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IN VITRO CULTURE OF SOMATIC EXPLANTS AND ANTHER CULTURES OF CANOLA (*Brassica napus* L.)

Sayed, A. I. H. *; Sh. I. El-Morsy* and R. A. R. El-Said **

*** Agric. Botany Dept., (Genetics),Fac. of Agric.,Al-Azhar Univ. at Cairo**

**** Home Economic(Biological and Environmental Science Dept.,) Al-Azhar University at Tanta**

ABSTRACT

This investigation was carried out at the Cell and Tissue Culture Lab. Agronomy Dept. Faculty of Agriculture ,Al-Azhar University, during the period from 2006 to 2008 aiming to study the main factors affecting callus induction and plant regeneration from cultured somatic explants and anther culture of canola cultivars (*Brassica napus* L) namely Sarow -4 ,Sarow-6, Pactol, N.A.51 and N.A.355. Experiments were designed to identify cultivars of canola and growth regulators most suitable for successful *in vitro*. Also, to develop an efficient method for shoot regenerations of canola . The regeneration capacity of different genotypes on MS medium with several combination of plant growth regulators were compared .

The experiments showed that the morphogenitical potential of canola depends on genotype, primary explants and hormonal structure . Highest significant value of plant regeneration was determined for N.A.51 while the lowest value of plant regeneration obtained from Sarow-4 cultivar . The highest value recorded when M.S medium with 0.2 mg/l NAA + 2 or 3 mg/l BAP were used . The cotyledons explants gave the highest values of plant regeneration compared with the hypocotyls explants (2.36 per 5 calluses).In the same time roots did not gave any plants.

Highest callus induction from anthers reported on MS or B5 ,while the lowest callus initiation recorded on N6 medium . The highest value of callus initiation recorded when 3% sucrose were added in the medium while, the lowest value obtained when 9% sucrose were added to the medium .

The genotype effect on callus initiation from anthers showed significance in this respect. Sarow 4 cultivar recorded the highest callus initiation followed by sarow 6 cultivar while, the lowest value recorded was for N.A.355 cultivar. Also, the stage of the microspores development at the time of culture was effective on plant regeneration percent. Uninucleate microspores stage was identified as producing the highest percentage of green plants (33.75 %) compared with the tetrad (23.5 %) stage.

The process of embryogenesis depend on the genotype , light levels, the method of thermal shock and the interaction between light and genotypes .

INTRODUCTION

It is well-known that Egypt is suffering from a great shortage in edible oils. The gap between production and consumption is estimated by about 90% (FAO,2004) . In this context, canola is considered as the most important oil crop worldwide. Canola (*Brassica napus* L.) is considered as the most important source of vegetable oil and protein-rich meal worldwide . It ranks the third among the oil crops, following palm oil and soya oil and the fifth among economically important crops, following rice, wheat, maize and cotton (Sovero, 1993;,Kazan *et al*.,1999; Cardoza and Stewart (2003) .

The term "canola" was adopted by Canada apparently as an acronym of the Canadian Oilseed Association in 1979,with the goal of branding , and to replace the terms oilseed rape and rapeseed . Unlike these, canola oil is defined as an oil that must contains less than 2% erucic acid and the solid components of the seed must contain less than 30 m mol of any one or a mixture of 3-butenyl glucosinolate,4- pentyle glucosinolate, 2- hydroxyl-3 butenyl glucosinolate and 2- hydroxyl 4- pentenyl glucosinolate per gram of the air-dry. Oil free solid.

Two important factors governing the efficiency of transgenic plant recovery are obtaining healthy shoots that are not hyper hydrated and having a good rooting efficiency (Cardoza and Stewart (2003)).

In development of new forms of transgenic plants by genetic transformation methods, shoot regeneration frequency has a great value. Considerable progress has been accomplished in the cellular and molecular biology of Brassica species in the recent years. Plant regeneration has been increasingly optimized via organogenesis and somatic embryogenesis using various explants and by tissue culture improvement focusing on factors such as age of explants, genotype and media additives. There are increased domestic and export market opportunities for canola oil that can be realized through the development of high- oleic acid canola to replace saturated palm oil in food service applications (Spector , 1999;Stoujesdijk *et al* ., 2000) .Additionally, high oleic acid oils are more nutritionally because oleic acid had cholesterol- lowering properties, whereas saturated fatty acids tend to raise blood cholesterol levels (Stoujesdijk *et al .,* 2000). On the other hand , in Egypt there are agricultural opportunities to increase canola production by expanding into the new reclaimed regions. Therefore, *Brassica napus* L has become an object of extensive tissue culture studies and breeding. Cell and tissue culture relating to variability and selection efficiency are two essential components of molecular breeding (Lichtenstein and Draper, 1985).

In total, during the past few years over 2000 new *Brassica napus* L genotypes have been created by different biotechnology methods, the best of which have been involved in spring rapeseed breeding programs (Natalija *et al*., 2007).

Anther cultures leading to callus production often generate genetic variation called gametoclonal variation, which describes phenotypically variant plants regenerated from gametophytic cells . Although the basis of gametoclonal and somaclonal variation is not understood, *in vitro* induced chromosomal aberrations may have contributed to such variation (Marburger and Jauhar 1989).

