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GENETIC STUDIES ON TOMATO ANTHER CULTURE. FACTORS AFFECTING INDUCTION OF ANDROGENESIS IN TOMATO ANTHER CULTURE

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ABSTRACT: This investigation aimed to study the effect of genotypes, bud lengths, growth regulators and cold pre-incubation on callus and somatic embryo induction from tomato anther cultures. Four tomato cultivars were chosen as an experimental material, i.e., Super Strain B (SSB), Super Marmand (SM), Castle Rock (CR) and Money Maker (MM). Many trials were carried out to study the androgenesis ability.

At the first trial, low callus induction frequency for all treatments, except without growth regulators treatment, which gave (20.379%) (twice other treatments). While in the media contain 2mg/l IAA + 1mg/l 2ip concentrations gave highest somatic embryo frequency (6.11%). In the present study, the flower bud length and anther length possessed stable relationship between them. Bud length (4mm) gave a high callus induction, followed by (6mm) bud length. Low response was with (2mm) bud length for callus induction. The same trend was reported for somatic embryo formation.

At the second trial Castle Rock cultivar considered as a better genotype for androgenesis under different concentrations of growth regulators, followed by Super Marmand and Super Strain B, while Money Maker possessed lower response of androgenesis. Regarding the effect of cold pre-incubation, insignificant differences were obtained between with and without cold pre-incubation. Meanwhile, the relationship between cultivars and cold pre-incubation was

significant. The cultivar (SM) was found to have high response for cold pre-incubation. In contrary, cultivars (SBB), and (CR) appeared not to have any response against cold pre-incubation. Meanwhile, cultivar (MM) gave lower callus response than others. 2mg/l NAA + 1mg/l 2ip considered as better growth regulators (10.6) concentrations than others, followed by 0.5 mg/l NAA + 0.5mg/l 2ip (9.48) concentrations. Lower embryos than callus induction frequencies were recorded. The same effects of cold pre-incubation were shown on somatic embryo as a callus induction frequency.

At the third trial, (factorial experiment) highly significant differences were recorded for genotypes (A), growth regulators (B) and interaction between them for callus and embryo frequencies.

INTRODUCTION

The cultivated tomato, *Lycopersicon esculentum*, is considered as an important crop plant in many parts of the world. Since its spread to Europe via Spain in the 16th century from Mexico, where it was originally domesticated, it has found good natural growing condition particularly in Mediterranean countries. This Crop has spread to countries in temperate zone, where it has become a popular diet (Esquinas Alcazar 1981).

The production of haploid plants, usually obtained in vitro by regeneration from a haploid cell of either the male or female gametophyte. It is an important means of producing and selecting new genotypes. In tomato, although parthenogenesis has been cited as the origin of haploid plant obtained in vivo (Lindstrom and koos 1931), this is not a readily obtained phenomenon and has not been applied to plant improvement in this species (Chlyah *et al.* 1990).

Induction of androgenesis in tomato is very difficult, and is influenced by many factors still poorly or not examined (Shtereva *et al.* 1998). A number of studies have shown that tomato androgenesis can be improved by manipulating donor plant growth conditions, controlling the microspore developmental stage, and optimizing the culture medium, especially growth regulators, as well as the cultivation conditions (Sharp *et al.* 1971; Gresshoff and Doy 1972; Debergh and Nitsch 1973;

Zagorska *et al.* 1982; Jaramillo and Summers 1990, 1991; Summers *et al.* 1992 and Bellamik and chlyah 1994).

Almost all of the researchers subsequently studyies haploid induction in anther or pollen cultures of tomato obtained only callus or rarely, shoots that died at a very early stage of development (Debergh and Nitsch 1973; Dao and Shamina 1978; Chlyah *et al.* 1990; Jaramillo and summers 1990, 1991 and Summers *et al.* 1992). Some authors obtained non haploid plants of high ploidy level, i.e. 2n, 4n (Ancora *et al.* 1977). Zamir *et al.* (1980) obtained diploid plants by anther cultures of tomatoes. Ziv *et al.* (1982, 1984) also obtained diploid plants and using a recessive marker (trifoliate leaf) proved the sporogenic origin of the regenerants.

