

Enhanced Haploids Regeneration in Anther Culture of Summer Squash (*Cucurbita pepo* L.)

M.F. Mohamed and E. F. S. Refaei

Department of Horticulture, Faculty of Agriculture, Assiut University, Assiut 71526, Egypt.

Abstract: The best callus induction and proliferation and plant regeneration responses in anther cultures of zucchini type summer squash cv. 'Eskandarani' were obtained when growing the donor plants during summer on 1st of March and during winter on 1st of November. However, the cultures of anthers prepared from donor plants grown during the winter season greatly enhanced callus induction and plant regeneration when compared with those of the summer season. About 60% of the regenerants were haploid ($2n = X = 20$). We concluded that growing conditions of donor plants are influential factor for optimizing the production of haploids via androgenesis in summer squash cv "Eskandrani".

Introduction: Production of double haploid plants has been studied in several vegetable species of the cucurbitaceae family [2, 4, 8]. One of the prominent key points in the induction of the androgenesis is the condition of the donor plants as influenced by the surrounding culture environment especially light and temperature [1, 5, 10]. However, no information is available on this factor as affecting the in vitro androgenesis of zucchini type summer squash. Such information would be important in optimizing tissue culture protocols of androgenesis and enhancing their repeatability and thereby the application towards the improvement of this species. The objective of the present study was, therefore, to investigate the anther culture responses of the summer squash to different planting seasons and dates for the donor plants.

Materials and Methods: Seeds of the summer squash cv 'Eskandrani' were sown

during the summer and the winter growing seasons in Malawy Experimental Research Station (27° 18' N latitude), El-Minia Governorate, Egypt. Sowing dates were on the first of March, April, and May during the summer season and on the first of September, October, and November during the winter season. Separate experiments were conducted for these planting dates in randomized complete-blocks with four replicates. Maximum, minimum, average day and average night temperatures along with relative humidity are presented in Table (1). Male flower buds were collected when they were about 9 to 10 mm and exposed to cold pretreatment at 4° C for 4 days. After surface disinfections of the buds, anthers were excised and placed on MS medium [9] containing 100g/l sucrose and 6 mg/l 2,4-D [8]. The culture was in 100 ml Erlenmeyer flasks containing 30 ml agar-solidified medium (8g/l). The anther cultures were kept in the dark at 35°C for a week followed by 4 weeks at 25°C. The induced callus was subcultured on the same medium for additional 4 weeks. The weight and diameter of the proliferated callus were determined. The proliferated callus was transferred onto MS medium with 0.05 mg/l of both kinetin and α -naphthalene acetic acid (NAA) and incubated under cool white light (16/8, day/night) at 25°C for 4 weeks.

The differentiated callus cultures were subcultured onto the medium without plant growth regulators (PGR) for 4 weeks. The number of the developed plantlets was recorded and expressed as per 100 anther explant cultures. The developed shoots and plantlets were cultured in the medium containing 1 mg indole-3-butyric acid

(IBA). Samples of root tips were used to examine the number of chromosomes in the regenerated plants following the procedure described by Darlington and Lacour [3]. The defined haploid plantlets were immersed in 1% aqueous colchicine solution for 2 h [2]. Shoot-tips from these plantlets were transferred into flasks containing PGR-free medium. Double haploid plantlets, with sufficiently developed roots, were hardened and were then transplanted into pots containing sterilized peat moss. The pots were covered with clear plastic bags for one week. The plants were grown in the soil and observed for the growth and the fruit and seed set. The data were subjected to the combined analysis of variance over years, growing season and the treatments of planting dates. Means were separated with the "Least Significance Difference" (LSD) and additional selected orthogonal comparisons were also tested.

