Abraha *et al*., 2008).

Conventional methods for breeding crop plants require more than six to seven years of continuous efforts to get true breeding lines after following hybridization approach, a time consuming process (Morrison and Evans, 1988). Hence, biotechnological tools including anther culture, hold a great promise in accelerating the pace of breeding programme (Guha and Maheshwari, 1964). In vitro technique of anther culture helps to achieve homozygosity very quickly (Snape, 1989). Anther culture of potential F_1 generation genotypes can be used to facilitate regeneration of stable recombinant inbreds in one to two years thereby saving time and resources for their further use directly as commercial cultivars and/or in structural and functional genomics. The object of this study was to investigate the response of different genotypes and their cross combinations for days to calli appearance.

MATERIALS AND METHODS

The anther culture work was carried out in the Molecular Cytogenetics and Tissue culture Laboratory of Department of Crop Improvement, CSK HPKV, Palampur during Rabi 2010-11. The material used and methodology adopted to achieve the objectives of the investigation are given below.The material used for anther culture studies comprised of four elite genotypes and their three cross combinations (Table 1). Sufficient numbers of plants of aforementioned four genotypes and their cross combinations were raised in the pots. In order to have availability of anthers over a long period of time, plants were raised in five lots at an interval of 15 days each. For anther culture, florets from plants were clipped off when the size of bud was about 2-4 mm. The bud size was earlier established on the basis of presence of majority of the microspores at late uninuclate to early binucleate stage as studied by squashing of anthers in a drop of 1% acetocarmine. The florets of appropriate size were collected in 50 ml test tubes containing distilled water.

The florets collected at aforementioned stages were treated with 70% ethanol for 10-15 seconds under aseptic conditions in a laminar air flow chamber. The florets were then surface sterilized with 0.1% $HgCl_2$ for 3-5 minutes with intermittent shaking followed by three washings with sterile distilled water. Florets were blot dried and opened under aseptic conditions with the help of sterile forceps and the six anthers were clipped off from each floret without damaging the anther wall. About 60 anthers were cultured in each pre-sterilized petri plate containing about 25 ml of culture medium. Two basal media *viz*. B_5 (Gamborg *et al*. 1968) and MS (Murashige and Skoog 1962) were used for callus induction. Each of these medium was supplemented with two different sucrose concentrations *i.e*., 3% and 4% sucrose and each of these sucrose concentrated media was also supplemented with three combinations of hormones *viz*. HM_1, HM_2 and HM_3 (Table 2). All the media were supplemented with 0.8% agar.The experiments on different callus induction media were replicated thrice involving different media and plant growth hormones. Anthers of all four genotypes and their crosses were plated in a replicated fashion. If there was any contamination, replating of the particular treatment was done to complete the experiment under uniform conditions. All the cultured plates were sealed with parafilm wax and kept under dark at 25 ± 1°C until calli were developed. The Days to calli appearance was calculated as follows: Days to calli appearance = Number of days taken for calli appearance

Table 1. List of genotypes and their cross combinations under anther culture study

Sr. No	Genotype	Parentage
1	Jayanti	Developed through irradiation from the parent variety HC-1
2	P-18	Advanced generation mutant obtained through treatment of Jayanti seeds with 0.3% EMS (Pre-Soaked)
3	P-51	Advanced generation mutant obtained through treatment of Jayanti seeds with 0.3% EMS (Pre-Soaked)
4	$P_{(2)2}$	Advanced generation mutant obtained through treatment of Jayanti seeds with 90 kR dose of gamma radiations
5	Jayanti X P-18	-
6	Jayanti X P-51	-
7	Jayanti X $P_{(2)2}$	-

Table 2. Different media, hormones and sucrose concentration used for calli index