Recent studies confirmed the importance of genotypes responsible for androgenesis. Zagorska *et al.* (1998) obtained 6000 regenerantes, by using genotypes possess (ms) male sterility in tomato, confirmed induction of organogenetic potential on the homologous (ms/ms) or heterozygous (ms/+) state of that gene. These genotypes are Roma, Pearson, San Marzano, Por, Sar, Vigapol, Day, David and Start cultivars. Most of regenerants showed different morphological alterations and variations in chromosome number (n, 2n, 4n); some of them are used as a material for tomato breeding programs. Therefore, the present study aimed to study factors affecting callus and somatic embryo induction and regeneration as genotype, flower bud length, growth regulators and other conditions, to enhancement androgenesis ability of tomato anthers, which has not yet been thoroughly investigated. This study consider as a first trial in Egypt at this area.

MATERIALS AND METHODS

The present investigation was carried out at the Biotechnology Laboratory and Greenhouse, Genetic Department, Faculty of Agriculture, and Laboratory of Plant Biotechnology, Institute for Efficient Productivity, University of Zagazig.

Plant Material

Four tomato cultivars (*Lycopersicon esculentum* Mil.) were chosen as experimental material (Table 1). These cultivars

were obtained from Vegetable Research Section, Horticulture Institute, Agriculture Research Center (ARC), Ministry of Agriculture, Egypt.

Manipulation of Plant under Greenhouse Condition

Seeds of cultivars were sown in multi-pot transplant trays filled with a mixture of peat-moss, and vermiculite (1:1, v/v) medium. After 35 days from sowing, transplants were transferred to bigger pots with the same mixture under greenhouse condition.

Manipulation of Anthers under Lab Condition

Flowers were collected from vigorously grown diploid plant at a bud length of (2- 6mm) at morning, and stored moistened in plastic bags. The chosen buds were kept in refrigerator at 4c° for 48hs.

Media preparation

Anthers were cultured on nutrient media contain mineral salts, vitamins of (MS) medium (Murashige and Skoog 1962), 20g/l sucrose, 8g/l agar and supplemented with different concentrations of growth regulators. Therefore, many trials were carried for studying androgenetic ability. First trial at summer 2001 carried out to study the effect of genotypes and growth regulators on callus induction and somatic embryo formation as well as bud length (2mm, 4mm, and 6mm). Balanced growth regulators were used in this trial as follows:-

1- Basic medium without growth regulators addition.

2- 2mg/l IAA (Indole-3-acetic acid) + 1mg/l 2ip (6-(γ, γ-Dimethylallylamino) purine. This balance according to Shtereva *et al.* (1998).

3- 2mg/l IAA + 2mg/l 2ip.

4- 2mg/l IAA + 3mg/L 2ip.

The second trial was carried at summer 2002 to study the effect of different genotypes on callus induction and somatic embryo formation with different concentrations of growth regulators. The study also includes the effect of cold pre-incubation on callus induction and somatic embryo formation. Growth regulator balance used in this trial was as follows:-

1- 2mg/l NAA (1-naphthaleneacetic acid) + 1mg/l 2ip.

2- 0.5mg/l NAA + 0.5mg/l 2ip.

3- 2mg/l NAA + 0.5mg/l 2ip + 0.5mg/l BAP (N^6-Benzylaminopurine).

Table (1): The origin and characterization of the cultivars.

Name	Origin	Characterization
Super Strain B (SSB)	U.S.A	Determinate, very firm, processing, fresh market
Super Marmand (SM)	France	Semi-determinate, very firm, processing, fresh market
Castle Rock (CR)	U.S.A	Determinate, medium firm, heat tolerant, fresh market
Money Maker (MM)	Holland	Indeterminate, for exportation

4- 1mg/l NAA + 0.5mg/l 2ip + 1.5mg/l Kinetin (*N*-(2-furfanylmethyl)-1*H*-purine-6-amine).