Results and Discussion: Greater weight and larger sized callus were obtained when the anther explants were prepared from plants sown earlier in the summer season as on March than those that were sown later especially on May (Table 2A). The squash plants sown late in the winter season (November) gave anthers producing enhanced callus weight and diameter comparing with the early planting in October and September (Table 2B). The number of the regenerated plants per 100 anthers exhibited similar response to the planting dates of the donor plants in the summer and the winter seasons. Therefore, significant positively correlated existed between the number of the regenerated plants per 100 anthers and the both of the callus weight ($r = 0.973 **$) and the callus diameter ($r = 0.978 **$). The percentage of the abnormal regenerated plants did not differ within each of the summer (Table 2A) and winter (Table 2B) planting dates.

Comparison of the average overall the winter vs. the summer revealed a significant difference towards enhanced callus weight,

diameter and the number of the regenerated plants per 100 anthers in the winter season. The percentage of the abnormal regenerated plants significantly reduced in the cultures of anthers prepared from plants grown in the winter season. The number of regenerated plants from anther-derived callus of the squash sown in November was more than twice as those planted in March. Heberle-Bors [5] indicated that short-day (8 h) and relatively low temperatures (15-18° C) were preferable for growing the anther donor *Nicotiana tabacum* plants. In a study conducted by Yong et al. [10], the explants from cauliflower plants grown in the winter and the spring were more responsive than those grown in the summer or early autumn. Furthermore, Matsubara et al. [7] reported that the best differentiation of the anther-derived embryoids of pepper was from plants grown during September and October (15 to 25° C). The enhanced responses of the tissue culture towards plant differentiation could be attributed to the slow maturity and synchronized development of the microspores and thus prolonging their responsive stage [1]. Also, surrounding environment of the donor plants could affect the indigenous hormonal level [6] and thereby the conditioning of the microspores towards stimulating their responses in tissue cultures. Our cytological observation indicated that about 60% of the regenerated plants were haploids ($2n = X = 20$) while 23% were diploid ($2n = 2X = 40$) and 17% were aneuploid ($2n = X = 21-39$). Spontaneous doubling was also observed by Dryanovska [4] occurring for the regenerants in anther culture of cucurbitaceae members. Dihaploid plants obtained by colchicine treatment developed normal fruit and seed set.

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Table 1. Maximum, minimum and average day and night temperatures along with the relative humidity recorded during the months of the summer (A) and the winter (B) growing season of the anther-explant donor summer squash (cv Eskandrani), Malawy Metreological Station, Egypt.^z

Months	Temperature (C)				Relative Humidity
	Maximum	Minimum	Average. Day	Average Night	
(A)					
March	26.4	8.4	22.4	15.4	75.4
April	36.2	15.6	30.3	20.2	68.3
May	36.6	18.4	30.2	20.5	68.6
June	35.2	20.3	32.3	23.4	65.3
(B)					
September	35.4	20.5	25.4	28.5	70.5
October	30.5	17.6	30.2	20.3	75.4
November	28.3	10.3	23.5	15.2	78.3
December	21.6	8.4	18.2	10.4	84.1

^z Average of 2 seasons, 1998 and 1999.

Table 2. Average diameter and weight of the proliferated callus from anther culture of summer squash cv 'Eskandrani' and the number of the regenerated plants and the percentage of the abnormal plants in those anther-derived callus^z.

Treatments	Callus proliferation		Plant regeneration	
	Diameter (cm)	Weight (g)	Plants/100 anthers (no.)	Abnormal plants (%)
(A)				
<i>A- Summer season</i>				
March	2.5	2.9	118	24.6
April	2.4	2.6	39	24.6
May	2.1	2.2	13	24.5
LSD _{0.05}	0.1	0.1	6	ns ^y
(B)				
<i>B- winter season</i>				
September	2.6	2.9	126	17.4
October	3.0	3.3	211	17.4
November	3.3	3.4	260	17.4
LSD _{0.05}	0.2	0.1	28	ns
	* x	*	*	*
Summer vs winter				

^z Data were pooled from 2 seasons, 1998 and 1999.

^{y,x} Nonsignificant and significant at 0.05 probability level, respectively.