Medium	Sucrose Conc.	Hormone	
		Designation	Name and Concentration
B_5	3%	HM_1	NAA (1.0 mg/l)
B_5	3%	HM_2	BAP (2.0 mg/l) + NAA (2.0 mg/l)
B_5	3%	HM_3	2, 4-D (0.5 mg/l) + NAA (1.0 mg/l)
B_5	4%	HM_1	NAA (1.0 mg/l)
B_5	4%	HM_2	BAP (2.0 mg/l) + NAA (2.0 mg/l)
B_5	4%	HM_3	2, 4-D (0.5 mg/l) + NAA (1.0 mg/l)
MS	3%	HM_1	NAA (1.0 mg/l)
MS	3%	HM_2	BAP (2.0 mg/l) + NAA (2.0 mg/l)
MS	3%	HM_3	2, 4-D (0.5 mg/l) + NAA (1.0 mg/l)
MS	4%	HM_1	NAA (1.0 mg/l)
MS	4%	HM_2	BAP (2.0 mg/l) + NAA (2.0 mg/l)
MS	4%	HM_3	2, 4-D (0.5 mg/l) + NAA (1.0 mg/l)

from the day of culturing of anthers. Data on days to calli appearance were analyzed in Factorial Completely Randomized Design (CRD) to obtain the effect of various treatments and their interactions using statistical CPCS software.

RESULTS AND DISCUSSION

Analysis of variance for days to calli appearance involving different parameters is presented in Table 3. Out of four factors, only genotypes had significant effect on

Table 3. ANOVA for days to calli appearance in different genotypes of *Brassica carinata* and their hybrids involving different media, hormones and sucrose concentration

Source of variation	df	Mean Squares	CD (5%)	CV (%)
Genotypes	6	28.23**	0.47	11.00
Hormones	2	1.38	NS	
Genotypes x Hormones	12	5.17**	0.82	
Media	1	0.25	NS	
Genotypes x Media	6	4.67**	0.67	
Hormones x Media	2	21.60**	0.44	
Genotypes x Hormones x Media	12	5.04**	1.16	
Sucrose	1	0.25	NS	
Genotypes x Sucrose	6	0.93	NS	
Hormones x Sucrose	2	63.65**	0.44	
Genotypes x Hormones x Sucrose	12	5.21**	1.16	
Media x Sucrose	1	1.02	NS	
Genotypes x Media x Sucrose	6	12.06**	0.94	
Hormones x Media x Sucrose	2	113.34**	0.62	
Genotypes x Hormones x Media x Sucrose	12	7.19**	1.64	
Error	168	1.02		

**Significant at $P \leq 0.01$

Table 4.1. Effects of media and genotypes on days to calli appearance

Media	Genotypes								
	Jayanti	$P_{(2)2}$	P-51	P-18	Jayanti x $P_{(2)2}$	Jayanti x P-51	Jayanti X P-18	Mean	CD (P≤0.05)
MS	10.50	8.39	9.39	8.44	8.39	9.50	8.44	9.01	NS (Media)
B_5	11.44	11.06	8.56	8.83	8.44	8.89	8.83	9.44	
Mean	10.97	9.72	8.97	8.64	8.42	9.19	8.64		

CD (P≤0.05) = 0.47 (Genotypes)
CD interaction = 0.67 (Genotypes x Media)

Table 4.2. Effects of hormones and genotypes on days to calli appearance

Hormonal combination	Genotypes								
	Jayanti	$P_{(2)2}$	P-51	P-18	Jayanti x $P_{(2)2}$	Jayanti x P-51	Jayanti x P-18	Mean	CD (P≤0.05)
HM_1	13.08	9.75	10.00	10.75	9.33	10.50	10.75	10.60	NS (Hormones)
HM_2	9.67	9.42	7.17	6.67	7.25	7.08	6.67	7.70	
HM_3	10.17	10.00	7.17	8.50	8.67	10.00	8.50	9.00	
Mean	10.97	9.72	8.11	8.64	8.42	9.19	8.64		

CD (P≤0.05) = 0.47 (Genotypes)
CD interaction= 0.82 (Genotypes x Hormones)

Table 4.3. Effects of sucrose and genotypes on days to calli appearance

Sucrose	Genotypes								
	Jayanti	$P_{(2)2}$	P-51	P-18	Jayanti x $P_{(2)2}$	Jayanti x P-51	Jayanti x P-18	Mean	CD (P≤0.05)
3 %	10.17	10.39	8.72	8.56	8.78	9.00	8.56	9.17	NS (Sucrose)
4 %	11.78	9.06	9.22	8.72	8.06	9.39	8.72	9.28	
Mean	10.97	9.72	8.97	8.64	8.42	9.19	8.64		