The third trial was carried out at summer 2003. This trial focused on best genotypes (SSB, SM, and CR) and best concentrations of growth regulators (2mg/l NAA + 1mg/l 2ip, 0.5mg/l NAA + 0.5mg/l 2ip, 1mg/l NAA + 0.5mg/l 2ip + 1.5mg/l Kinetin) for analysis in a Factorial Experiment with three replicates at Randomized Complete Block design (RCB) of callus and somatic embryo frequencies. For plant regeneration growth regulators balance was as follows:-

1- 0.25mg/l 2ip + 0.25mg/l NAA.

2- 0.5mg/l 2ip + 0.25mg/l NAA.

3- 0.25mg/l 2ip + 0.25mg/l BAP + 0.5mg/L NAA.

Medium pH was adjusted to 5.8 with 0.1N NaOH before Agar was added. Each medium was autoclaved for 20 min at 1.5 bars.

Surface sterilization of flower buds

Buds were surface-sterilized for 15 to 20 sec in 70% ethanol, followed by immersion in solution contains 25% commercial Clorox for 5 min, and rinsed four times with sterilized distilled water.

Dissection and culture of anthers

Anthers were dissected, and plated on nutrient medium. Cultures were wrapped with Para film covered with aluminum foil. The relation between anther size and bud length was checked, and considered during the experiments.

Pre-incubation

Some trials were exposed to cold pre-incubation at 4c° for two days.

Incubation of cultures

Cultures were placed in a dark growth chamber programmed at 26 ± 1.5 for two weeks at first trial and three weeks at the others trials. After dark treatment, cultures were exposed to a 16/8h photoperiod (3000 lux) provided by white fluorescent lamb until formation of callus and somatic embryo.

Data Collection

The numbers of anthers with calli, or the numbers of anthers with somatic embryos were recorded after 8 weeks following culture on nutrient medium. The callus frequencies were calculated as using the following equation:-

$$\frac{\text{No. of response anthers for callus formation}}{\text{Total no. of anthers}} \times 100$$

The same equation was used for somatic embryo frequencies.

Statistical Analysis

Data on callus and somatic embryo frequencies were analyzed by Chi-square test for test the effect of different genotypes, growth regulators balance, bud lengths and pre-incubation on responses for callus induction and somatic embryo formation, as well as for independence of these factors in the effect on the response of anthers for androgenesis. Factorial experiment was applied in this study with two factors, i.e., growth regulators and genotypes, for studying the interaction between them (Gomez and Gomez 1984). Heritability estimates in broad sense for callus and somatic embryo frequency were calculated according to Singh and Chaudhary (1977).

RESULTS AND DISCUSSION

Development of tomato anthers in vitro proceeds via callus formation followed by organogenesis. A similar development has already been observed in anther culture of rice, barley and wheat (Zagorska 1986). Androgenesis of tomato anthers developed into somatic embryos or calli at present study. It confirmed with the findings of Varghese and Gulshan Yadav (1986). They found that the microspores developed into haploid somatic embryos or into compact or friable calli from tomato anthers. The same results were reported by Sangwan and Sangwan Norreel (1987). In this investigation, the initial growth of the anthers occurred within a few weeks. Four weeks later they burst, forming yellow-green callus. Developmed callus and somatic embryo showed in (Figure 1, 2). After 6-8 weeks later the callus arrived into optimum stage for transferring to plant regeneration media. Some of the calli were green and compact, grew relatively slowly and did not regenerate. Somatic embryos were obtained at the same time of calli formation and they were transferred to regeneration media. The differences between optimum size of callus and somatic embryo was shown in (Figure 3). The present study is focused on the factors that influence calli and somatic embryos induction. These factors are genotypes, growth regulators, flower bud lengths (developed stage of anthers) and cold pre-incubation.