One of the important techniques that is increasingly used in plant breeding programmes is the production of doubled haploids. The artificial production of haploid plants followed by chromosome doubling offers the quickest method for developing homozygous breeding lines from heterozygous parental genotypes in a single generation. Through anther culture, considerable progress has been achieved for a large number of economically important crop species, such as barley, wheat, maize,

rapeseed and rice. For the Brassica species the first attempt was by Keller and Armstrong 1977.

Genetic variation in canola is required to breed cultivars that are high yielding, and resistant to several biotic and abiotic stress conditions. It is well known that improvement of plant through conventional breeding methods is slow, time- consuming and labor- intensive. Non – conventional genetics improvement programs based on tissue culture and molecular genetics is essential as a complement to standard breeding (Lichtenstein and Draper, 1985).

Since the early observation by (Skoog and Miller 1957) that the balance of auxin and cytokinin in the culture medium regulates organogenesis, much progress has been made in identifying factors that control plant morphogenesis. These regulatory factors include both naturally occurring and synthetic plant growth substances as well as various environmental stimulates (Lakshmanan *et al*., 1997). In earlier studies, attention had been focused on determining the requirements of various plant growth substances and mineral nutrients for different organogenic processes (Murashige, 1974; Gamborg *et al*., 1977). More recently a number of investigations on organogenesis have been conducted from a physiological perspective to analyze various cellular processes associated with organogenesis (Thrope , 1993). Regeneration in *B. napus* is highly variable and genotype specific. Several papers have reported regeneration of shoots from seedling or mature plant derived explants of *B. napus* (Dunwell , 1981). To date organogenesis has been achieved in a variety of explants such as stem sections (Pua *et al*., 1991) , stem thin-cell layer (Klimaszewska and Keller, 1985), leaf discs (Dunwell, 1981), roots (Sharma and Thorpe, 1989), cotyledons (Moloney *et al*., 1989) and hypocotyls (Phogat *et al*., 2000) . It is well documented that efficient Agrobacterium mediated transformation methods require a reliable and efficient callus induction and plantlet regeneration procedures (Riemenschiender *et al*., 1988).

Pollen development can also influence embryogenesis. The most embryos in *B. napus* came from late uninucleate to early binucleate stages (Pechan and Keller 1988, Hansen and Svinnset 1993). In certain species, bud size can be correlated to the development stage of the pollen. Bud size were separated by bud length in *B. napus* and *B. campestris* which influenced embryogenesis.

The aim of this study was to develop a procedure for the regeneration of viable shoots from hypocotyls, cotyledons, roots and anther culture explants of commercial Egyptian canola varieties.

MATERIALS AND METHODS

The present investigation was carried out at the Cell and Tissue culture lab., Agronomy Dept. Faculty of Agriculture, Al-Azhar Univ. During the period from 2006 to 2008.; Five cultivars (Sarow-4,-Sarow-6,Pactol, N.A.51 and N.A.355) , of Canola were used. Each experiment was set up with these five genotypes,

Canola seeds were surface sterilized by immersion in 70% ethanol followed by immersion in 3% (v/v) sodium hypochlorite, and rinsed in sterile distilled water. The sterilized seeds were germinated in flasks on 0.7% agar (w/v). The cultures were incubated as 25˚C under a 16/8 h day/night photoperiod. The seeds were then germinated on MS basal medium (Murashige and Skoog, 1962). After 6 days, the seedlings were collected and the hypocotyls , cotyledons and roots segments (0.5 cm in length) were excised from 6-day-old canola seedlings. Cotyledon, hypocotyls and root segments from 6 days old seedlings were used as explants. These explants were carefully excised from the seedling excluding meristematic axillary bud. The explants were transferred to the callus induction medium containing MS, 3% (w/v) sucrose, 0.8% (w/v) agar in addition to different concentrations of 2,4-D,Kin,BAP and NAA. Two weeks later the explants were transferred onto shoot induction (MS) medium (Murashige and Skoog, 1962).

Shoot induction medium:

Two weeks later the explants were transferred onto shoot induction (MS) medium (Murashige and Skoog, 1962), supplemented with 0.2mg/l NAA and BAP at graded levels (1.0, 2.0, 3.0, 4.0 and 5.0 mg/l),PH 5.8.Each jar contained 5 segments and all the treatments were performed with 5 replications. The jars were sealed with aluminum foil and incubated at 25˚ C under a 16/8 h light/dark photoperiodic regime. The explants were sub-cultured weekly on corresponding medium freshly prepared. Callus induction and the shoot induction frequencies (CIF and SIF) were calculated as follows:

$$CIF = \frac{\text{Number calli-producing explants}}{\text{Total number explants in the culture}} \times 100$$

$$SIF = \frac{\text{Number shoots-producing explants}}{\text{Total number explants in the culture}} \times 100$$

The regenerated explants transferred into rooting medium. The rooted plantlets were washed and transferred to the autoclaved soil in pots. The pots were covered with clear bags to provide 100% relative humidity.

