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Original Article

ANDROGENESIS-MEDIATED RESPONSE OF DIFFERENT GENOTYPES AND THEIR CROSS COMBINATIONS FOR CALLI INDEX IN ETHIOPIAN MUSTARD (*BRASSICA CARINATA* A. BRAUN)

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Abstract: ***The effects of seven genotypes and their cross combinations, two basal media i.e., B_5 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 calli index in Brassica carinata were analyzed by using CPCS software. Mean sum of squares due to all factors were significant revealing thereby significant effects of genotypes, media, hormones, sucrose and their interactions on calli index. Out of all factors and their interactions, the genotype P-51 performed better in B_5 medium supplemented with HM_2 (0.2mg/l BAP + 2.0 mg/l NAA) and 3% sucrose concentration for high calli index.***

Key words: *Brassica carinata* , Calli index

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 [1].

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 6.80 million tonnes and productivity of 1046 kg/ha [2].

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 [3].

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 [4]. Hence, biotechnological tools including anther culture, hold a great promise in accelerating the pace of breeding programme [5]. In vitro technique of anther culture helps to achieve homozygosity very quickly [6]. 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 androgenesis-mediated response of different genotypes and their cross combinations.

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.

Experimental material: The material used for anther culture studies comprised of four elite genotypes and their three cross combinations (Table 1).

Plant material for anther culture: 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.

Stage of explants: 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 micros-pores 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.

Plating of anthers in callus induction media: 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 [7] and MS [8] 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.

Statistical analysis: The Calli index was calculated as follows: Calli index = Growth score (G) X % callus induction frequency, Where, G = average weight of callus rating on explant, and

$$\% \text{ callus induction frequency} = \frac{\text{Number of calli forming anthers}}{\text{Number of anthers plated}} \times 100$$

Data analysis: Data on calli index 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

Effects of different parameters on calli index: Analysis of variance for calli index in anthers of seven genotypes cultured in vitro on two media supplemented with three hormonal combinations and two different sucrose concentrations, is presented in Table 3. Mean sum of squares due to all factors were significant revealing thereby significant effects of genotypes, hormones, media, sucrose and their interactions on calli index.

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)

Table 3: ANOVA for calli index in different genotypes of *Brassica carinata* and their hybrids involving different media, hormones and sucrose concentrations . *Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$

Sr. no.	Source of variation	df	Mean Squares	CD 5%)	CV (%)
1	Genotypes	6	233.54*	4.22	15.6
2	Hormones	2	2029.45**	2.76	
3	Genotypes X Hormones	12	1080.43**	7.30	
4	Media	1	16382.76**	2.25	
5	Genotypes X Media	6	1127.07**	5.96	
6	Hormones X Media	2	407.57**	3.90	
7	Genotypes X Hormones X Media	12	434.24**	10.33	
8	Sucrose	1	2277.56**	2.25	
9	Genotypes X Sucrose	6	250.43**	5.96	
10	Hormones X Sucrose	2	3453.60**	3.90	
11	Genotypes X Hormones X Sucrose	12	446.90**	10.33	
12	Media X Sucrose	1	1669.55**	3.19	
13	Genotypes X Media X Sucrose	6	1552.63**	8.43	
14	Hormones X Media X Sucrose	2	25199.98**	5.52	
15	Genotypes X Hormones X Media X Sucrose	12	507.45**	14.61	
16	Error	168	81.23		

Table 4a: Effects of media and genotypes on calli index. CD ($\leq$ 0.05) = 4.22 (Genotypes);CD interaction= 5.96 (Genotypes X Media)

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	50.81	63.90	48.37	47.78	49.28	55.84	59.52	53.64	2.25 (Media)
B_5	59.68	50.31	76.64	66.91	60.93	60.60	59.03	62.01	
Mean	55.24	57.11	62.50	57.34	55.11	58.22	59.27		

Table 4b: Effects of hormones and genotypes on calli index. CD ($\leq$ 0.05) = 4.22 (Genotypes); CD interaction = 7.30 (Genotypes X Hormones)

Hormonal. Comb.	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	41.34	52.38	51.28	48.89	39.74	32.08	37.01	43.25	2.76 (Hormones)
HM_2	72.00	68.02	74.86	63.52	62.13	69.48	67.25	68.18	
HM_3	52.39	50.92	61.37	59.62	63.44	73.10	73.56	62.06	
Mean	55.24	57.11	62.50	57.34	55.11	58.22	59.27		

Table 4c: Effects of sucrose and genotypes on calli index. CD ($\leq$ 0.05) = 4.22 (Genotypes); CD interaction = 5.96 (Genotypes X Sucrose)

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%	55.98	55.97	64.86	58.94	56.08	58.62	59.23	58.52	2.25 (Sucrose)
4%	55.37	56.92	62.90	57.61	55.27	58.29	59.27	57.94	
Mean	55.68	56.44	63.88	58.27	55.67	58.45	59.25		

Table 5a: Effects of sucrose and hormones on calli index. CD ($\leq$ 0.05) = 2.76 (Hormone); CD interaction = 3.90 (Hormone X Sucrose)

Sucrose	Hormonal combination				
	HM_1	HM_2	HM_3	Mean	CD (P=0.05)
3%	10.88	7.67	8.95	9.17	2.25 (Sucrose)
4%	10.31	7.74	9.79	9.28	
Mean	10.60	7.70	9.37		