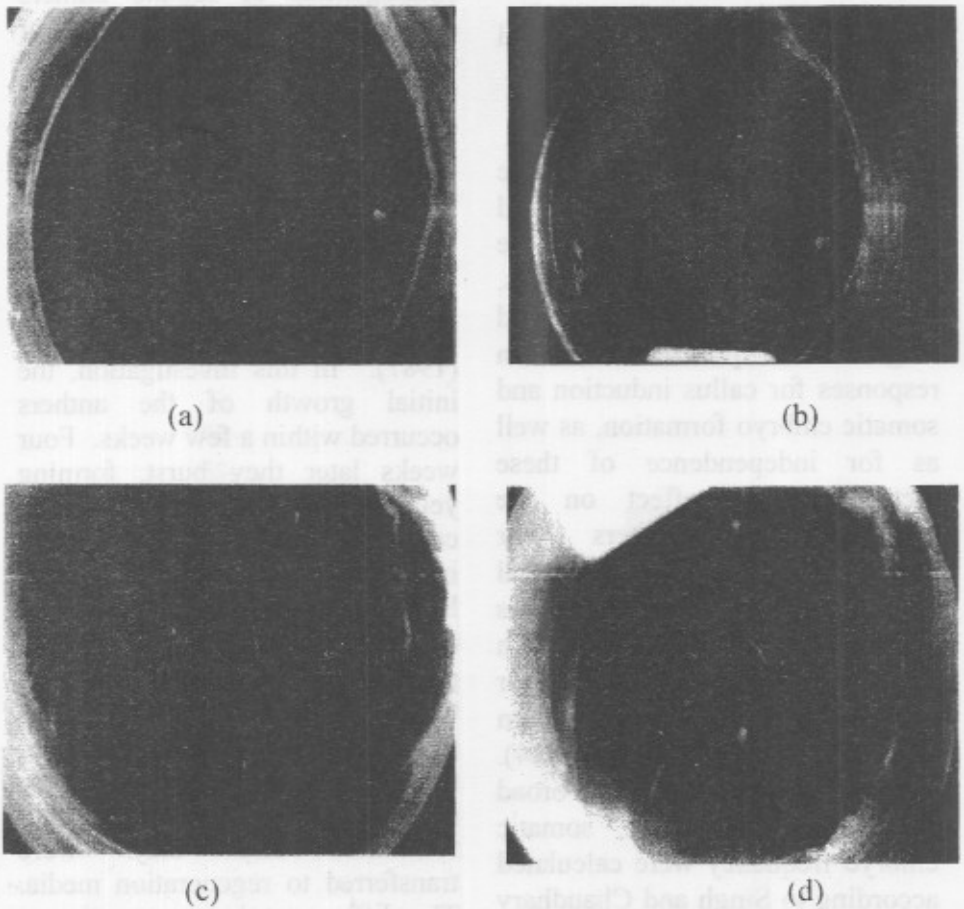
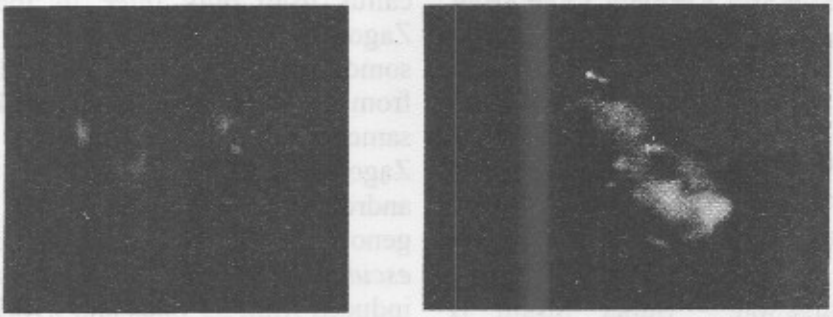
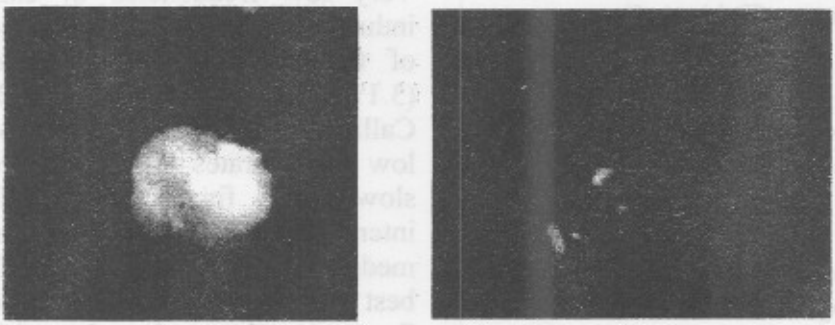


Figure (1): Developmental stages of callus induction for anthers from different tomato genotypes on MS media contain 2mg/l NAA + 1 mg/l 2ip concentrations (a) Super Marmand (SM) cultivars after two weeks of inoculation, (b) (SM) after six weeks later , (c) Super Strain B (SSB) after two weeks of inoculation, (d) (SSB) after six weeks later.