They were placed in an acclimatization room under a 16/8 h photoperiod at 20 – 30˚ C . After 2 weeks , acclimatized plants were transferred to greenhouse and allowed to grow to maturity. They normally passed generative phase and produced seeds . Regeneration frequency was calculated 21 days after precultivation as percentage of explants capable to shoot regeneration on the media. The data were analyzed by ANOVA (analysis of variance).

Anther culture:

Different induction media were used for each experiment. The induction media was solidified with 0.8% agar agar . immature tellers flower placed in a beaker containing enough distilled water to cover the stem at or just below the node prior to the flower , covered with plastic wrap, labeled , and refrigerated at 4˚ C or chocked by heat (35˚ C) for two days. After cold or heat treatments, flowers were sterilized with 70% ethyl alcohol for 1 min,

followed by 1% sodium hypochlorite and 0.1% with Tween 20 , then rinsed with sterile double-distilled water four to five times.

Each flower was staged by squashing its centermost anther in 1% acetocarmine. Anther containing microspores at tetrad or the mid-uninucleate stage were cultured on MS , B5(Gamborg *et al* 1968) and N6 medium supplied with 1. mg/l NAA and 0.5 mg/l BAP on callus formations with 3,6,9,12 and sucrose concentrations.

Twenty anthers were cultured in jars containing 20 ml of one of the induction media which contend MS,B5 or N6 (Chen *et al* 1998) medium supplemented with different concentration of sucrose concentration. Cultures were sealed with aluminum foil and kept in an incubator at 28˚ C in the dark. Culture were observed weekly for callus and/or embryoid development. Cultured were automatically timed fluorescent lights with a 16 h photoperiod. Embryos was achieved on MS ,B5 and N6 medium supplied with 0.1 mg/l NAA and 1.0 mg BAP/1 with 3,6,9 12 and 15 sucrose concentrations.

The cultures were transferred to jars containing 25 ml of modified MS medium without growth regulators and 30 g/L sucrose (Murashige and Skoog 1962) and kept in the same incubation room.

RESULTS AND DISCUSSION

1-Somatic explants studies:

A- Callus induction:

Results recorded in Table (1) indicated that the hypocotyls, cotyledons and the roots had significant effect on callus induction .

Table (1): Mean of callus induction from 15 pieces of hypocotyls, cotyledon and roots cultured in MS medium with different growth regulators

Growth regulators	Genotypes	Explants			Mean
		Hypocotyls	Cotyledons	Roots	
0.5mg/l BAP+2mg/l NAA	Sarow 4	11.733	14.467	11.467	12.556
	Sarow 6	11.200	14.000	11.267	12.156
	Pactol	11.800	14.533	13.600	13.311
	N.A.51	11.067	14.733	11.333	12.378
	N.A.355	11.400	14.400	10.600	12.133
Mean		11.440	14.427	11.653	12.507
0.5 mg/l Kin+2mg/l 2,4-D	Sarow 4	11.000	13.067	8.933	11.000
	Sarow 6	11.200	12.067	6.600	9.956
	Pactol	12.200	12.267	6.600	10.356
	N.A.51	10.867	12.267	8.400	10.511
	N.A.355	10.200	12.600	7.667	10.156
Mean		11.093	12.453	7.640	10.396
Overall mean	Sarow 4	11.367	13.767	10.200	11.778
	Sarow 6	11.200	13.033	8.933	11.056
	Pactol	12.000	13.400	10.100	11.833
	N.A.51	10.967	13.500	9.867	11.444
	N.A.355	10.800	13.500	9.133	11.144
Mean		11.267	13.440	9.647	

L.S.D at 5% for genotypes (G) N.S
for growth regulator (R) 0.491
for explants (E) 0.603
(R)x (E) 0.845

The highest callus induction recorded when cotyledon explants were cultured (13.44 about 89.6%) while the roots reported the lowest value (9.64 about 64.31%) . The growth regulators also had significant effect on callus induction , highest values of callus obtained (12.507) when 0.5 mg/l BAP+2 mg/l NAA were used compared with 2 mg/l 2,4-D+0.5mg/l Kin. The interaction between growth regulators and explants had significant effect. The highest value obtained when cotyledons cultured on medium containing 0.5 mg/L BAP + 2mg/L NAA . The effect of genotypes was non significant on callus induction.

These results are in harmony with those reported by Turget *et al.* (1998) Muhammad *et al.* (2002), Chamandosti *et al*. (2006) and Al-Naggar *et al*. (2008).

B- Plant regeneration:

The morphogenesis potential of cotyledon, hypocotyls and root explants of canola cultivars was estimated. Occurrence of the first regenerated shoots was observed 10 – 15 days after subculture, irrespective of the studied genotypes. The result established that for shoot regeneration of canola, cotyledons isolated from 6-day old seedlings were the best explants (Table 2) and fig. (1).

Table (2): Shoot regeneration frequencies of the hypocotyls and cotyledon of canola cultivars obtained from five callus in MS medium with different growth regulator levels.