Table 5b: Effects of hormones and media on calli index. CD ($\leq$ 0.05) = 2.25 (Media); CD interaction = 3.90 (Media X Hormone)

Hormonal comb.	Callusing Media			
	MS	B_5	Mean	CD (P=0.05)
HM_1	30.86	55.63	43.25	2.76 (Hormone)
HM_2	70.12	66.24	68.18	
HM_3	59.95	64.17	62.06	
Mean	53.64	62.01		

Table 5c: Effects of media and sucrose on calli index. CD ($\leq$ 0.05) = 2.25 (Sucrose); CD interaction = 3.19 (Sucrose X Media)

Media	Sucrose			
	3%	4%	Mean	CD (P=0.05)
MS	54.35	52.94	53.64	2.25 (Media)
B_5	67.47	58.10	62.79	
Mean	60.91	55.52		

Effects of media and genotypes on calli Index: Out of the two media tested, the anthers plated on B_5 medium recorded significantly highest calli index (62.01) than MS medium (Table 4a). Out of the seven genotypes tested, P-51 recorded highest calli index (62.50) and was statistically at par with Jayanti X P-18. On the other hand Jayanti X $P_{(2)2}$ recorded least calli index (55.11). The calli index was also significantly affected by the genotype X media interaction. Best calli index of cultured anthers was recorded for P-51 on B_5 medium (76.64) followed by P-18 (66.91) and Jayanti X $P_{(2)2}$ (60.93). Overall, culturing anthers of genotype P-51 on B_5 medium exhibited significantly better calli index.

Effects of hormones and genotypes on calli Index: Out of three hormonal combinations tested, HM_2 gave significantly highest calli index (68.18) in comparison to HM_1 and HM_3 (Table 4b). Out of seven genotypes used for anther culture, P-51 gave highest calli index (62.50) followed by Jayanti X P-18 (59.27) being statistically at par with each other. Jayanti X $P_{(2)2}$ gave lowest calli index (55.11). The interaction genotypes X hormones had significant effect on calli index. Best calli index of cultured anthers was recorded for P-51 on HM_2 (74.86). Overall, culturing anthers of P-51 in HM_2 (0.2mg/l BAP + 2.0mg/l NAA) exhibited significantly better calli index.

Effects of sucrose and genotypes on calli Index: The data pertaining to effects of sucrose and genotypes on calli index is presented in Table 4c. Out of two different sucrose concentrations i.e., 3% and 4% sucrose tested, 3% sucrose gave highest calli index (58.52%) and was statistically at par with 4% sucrose. Out of the seven genotypes tested, P-51 recorded highest mean calli index (63.88). On the other hand Jayanti X $P_{(2)2}$ recorded least calli index (55.67). The calli index was also significantly affected by the sucrose X genotype interaction. Best calli index of cultured anthers was recorded for P-51 on 3% sucrose (64.86) followed by 4% sucrose concentrations (62.90). Overall, culturing anthers of genotype P-51 on 3% sucrose concentration exhibited better calli index.

Effects of sucrose and hormones on calli index: The perusal of data presented in Table 5a indicated that out of two different sucrose concentrations i.e., 3% and 4% sucrose tested, 4% sucrose gave highest calli index (9.28) than 3% sucrose and both were statistically at par with each other. Out of three hormonal combinations tested, HM_1 (1.0 mg/l NAA) gave highest calli index (10.60) and was statistically at par with HM_3. The interaction between two factors i.e., sucrose X hormones had significant effect on the calli index. Considering interaction, the highest calli index was observed in 3% sucrose supplemented with HM_1 (10.88) followed by 4% sucrose supplemented with HM_1 (10.31).

Effects of hormones and media on calli index: The data pertaining to effects of hormones and media on calli index is presented in Table 5b. Out of the three hormonal combinations used for anther culture, HM_2 gave significantly highest calli index (68.18) than HM_3 and HM_1. Out of two media tested, B_5 medium gave significantly highest calli index (62.01) in comparison to MS medium. The calli index was also significantly affected by media X hormones interaction. Significantly higher calli index of cultured anthers were recorded in MS medium supplemented with HM_2 (70.12) followed by B_5 medium also supplemented with HM_2 (66.24). Overall, B_5 medium supplemented with HM_2 (0.2mg/l BAP + 2.0mg/l NAA) was best for calli index.

Effects of media and sucrose on calli index: The perusal of data presented in Table 5c indicated that out of two media tested, B_5 gave highest calli index (62.79) and was found to significantly superior than MS medium. Out of two different sucrose concentrations, 3% sucrose gave highest calli index (60.91) and was found to significantly superior than 4% sucrose. The interaction between two factors i.e., media X sucrose had significant effect on the calli index. Considering interaction, the highest calli index was observed in B_5 medium supplemented with 3% sucrose (67.47).

In summary, the quality and calli index of cultured anthers ultimately depend on genotypes, culture medium, hormonal combination and sucrose concentration. Therefore, selection of efficient culture medium and culture conditions like B_5 medium supplemented with HM_2 (0.2mg/l BAP + 2.0 mg/l NAA) and 3% sucrose concentration and cultivation at 25 ± 1°C under dark condition for genotype P-51 would offer great promise for the higher calli index.

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