(a) (b)

Figure (2): Developmental stages for somatic embryo induction from anther of tomato cultivar Super Marmand (SM) on MS media contain 2mg/l NAA + 1mg/l 2ip concentrations (a) the somatic embryo after two weeks of culture, (b) the same somatic embryo after six weeks later.



(a) (b)

Figure (3): The differences between optimum size of callus and somatic embryo (a) callus from anther of tomato cultivar Super Marmand (SM), (b) somatic embryo from anther of tomato cultivar (SM).

Influence of Genotype on Callus and Somatic Embryo Formation in Tomato Anther Culture

The genotypes proved to be one of the most important factors affecting induction of androgenesis in tomato. Anthers from four different genotypes (cultivars) were tested at present investigation. Super Strain B (SSB) cultivar possessed significant frequency of callus and somatic embryo induction than Super Marmand (SM) cultivar (Table 2, 3) in the first trial. However, in the second trial Castle Rock (CR) was considered as a better genotypes than other three cultivars for androgenesis under different concentrations of growth regulators (Table 4, 5).

The genotypes possessed the same trend for callus and somatic embryo induction as follows: CR > SM > SSB > MM at the second trial, while SSB > SM at the first trial. Therefore, the Castle Rock consider as a better genotype for androgenesis under these concentrations of growth regulators, followed by Super Marmand and Super Strain B, while Money Maker possessed lower response of androgenesis. These results confirmed with many investigators (Gresshoff and Doy, 1972), tested the androgenetic ability of 43 lines and obtained callus from only three of them. Zagorska *et al.* (1982a, b) had some success in obtaining callus from 22 out of 100 varieties. The same findings were reported by Zagorska *et al.* (1998) studying the androgenetic ability of 85 genotypes of *Lycopersicon esculentum* Mill. Callus was induced from 53 lines and hybrids. The highest callus induction was achieved from anthers of hybrid cv. Cristi (100%) and cv. Roma ms/ms (66.2%), while 20 hybrids from Netherlands showed quite weak ability to form callus. Relatively high numbers of calli were initiated by anthers of hybrids 5770 x Chilecs (34.5%) and 5770 x Apolchi (33.65%). Very low frequencies of callus induction were observed in anthers of the hybrids 3267 x Chilecs (3.1%) and 5016 x 7068 (4.7%). Calli of these hybrids also showed low growth rates. The calli grew slowly and finally died. The interaction between genotypes and media were reported also. The best results were provided by cv. Sar ms/+ when cultured on MS2 medium (22.0%) and cv. Pearson ms/+ grown on MS1 medium (16.0%). Recent study by Shtereva and Atanassova (2001) found that the frequency of callus induction in the mutant anthers was significantly higher than in the

Table (2): Chi-square test of callus induction frequency from anthers of two tomato genotypes with three bud lengths on MS medium contains different concentrations of growth regulators.

Growth regulators (mg/l)	SSB			SM			Growth regulators mean
	2mm	4mm	6mm	2mm	4mm	6mm	
Without growth regulators	13.33	23.53	16.67	0.00	2.08	66.67	20.38
2 IAA+ 1 2ip	0.00	30.00	0,00	8.33	20.83	0.00	9.86
2 IAA+ 2 2ip	19.05	0. 00	0. 00	0. 00	0. 00	0.00	3.18
2 IAA+ 3 2ip	20.00	25.00	0.00	6.25	10.00	0.00	10.21
Cultivars mean	12.30			9. 51			
Bud lengths mean	2mm		4mm		6mm		
	8.37		13.93		10.42		

Cultivar effect (C)
$X^2_c = 4.27$ $X^2_t = 3.841$

Bud length effect (B)
$X^2_c = 11.60$ $X^2_t = 5.991$

Growth regulator effect (G)
$X^2_c = 83.13$ $X^2_t = 7.815$

Independent for (C) x (B)
$X^2_c = 67.57$ $X^2_t = 5.991$

Independent for (C) x (G)
$X^2_c = 30.68$ $X^2_t = 7.815$

Table (3): Chi-square test of somatic embryo induction frequency from anthers of two tomato genotypes with three bud lengths on MS medium contains different concentrations of growth regulators.