Explants	Genotypes	Growth regulators levels					Mean
		MS+0.2mg/l NAA+1mg/l BAP	MS+0.2mg /lNAA+2mg/l BAP	MS+0.2mg/lNAA+3mg/l BAP	MS+0.2mg/lNAA+4mg/l BAP	MS+0.2mg/lNAA+5mg/l BAP	
Hypocotyls	Sarow 4	2.00	1.80	3.40	2.60	1.40	2.24
	Sarow 6	1.80	2.20	3.20	2.60	2.80	2.52
	Pactol	2.20	2.20	3.40	3.00	3.00	2.76
	N.A.51	2.60	2.60	3.80	3.20	2.80	3.00
	N.A.355	2.00	2.20	3.00	3.80	2.20	2.64
		2.12	2.20	3.36	3.04	2.44	2.63
Cotyledons	Sarow 4	1.60	2.00	2.60	3.00	2.00	2.24
	Sarow 6	1.60	2.20	2.80	3.00	2.40	2.40
	Pactol	1.60	2.60	2.20	2.80	2.20	2.28
	N.A.51	2.00	2.00	3.20	3.00	2.20	2.48
	N.A.355	1.80	2.40	2.40	2.80	2.60	2.4
		1.72	2.24	2.64	2.92	2.28	2.36
Overall mean	Sarow 4	1.80	1.90	3.00	2.80	1.70	2.24
	Sarow 6	1.70	2.20	3.00	2.80	2.60	2.46
	Pactol	1.90	2.40	2.80	2.90	2.60	2.52
	N.A.51	2.30	2.30	3.50	3.10	2.50	2.74
	N.A.355	1.90	2.30	2.70	3.30	2.40	2.52
		1.92	2.22	3.00	2.98	2.36	

L.S.D at 5% for genotypes (G) 0.337
for growth regulator 0.337
for explants 0.019

Fig. (1): Plant regeneration obtained from N.A.355 Cotyledons on MS medium +0.2mg/l NAA+ 3mg/l BAP

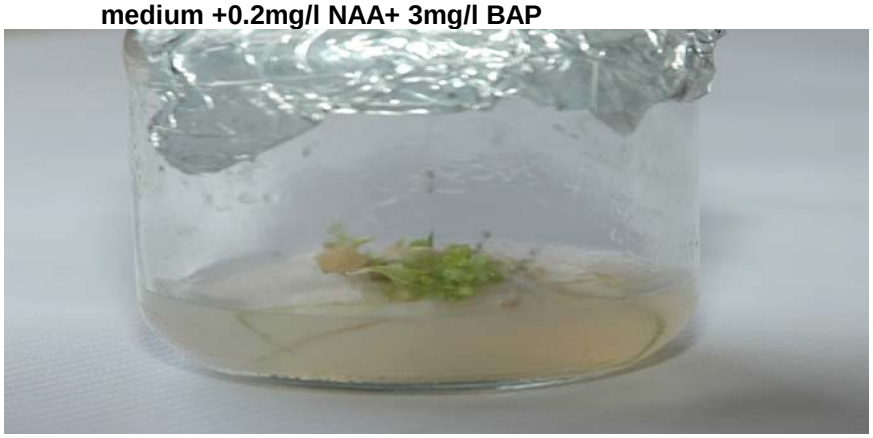

Fig.(2) : Plant regeneration with rooting obtained from N.A.51 hypocotyl on MS medium +0.2mg/l NAA+ 3mg/l BAP

Results in Table 2 showed that the genotypes showed significant effect on plant regeneration. Significant value of plant regeneration recorded for N.A.51 (2.74 plant per 5 calluses) while the lowest value of plant regeneration obtained from Sarow 4 cultivar. The growth regulators had significant effect on plant regeneration. The highest values recorded when MS medium with 0.2 mg/l NAA + 2 or 3 mg/l BAP were used (3.0 and 2.98 plant per 5 calluses) while the lowest value of plant regeneration recorded on MS medium with 0.2 mg/l NAA+ 1 mg/l BAP. Explants had significant effects on plant regeneration. The cotyledons explants gave the highest value of plant regeneration compared with the hypocotyle (2.36 per 5 calluses) fig.(2) in the same time roots did not gave any plants.

Similar results were obtained by Sharma and Thorpe,(1989),Molony *et al.* (1989), Phogat *et al*. (2000), Zhang and Bhalla (2004), Slesak *et al. (*2005), Chamandosti *et al*. (2006), Moghaieb *et al*. (2006) and Kamal, *et al*. (2007).

2- Anther culture studies:

A- Callus induction:

Results in Table 3 showed effect of three media and different sucrose concentrations on callus induction for the tested genotypes. Genotypes were used to study the effect of pre-culturing anthers on a high sucrose medium for a period of time before transfer to a low sucrose medium. The total callus initiation was determined.

Table (3): Effect of MS, B5 and N6 medium on callus formation from anthers with 3,6,9,12 and 15 sucrose concentrations.

