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Short Note

Response of corn genotypes to anther culture

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Development of inbred lines is an integral part of corn hybrid breeding programme. Corn or maize, a cross pollinated crop, is highly heterozygous and 6-7 generations of selfing or inbreeding are required to obtain the desired level of homozygosity in inbreds, which is a time taking and laborious process. Development of doubled haploid corn lines through anther culture (androgenesis) is a potentially time saving alternative to this conventional breeding approach. Through androgenesis, completely homozygous doubled haploids can be obtained in one generation (one year) by artificially doubling the haploid chromosome compliment of the regenerates. The homozygous lines produced by anther culture system have fixed genotypes so that continued selection for a certain gene combination is not necessary, i.e., the desired combination is either present or absent and will remain so upon further selfing, barring mutation. Anthers of corn were first successfully cultured in 1975 (Anonymous) and since then many reports describing anther studies have been published (Wan and Widholm, 1992). Like recombinant inbred line (RIL), doubled haploid (DH) population is also advantageous for gene mapping instead of F_2 or BC populations (Bentolila *et al.*, 1992). The chief advantage of DH population is that only one generation is required for its production instead of six generations that are normally required for the production of RIL population.

The present investigation was carried out in the Department of Plant Breeding & Genetics and the Advanced Centre of Hill Bioresources & Biotechnology, CSK HPKV, Palampur during 2005-06 to investigate the response of corn genotypes to anther culture. Seven maize genotypes comprising two local land races, three hybrids and two composites, *viz.*, (i) Chadhiar Local (Palampur), (ii) Palampur Local (Palampur), (iii) KH-2001 (hybrid) from Kanchan Ganga Seed Company, (iv) PMZ-4 (hybrid) and (v) Hishell (hybrid) from Monsanto Seed Company, (vi) Early composite and (vii) Girija composite from CSK HPKV, Palampur were evaluated for their response to anther culture. The tassels from the plants, grown in the farm (during Kharif) and poly/glass house (during Rabi) were harvested just before their emergence from the whorl when the microspores were in the mid-uninucleate stage of development. The excised tassels were covered with aluminium foil and stored at 7^0 C for one week. Anthers from the main branch and from the lateral branches of the tassel were used for making squashes and stained with acetocarmine. Microspores released from the anthers were observed under a light microscope. Microspores in the mid-uninucleate stage reached the late-uninucleate stage by the end of cold pretreatment. The tassels having anthers at late-uninucleate and premitotic stages were selected for *in vitro* culture. For the induction of microspores derived structures (calli / embryos), three basal media, *viz.*, Yu-Pei, N_6 and $6N_1$ supplemented with other organic additives (Table 1) and different hormones (Table 2) were used. The media were autoclaved for 20 minutes at 120^0C. Twenty five ml of the sterilized medium was distributed into petri dishes (60 mm in diameter). The whole procedure was carried out in a laminar air flow cabinet. Parts of the tassels, which contained late-uninucleate or premitotic microspores, were sterilized with 20% sodium hypochlorite for 30 minutes and then washed thrice with sterile double distilled water.

About 40 isolated anthers were inoculated on to the surface of the induction medium in each petri dish. Parafilm sealed anthers inoculated petri dishes were incubated at a constant temperature of 28 ± 1^0C in the dark for 8 weeks. Data on different components of anther culture response *viz.*, callusing frequency (%) and regenerating calli (%) were recorded on 7, 15, 30, 45, 60, 85 and 100 days after

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incubation. Based on the anther availability, 560 to 1400 anthers were inoculated in each hormone combination.

Table 1. Composition of callus induction media used in the study

Ingredients (mg/l)	Different media		
	Yu – Pei (Ku *et al.*, 1978)	N_6 (Chu, 1981)	$6N_1$ (Genovesi, 1990)
Major nutrients			
KNO_3	2500.00	2830.00	2022.00
$NHNO_3$	165.00	-	-
$(NH_4)_2SO_4$	-	463.00	-
$NH_4H_2PO_4$	-	-	384.00
$CaCl_2.2H_2O$	176.00	166.00	166.00
KH_2PO_4	510.00	400.00	-
$MgSO_2.7H_2O$	370.00	185.00	185.00
Minor nutrients			
$MnSO_4.4H_2O$	4.40	4.40	4.40
$ZnSO_4$	1.50	1.50	1.50
A_3BO_3	1.60	1.60	1.60
KI	0.80	0.80	0.80
Chelated iron			
Na_2EDTA	37.30	37.30	37.30
Fe_2SO_4	27.80	27.80	27.80
Vitamins			
Glycine	7.70	7.70	7.70
Thiamine-HCl	0.25	0.25	0.25
Pyridoxine	0.25	0.25	0.25
Nicotinic acid	1.30	1.30	1.30
Organic additives (g/litre)			
Sucrose	120.00	-	-
Casein hydrolysate	0.50	0.50	0.50
Activated charcoal	5.00	5.00	5.00
Agar	8.00	8.00	8.00
Proline	0.125, 0.25	0.125, 0.25	0.125, 0.25

The anthers were deep yellow in colour after 7 days of incubation and turned brown after 15 days. Similarly, after 30, 45, 60, 85 and 100 days of incubation the anthers were still brownish in colour and there was no callus induction in any of the hormonal-medium combinations.

Anther culture response in maize is highly genotype–dependent (Wan and Widholm, 1993). Miao *et al.* (1978) reported that only 9 of 159 genotypes (5-6%) were responsive to anther culture. Dieu and Beckert (1986) found 5 of 94 genotypes (5.3%) responsive from the germplasm obtained from Europe. Petolino and Jones (1986) found 12 of 39 maize genotypes (30.8%) responsive to anther culture.

The anther culture response is a complex character including callus or embryo induction, plantlet regeneration or plant development and probably requires specific components (Lashermes and Beckert, 1988). Vergne *et al.* (1993) observed a positive correlation between the rate of embryo formation *via* anther culture and the level of the protein 'MAR 32' in anthers during cold pretreatment of the tassel, and that this protein may be used as a marker to identify maize genotypes responsive to anther culture. Barret *et al.* (2004) identified four sequences including a new HSP 70 – like candidate gene which could be employed to understand the genetic control of *in vitro* androgenesis.

Table 2. Hormonal combinations and their concentrations used in anther culture

Hormones		Concentrations (mg/l)	
H_1	H_2	H_1	H_2
2,4-D	Kinetin	1.0	2.0
2,4-D	Kinetin	2.0	3.0
2,4-D	Kinetin	3.0	1.0
Kinetin	NAA	1.0	2.0
Kinetin	NAA	3.0	2.0
Kinetin	TIBA	3.0	0.1
Kinetin	TIBA	3.0	0.2
TIBA	2,4-D	0.1	2.0
TIBA	2,4-D	0.2	3.0
TIBA	NAA	0.1	1.0
Kinetin	–	1.0	–
Kinetin	–	3.0	–
TIBA	–	0.1	–
TIBA	–	0.2	–

From this study, it may be concluded that (i) a large number of corn genotypes need to be screened to identify responsive genotypes using a known genotype responsive to anther culture as a standard, (ii) anther culture response may be transmitted to elite germplasm from known responsive androgenic lines through hybridization and (iii) protocol used in the present study may be fine tuned and optimized further as far as culture conditions and other environmental factors are concerned.

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