Growth regulators (mg/l)	SSB			SM			Growth regulators mean
	2mm	4mm	6mm	2mm	4mm	6mm	
without Growth regulators	6.67	0. 00	8.33	0. 00	0. 00	0. 00	2. 50
2 IAA+ 1 2ip	0. 00	20. 00	8.33	0. 00	8.33	0. 00	6.11
2 IAA+ 2 2ip	0. 00	0. 00	0. 00	0. 00	0. 00	0. 00	0. 00
2 IAA+ 3 2ip	5. 00	0. 00	0. 00	0. 00	0. 00	0. 00	5. 00
Cultivars mean	4.03			0.69			
Bud lengths mean	2mm		4mm		6mm		
	1.46		3.54		2.08		

Cultivar effect (C)
$X^2_c = 28.235232$ $X^2_t = 3.841$
Bud length effect (B)
$X^2_c = 7.745149$ $X^2_t = 5.991$
Growth regulator effect (G)
$X^2_c = 55.88228$ $X^2_t = 7.815$
Independent for (C) x (B)
$X^2_c = 9.77005$ $X^2_t = 5.991$
Independent for (C) x (G)
$X^2_c = 5.329$ $X^2_t = 7.815$

Table (4): Chi-square test of callus induction frequency from anthers of four tomato genotypes treated with or without cold pre-incubation with different concentrations of growth regulators.

Growth regulators (mg/l)	SSB		SM		CR		MM		Growth regulators mean
	without cold pre-incubation	with cold pre-incubation	without cold pre-incubation	with cold pre-incubation	without cold pre-incubation	with cold pre-incubation	without cold pre-incubation	with cold pre-incubation	
2 NAA+ 1 2ip	17.39	0.00	15.00	17.21	20.00	6.11	9.09	0.00	10.60
0.5 NAA+ 0.5 2ip	16.67	6.21	0.00	21.23	0.00	31.78	0.00	0.00	9.48
2 NAA+ 0.5 2ip+ 0.5BAP	20.00	0.00	0.00	8.33	0.00	0.00	0.00	0.00	3.54
1NAA+ 0.5 2ip+ 1.5 Kinetin	7.68	0.00	0.00	14.34	33.33	11.45	0.00	6.25	9.13
Cultivars x Pre-incubations mean	19.78	1.55	3.75	15.28	13.33	12.33	2.27	1.56	
Cultivars mean	8.49		9.51		12.84		1.92		
Pre-incubations mean	Without cold pre-incubations				With cold pre-incubation				
	8.7				7.68				

Cultivar effect (C)

$X^2_c = 61.30325$ $X^2_t = 7.815$

Growth regulator effect (G)

$X^2_c = 29.28967$ $X^2_t = 7.815$

Independent for (C) x (G)

$X^2_c = 66.90547$ $X^2_t = 16.919$

Pre-incubation effect (P)

$X^2_c = 1.00268$ $X^2_t = 3.841$

Independent for (C) x (P)

$X^2_c = 73.26128$ $X^2_t = 7.815$

Table (5): Chi-square test of somatic embryo induction frequency from anthers of four tomato genotypes treated with or without cold pre-incubation with different concentration of growth regulators.