Sucrose concentrations	Genotypes	Culture media			Mean
		MS	B5	N6	
3%	Sarow 4	15.00	15.00	14.60	14.867
	Sarow 6	14.80	14.40	14.00	14.40
	Pactol	13.80	15.20	12.40	13.80
	N.A.51	13.80	14.00	13.40	13.733
	N.A.355	11.40	14.40	12.80	12.867
Mean		13.76	14.60	13.44	13.933
6%	Sarow 4	14.20	15.00	13.20	14.133
	Sarow 6	12.80	14.40	11.00	12.733
	Pactol	12.60	14.00	13.60	13.400
	N.A.51	15.00	14.00	13.20	14.067
	N.A.355	13.60	15.00	11.80	13.467
Mean		13.64	14.48	12.56	13.560
9%	Sarow 4	15.00	16.60	13.60	15.067
	Sarow 6	13.40	12.60	13.60	13.200
	Pactol	11.60	10.80	12.60	11.667
	N.A.51	13.60	10.60	11.20	11.800
	N.A.355	11.80	12.60	11.40	11.933
Mean		13.08	12.64	12.48	12.733
12%	Sarow 4	13.80	13.80	12.80	13.467
	Sarow 6	13.60	12.20	11.80	12.533
	Pactol	14.40	11.40	11.00	12.267
	N.A.51	14.00	14.80	12.80	13.867
	N.A.355	12.80	11.63	12.00	12.133
Mean		13.72	12.76	12.08	12.853
15%	Sarow 4	13.20	16.20	13.80	14.400
	Sarow 6	16.40	12.60	13.40	14.800
	Pactol	13.40	13.60	12.40	13.133
	N.A.51	13.20	14.00	13.20	13.733
	N.A.355	13.60	12.40	14.00	13.067
Mean		13.96	14.16	13.20	13.827
Overall mean	Sarow 4	14.24	15.32	13.36	14.387
	Sarow 6	14.20	13.64	13.60	13.533
	Pactol	13.16	13.00	12.76	12.853
	N.A.51	13.92	13.48	12.40	13.440
	N.A.355	12.64	13.20	12.92	12.693
Mean		13.63	13.72	12.24	

L.S.D at 5% for Genotypes (G)	**0.558**
For culture media (C)	**0.432**
For sucrose concentration(S)	**0.558**
(G)x(S)	**1.250**
(G) x (S)x(C)	**2.169**

Results indicated that media had significant effect on callus initiation from anthers. Highest callus initiation reported on MS or B5 Table (3) and

fig.(3). The effect of medium and pre-culture of anthers on a high sucrose medium on callus initiation medium (13.632 and 13.728 per 20 anthers) for Ms and B5 medium respectively. While the lowest callus initiation recorded on N6 medium(12.78 per 20 anthers). Sucrose concentration also, had significant effect on callus initiation from anthers. The highest value recorded when 3% sucrose were added in the medium (13.933 per 20 anthers). While the lowest value obtained when 9% sucrose were added to the medium. The genotype effect on callus initiation from anthers had significant. Sarow 4 cultivar recorded the highest callus initiation (14.387 per 20 anthers) followed by Sarow 6 cultivar. While the lowest value recorded was N.A.355 cultivar(12.69 per 20 anthers).

These results are in harmony with those reported by Siebel and Pauls (1989) Johnson, *et al*. (1997). They found differences between the genotype tested and culture media on callus initiation from anthers.

The interaction between genotype and sucrose concentration had significant effect on callus initiation from anthers. The highest callus recorded when Sarow 4 cultured on medium contained 3% sucrose. While the lowest value of callus recorded for Pactol cultivar cultured on medium contained 9% sucrose concentration.

The interaction Genotypes x sucrose x Culture media had significant effect on callus initiation. The highest callus recorded for Sarow 4 on B5 medium contained 9% sucrose. While the lowest value recorded for N.A.51 cultivar cultured on B5 medium contained 9% sucrose.

B-Embryos formation:

Results in Table (4) indicated the effect of genotypes, culture media and sucrose concentration on embryos formation. The induced calluses were transferred to the regeneration medium with the same levels of sucrose for determine of sucrose on embryogenesis.

Results indicated that sucrose concentration had significant effect on embryos formation. The highest embryos formation recorded when 12% sucrose were added (6.627 per 20 anthers) compared with the other concentrations. While the lowest embryos formed when 3% sucrose were added to the medium (4.107 per 20 anthers). Genotypes had significant effect on embryos formation. The highest embryos formed for N.A.355 (5.907 per 20 anthers). While the lowest value recorded from Pactol cultivar.

The results obtained coincides with those reported by Siebel and Pauls (1989) Johnson, *et al.* (1997). They found that donor plant genotype has effect on embryo production. Also, superior embryogenic potential among donor material was not always coincident with superior plantlet production.

The interaction between Genotypes x sucrose had significant effect on embryos formation. The highest embryos obtained from N.A.355 with 12% sucrose while, the lowest embryos formed for N.A.51 with 3% sucrose (3.667 per 20 anthers).

The interaction between genotypes x culture media also had significant effect on embryos formation. The highest value recorded on MS medium for N.A.355 cultivar. While the lowest value recorded (4.720 per 20 anthers)for Pactol cultured on the same medium.

Table (4): Effect of MS,N6 and B5 medium supplied with 0.1 mg/l NAA and 1.0mg /l BAP on embryos formations from anthers with 3,6,9,12 and 15 sucrose concentrations.