CD (P≤0.05) = 0.47 (Genotypes)
CD interaction = NS (Genotypes x Sucrose)

days to calli appearance. Nine out of eleven interactions *viz*., genotypes x hormones, genotypes x media, hormones x media, genotypes x hormones x media, hormones x sucrose, genotypes x hormones x sucrose, genotypes x media x sucrose, hormones x media x sucrose and genotypes x hormones x media x sucrose showed significant effect on days to calli appearance. From the Tables 4.1, 4.2 and 4.3, it is pertinent that the effects of media, hormones and sucrose were found to be non-significant on all seven genotypes which indicated that different genotypes behaved similar in different media, hormonal combinations and sucrose concentrations for days to calli appearance. However, the genotype Jayanti x $P_{(2)2}$ recorded lowest days to calli appearance on different media and sucrose concentrations. Likewise, the genotype P-51 took lowest days to calli appearance on different hormonal combinations. The effects of sucrose and hormones, hormones and media and media and sucrose on days to calli appearance are presented in Tables 5.1, 5.2 and 5.3, respectively. The results revealed that the effects of sucrose and hormones, hormones and

Table 5.1. Effects of sucrose and hormones on days to calli appearance

Sucrose	Hormonal Combination				
	HM_1	HM_2	HM_3	Mean	CD (P≤0.05)
3 %	10.88	7.67	8.95	9.17	NS (Sucrose)
4 %	10.31	7.74	9.79	9.28	
Mean	10.60	7.70	9.37		

CD (P≤0.05) = NS (Hormones)
CD interaction = 0.44 (Hormones x Sucrose)

Table 5.2. Effects of hormones and media on days to calli appearance

Hormonal combination	Callusing Media			
	MS	B_5	Mean	CD (P≤0.05)
HM_1	10.05	10.95	10.50	NS (Hormones)
HM_2	7.40	8.00	7.70	
HM_3	9.38	9.36	9.37	
Mean	8.94	9.44		
CD (P≤0.05) = NS (Media) CD interaction = 0.44 (Media x Hormones)				

Table 5.3. Effect of media and sucrose on days to calli appearance

Media	Sucrose			
	3%	4%	Mean	CD (P≤0.05)
MS	8.98	9.03	9.01	NS (Media)
B_5	9.35	9.52	9.44	
Mean	9.17	9.28		
CD (P≤0.05) = NS (Sucrose) CD interaction = NS (Sucrose x Media)				

media and media and sucrose were found to be non-significant which indicated that days to calli appearance were not affected significantly by different media, hormonal combinations and sucrose concentrations.

CONCLUSION

The factors such as media, hormones, sucrose and their interactions *viz.,* genotypes x sucrose and media x sucrose had non-significant effects on days to calli appearance which indicated that the genotypes behaved similar in different media, hormonal combinations and sucrose concentrations.

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REFERENCES

Abraha E, Bechyne M, Klima M, Vyvadilova M. (2008). Analysis of factors affecting embryogenesis in microspore cultures of *Brassica carinata*. Agricultura Tropica et Subtropica 41: 2.

Gamborg OL, Millar RA, Ojima L. (1968). Nutrient requirements of suspension cultures of soybean root cells. Exp Cell Res 50: 151-158.

Guha S, Maheshwari SC. (1964). In vitro production of embryos from anthers of *Datura*. Nature 204: 497.

Morrison RA, Evans DA. (1988). Haploid plants from tissue culture: new plants varieties in a shortened time frame. Biotechnology 6: 684-690.

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

Priyamedha, Kumar A, Haider ZA. (2017): Stability for seed yield and component traits in Indian mustard (*Brassica juncea* L.) under Jharkhand condition. J Oilseed Brassica 8(1): 37-42.

Shekhawat N, Jadeja G.C, Singh J, Ramesh (2014). Genetic diversity analysis in relation to seed yield and its component traits in Indian mustard (*Brassica juncea* L. Czern & Coss). Int Q J Life Sci. 9: 713-717.

Snape JW. (1989) In: Review of Advance in Plant Biotechnology. International Maize and Wheat Improvement Center, Mexico/ International Rice Research Institute. Pp 19-30 (Ed) Mujeeb-Kazi A, Sitch LA, Doubled haploid breeding: theoretical basis and practical applications, Manila, Philippines.