Growth regulators (mg/l)	SSB		SM		CR		MM		Growth regulators mean
	without cold pre-incubation	with cold pre-incubation	without cold pre-incupation	with cold pre-incubation	without cold pre-incubation	with cold pre-incubation	without cold pre-incubation	with cold pre-incubation	
2 NAA+ 1 2ip	8.28	0.00	5.00	10.81	3.34	3.34	9.09	0.00	4.98
0.5 NAA+ 0.5 2ip	0.00	12.44	0.00	4.12	2.35	2.35	0.00	0.00	2.66
2 NAA+ 0.5 2ip+ 0.5 BAP	0.00	0.00	0.00	4.17	10.00	0.00	0.00	0.00	1.77
1NAA+ 0.5 2ip+ 1.5 Kinetin	3.66	3.66	5.68	4.76	0.00	12.61	0.00	0.00	3.80
Cultivars x Pre-incubations mean	2.99	4.03	2.67	5.97	3.92	4.58	2.27	0	
Cultivars mean	3.51		4.32		4.25		1.14		
Pre-incubations mean	without cold pre-incubation				with cold pre-incubation				
	2.96				3.64				

Cultivar effect (C)

$X^2_c = 16.13025$ $X^2_t = 7.815$

Growth regulator effect (G)

$X^2_c = 14.11569$ $X^2_t = 7.815$

Pre-incubation effect (P)

$X^2_c = 1.11691$ $X^2_t = 3.841$

Independent for (C) x (G)

$X^2_c = 40.2777$ $X^2_t = 16.919$

Independent for (C) x (P)

$X^2_c = 13.96708$ $X^2_t = 7.815$

wild type, in addition to the ms mutants, solanifolia and trifoliate also affected callus induction frequency.

Influence of Developmental Stage of Male Gametophyte Cells on Callus and Somatic Embryo Induction in Tomato Anther Culture

Microspore developmental stage is one of the factors which determine the induction of androgenesis. Some investigations indicate early meiosis as an optimal stage, while according to others it is the uninucleate stage (Gressoff and Doy 1972; Dao and Shamina 1978; Zamir *et al.* 1980; Gulshan *et al.* 1981; Zagorska *et al.* 1982 and Smmers *et al.* 1992).

In the present study, the flower bud length and anther length possessed stable relationship between them (Figure 4). The (2mm) bud length indicate the pre-meiotic prophase, (4mm) bud length indicate the prophase-metaphase I and (6mm) bud length indicate the uninucleate stage of pollen mother cell (PMC) (Shtereva *et al.* 1998). The effect of developmental stages on callus and somatic embryo induction were significant (Table 2, 3) frequencies. Bud length (4mm) gave high callus induction frequency, followed by (6mm) bud length. Low response was with (2mm) bud length for callus induction frequency. The same trend were reported for somatic embryo formation therefore, prophase to metaphase I was beneficial than the other stages for callus and somatic embryo induction from tomato anthers, followed by uninucleate stage. These results were in harmony with the finding of Shtereva *et al.* (1998) who reported that the period between prophase to telophase II is optimal for tomato anther implantation. Dao and Shamina (1978) found that callus induction when microspores were at the meiotic stage developed. Whereas embryogenesis take place in microspores at the uninucleate stage. Summers *et al.* (1992) found that some callus formation could be obtained at the meiotic phases, prophase I being the most appropriate. Significance relationship between cultivars and bud lengths were recorded at present study, therefore the selection genotypes which possess highly response of androgenetic ability may be selected at the optimum stage.