Sucrose concentrations	Genotypes	Culture media			Mean
		MS	B5	N6	
3%	Sarow 4	4.20	4.00	4.60	4.267
	Sarow 6	3.60	4.80	4.00	4.133
	Pactol	3.20	4.20	4.20	3.867
	N.A.51	4.20	3.60	3.20	3.667
	N.A.355	5.20	4.40	4.20	4.600
Mean		4.08	4.20	4.04	4.107
6%	Sarow 4	4.80	4.00	4.40	4.400
	Sarow 6	4.20	4.60	4.40	4.400
	Pactol	4.40	4.80	5.20	4.800
	N.A.51	5.40	5.20	5.00	5.200
	N.A.355	6.00	5.20	5.20	5.467
Mean		4.96	4.76	4.84	4.853
9%	Sarow 4	6.00	5.80	6.00	5.933
	Sarow 6	6.40	7.20	5.40	6.333
	Pactol	5.20	5.00	6.00	5.400
	N.A.51	6.00	5.60	6.00	5.867
	N.A.355	6.20	6.00	6.00	6.067
Mean		5.96	5.92	5.88	5.920
12%	Sarow 4	7.40	8.00	6.80	7.400
	Sarow 6	6.20	6.20	5.60	6.000
	Pactol	6.00	6.60	5.80	6.133
	N.A.51	6.00	6.00	6.20	6.267
	N.A.355	7.60	7.20	7.20	7.333
Mean		6.76	6.80	6.32	6.627
15%	Sarow 4	6.20	7.20	7.00	6.800
	Sarow 6	5.80	5.60	5.40	5.600
	Pactol	4.80	5.60	6.00	5.467
	N.A.51	6.40	6.00	6.40	6.267
	N.A.355	7.00	6.00	5.20	6.067
Mean		6.04	6.08	6.00	6.040
Overall mean	Sarow 4	5.72	5.80	5.76	5.760
	Sarow 6	5.24	5.68	4.96	5.293
	Pactol	4.72	5.24	5.44	5.133
	N.A.51	5.72	5.28	5.36	5.453
	N.A.355	6.40	5.76	5.56	5.907
Mean		5.560	5.552	5.416	

L.S.D at 5% for Genotypes (G) 0.388
For culture media (C) N.S
For sucrose concentration(S) 0.388
(G)x(S) 0.868
(G)x(C) 0.298

C-Shocking temperature of anthers:

The first step after preparing and isolation of anthers is the treatment of the microspores within anthers in order to induce embryogenesis. This has been achieved using different methods, in most cases heat or cold shock were applied.

We have applied anther culture thermal shock, effect of genotypes and light were studied in cultivars tested. The processes of embryogenesis, which proceeded in the culture of isolated anthers, depended on the genotype, light

levels, the methods of thermal shock and the interaction between light and genotypes.

Results in Table (5) showed that N.A.51 proved to be the most suitable cultivar among the cultivar tested (6.833 embryos per 20 anthers) followed by N.A.355 cultivar (6.667 embryos per 20 anthers). While the lowest embryos recorded for the Sarow 6 cultivar (4.700 embryos per 20 anthers). Shocking temperatures also, influence on the canola embryogenesis in the culture of isolated anthers. Shocking temperatures 4˚ C for 48 h was the most suitable for embryogenesis (6.280 embryos per 20 anthers) than the other shocking temperatures.

Table (5): Embryos initiation from 20 anthers shocked by cold (4˚C) or heat treatments (35C) for two days before inoculation and cultured on medium at uninucleat stages after eight weeks compared with the control (non shocked).

Condition cultures	Genotypes	22C (control)	4C + 22C	35C+ 22C	Mean
Dark	Sarow 4	4.80	6.60	5.20	5.533
	Sarow 6	5.00	4.40	5.00	4.800
	Pactol	5.80	6.20	6.00	6.000
	N.A.51	6.20	7.20	7.00	6.800
	N.A.355	8.40	8.20	6.60	7.733
Mean					6.173
Light	Sarow 4	4.20	5.60	4.40	4.733
	Sarow 6	4.20	5.40	4.20	4.600
	Pactol	5.20	6.00	5.20	5.467
	N.A.51	6.20	7.80	6.60	6.867
	N.A.355	6.40	5.40	5.00	5.600
Mean					5.453
Overall mean	Sarow 4	4.50	6.10	4.80	5.133
	Sarow 6	4.60	4.90	4.60	4.700
	Pactol	5.50	6.10	5.60	5.733
	N.A.51	6.20	7.50	6.80	6.833
	N.A.355	7.40	6.80	5.80	6.667
Mean		5.64	6.28	5.52	

L.S.D at 5% for Genotypes (G) 0.675
For shocking temperatures(T) 0.522
For light (L) 0.427
(G)x(L) 0.954

Also, the dark was significant for embryogenesis than the light. The highest value recorded for dark was (6.173 embryos per 20 anthers) while the light recorded the lowest value (5.453 embryos per 20 anthers).

The interaction between genotype and light levels was significant on embryogenesis. The highest value of embryos (7.733 embryos per 20 anthers) formed when N.A.355 cultured on the dark.

The results are in agreement with those of Custers *et al* (1996) Schulze, and Pauls (1998), Zhang-*et al* (2003). Kupriene, *et al* (2004).

D-Effect of microspore stages

Callus induction and plant regeneration in cultures from the different stages in the canola genotypes are presented in Table (6) and Fig.(4-6).

Results indicated that microspore stages had significant effect on callus initiation and plant regeneration from anthers. The ideal microspore development stage is the first important step of androgenesis induction in microspore culture. The collected donor flowers consisted of microspore with tetrad uninucleate stages in middle part of branches.

Table (6): Effect of microspore stages on callus induction and plant regeneration of B5 medium with 1 mg/l 2,4-D + 0.5 mg/l BAP

Microspore stages	Genotypes	Callus induction	Callus induction(%)	Plant regeneration	Plant regeneration(%)
Tetrad stage	Sarow 4	15.167	75.835	4.750	23.750
	Sarow 6	13.167	65.835	4.417	22.085
	Pactol	12.083	60.415	3.417	17.085
	N.A.51	12.750	63.750	4.833	24.165
	N.A.355	10.000	50.000	6.083	30.415
Mean		12.633	63.165	4.700	23.500
Uninucleate stage	Sarow 4	15.083	75.415	6.083	30.415
	Sarow 6	15.083	75.415	4.917	24.585
	Pactol	12.250	61.250	6.333	31.665
	N.A.51	15.333	76.665	8.750	43.750
	N.A.355	12.250	61.250	7.667	38.335
Mean		14.000	70.000	6.750	33.750
Overall mean	Sarow 4	15.125	75.625	5.417	27.085
	Sarow 6	14.125	70.625	4.667	23.335
	Pactol	12.167	60.835	4.875	24.375
	N.A.51	14.042	70.210	6.792	33.960
	N.A.355	11.125	55.625	6.875	34.375
Mean					

	Callus	Plant regeneration
L.S.D at 5% for Genotypes (G)	**0.979**	**0.988**
For Microspore stages (M)	**0.619**	**0.625**
(G)x (M)	**1.384**	**1.398**

The stage of development of the microspores at the time of culture affected callus initiation percent. Uninucleate microspores stage was identified as producing the highest percentages of callus induction (70.00%) compared with the tetrad (63.165%) stage. Also, the stage of development of the microspores at the time of culture affected plant regeneration percent. It is also indicated that the genotype Sarow-4 showed the highest percentage of callus induction from the uninucleate stage (75.625%). Uninucleate microspores stage was identified as producing the highest percentages of green plants (33.75%) stage.

While N.A 51 and N.A 355 recorded the highest plant regeneration percent (33.96 and 34.375%) respectively. These results agreement with those reported by Martha *et al* (1991) and Abraha *et al* (2008).

The interaction between genotypes and microspore stages had significant effect in callus initiation from anthers. The highest callus recorded when Sarow-4 cultured on tetrad stages (75.835%). While the lowest value (50.00%) of callus recorded for cultivar N.A 355 cultured on tetrad stages.

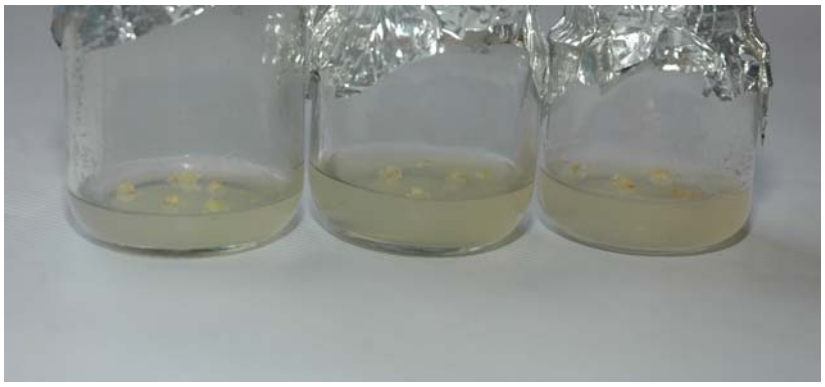

Fig. (3): Callus formation from anthers after 8 weeks

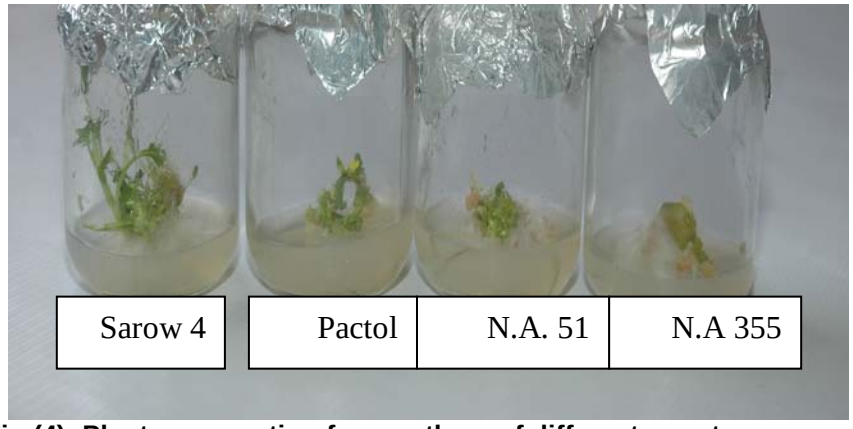

Fig.(4): Plant regeneration from anthers of different genotypes

Fig. (5): Plant regeneration developed from uninuclate stage of sarow- 4 microspore

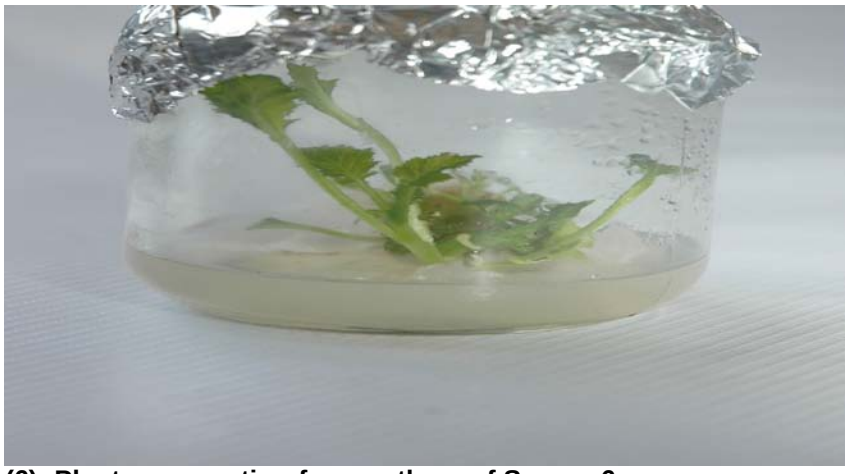

Fig.(6): Plant regeneration from anthers of Sarow- 6

Also, the interaction between genotypes and microspore stage had significant effect on plant regeneration from anthers. The highest percent of plant regeneration recorded when N.A 355 cultured in uninucleate stage (38.335%). While the lowest value (17.085%) of plant regeneration recorded for Pactol cultivar cultured on tetrad stages.

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زراعة المتوك و الأجزاء الجسمية للكانولا فى أنابيب

عبد الهادى ابراهيم حسن سيد*، شفيق ابراهيم المرسى* و رانيا أحمد رشاد السعيد**

*** قسم النبات الزراعي(وراثة)- كلية الزراعة- جامعة الأزهر بالقاهرة.**

****قسم العلوم البيولوجية والبيئية- كلية الاقتصاد المنزلي- جامعة الأزهر.**

أجريت هذه الدراسة فى معمل مزارع الأنسجة والخلايا بقسم المحاصيل كلية الزراعة – جامعة الأزهر بالقاهرة خلال الفترة من 2006- 2008 على خمس تراكيب وراثية من الكانولا وهى:

سرو 4، سرو 6 ، باكتول، N.A 51 وN.A 355 تزرع تحت الظروف المصرية حيث بدأ فى الآونة الأخيرة استخدام أسلوب زراعة حبوب اللقاح كوسيلة جديدة فى تربية النبات حيث أمكن بواسطة هذه التقنيات استنباط سلالات نقية و متماثلة تماما تصلح فى إنتاج الهجن، وكذلك أمكن الوصول إلى تغيرات وراثية ومظهرية بين السلالات الناتجة مما يتيح الفرصة للانتخاب للعديد من الصفات المرغوبة.

وقد أجريت الدراسة بغرض دراسة العوامل التى تؤثر فى نمو الكالس و تجديد النباتات و ذلك من بعض الأجزاء الجسمية لنبات الكانولا(الفلقات – السويقة الجنينية – الجذور) و كذلك من زراعة المتوك.

و قد أوضحت الدراسة أن هناك تباينات ترجع إلى كل من استخدام منظمات النمو و التركيب الوراثى و كذلك الجزء النباتى المستخدم . فقد سجل التركيز 0.2mg/l NAA+ 3mg/l BAP أعلى قيم لتجديد النباتات و كذلك التركيب الوراثى N.A51 قد سجل أعلى معدل لتجديد النباتات أما الفلقات قد سجلت أعلى قيم لتجديدالنباتات بالمقارنة بالسويقة الجنينية.

وقد أوضح استخدام بيئات مختلفة فى زراعة المتوك أن بيئة M.S وكذلك B5 قد أظهرت استجابات عالية لتحفيز الكالس بالمقارنة ببيئة N6.وكان لزيادة السكروز تأثير مثبط على تحفيز الكالس بينما كان له تأثير محفز لتكوين أجنة جسمية . وقد أوضحت النتائج أيضا أن عمر حبوب اللقاح له تأثير هام على مدى استجابة تجدد النباتات فقد وجد أن حبوب اللقاح غير الناضجة فى مرحلة Uninucleate كانت ذات نتائج أفضل و أعلى من مرحلة Tetrad و كان للظلام تأثير محفز على تكوين الأجنة كذلك فإن عمل صدمة حرارية للمتوك عند إعدادها ا للزراعة بتعريضها لدرجة 4 درجات مئوية لمدة يومين أدى الى زيادة تكوين الأجنة الجسمية.

قام بتحكيم البحث

أ. د/ محمد سعد إبراهيم حمادة	**كلية الزراعة – جامعة المنصورة**
أ. د/ سمير سيد بيومى مراد	**كلية الزراعة – جامعة الأزهر**