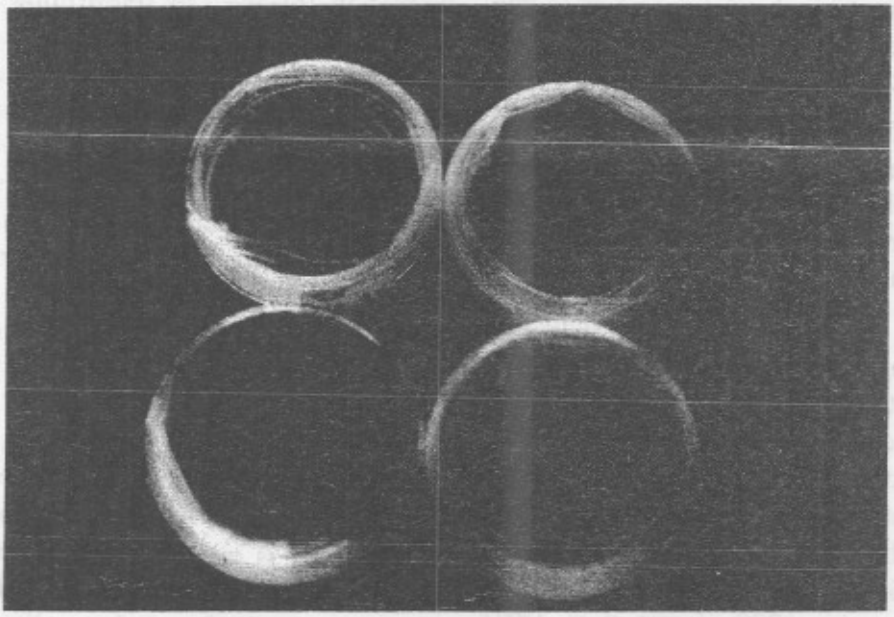


Figure (4): Developmental st ages for tomato as indicator for anther lengths i.e. 1, 2, 4, 6mm of Super Marmand (SM) cultivar.

Influence of Growth Regulators Concentrations on Callus and Embryo Induction in Tomato Anther Culture

At the first trial (Table 2, 3), showed significant differences between growth regulators concentrations on callus and somatic embryo induction. Low callus induction frequency for all treatments, except without growth regulators treatment, which gave (20.379%) (twice other treatment). This phenomenon may be explained on the basis of the fact that tomato had a high level of endogenous auxin, because shoot cultures produced roots easily without the addition of auxin (Sink and Reynolds 1986; and Asakura *et al.* 1995). In general, auxins added to the media to stimulate cell enlargement and cytokinins enhance cell division (Torres 1989).

At the present study correlation between cultivars (C) and growth regulators concentrations (G) effects callus frequency were recorded. Many investigators reported that low callus frequency of many genotypes with different concentrations of growth regulators depend on the interaction between genotypes and growth regulators (Gresshoff and Doy 1972; Summers *et al.* 1990 and Shtereva *et al.* 1998).

Somatic embryo frequency, at the first trial, was lower than callus frequency (Table 3). Highest somatic embryo frequency was obtained on the media contain 2mg/l IAA + 1mg/l 2ip (6.11%). The interaction between cultivars and growth regulators balance were recorded at the present study. The same concentrations gave (20%) and (8.33%) somatic embryo frequency for (SSB) and (SM) cultivars respectively. For that, the interaction between genotypes and growth regulators balance is considered as a general phenomenon for androgenetic ability with tomato anthers. The low frequencies of callus and somatic embryo induction at the first trial may be due to the lower effect of IAA as auxin source and it's rapidly degradation in culture.

Therefore, in the second trial replacement of IAA by NAA has occurred. Pre-incubation was done for four cultivars and four different concentrations of growth regulators (Table 4). Insignificant differences were recorded between cold and without cold pre-incubation. But the relationship between cultivars and cold pre-

incubation was significant. Subsequently, correlation was found between cultivars and cold pre-incubation concerning callus induction. The means of callus induction frequency for (CR) cultivar were (12.38) and (13.33) cold pre-incubation and without cold pre-incubation respectively. While (SM) cultivar possessed callus frequencies mean (15.28) and (3.75) under cold or without cold pre-incubation. In contrary (SSB) cultivar appeared to have (1.55) and (19.78) means of callus frequencies under cold and without cold pre-incubation respectively. For that 2mg/l NAA + 1mg/l 2ip concentrations considered as better growth regulators than others, followed by 0.5mg/l NAA + 0.5mg/l 2ip concentration. Regarding somatic embryo induction frequency, lower embryos than calli induction frequencies were recorded. While 2mg/l NAA + 1mg/l 2ip concentrations gave (4.98) mean of embryo frequency. The same effects of cold pre-incubation were shown on somatic embryos and calli induction frequencies.

Analysis of variance of callus and somatic embryo induction frequencies were carried out by using Factorial Experiment with two factors i.e. three genotypes and three different concentrations of growth regulators as a third trial (Table 6, 7). Highly significant differences were recorded of genotypes (A), growth regulators (B) and interaction between them. These results were in agreement with many investigators (Gresshoff and Doy 1972; Zamir *et al.* 1980; Zagorska *et al.* 1982 and Park *et al.* 2001). Callus formation frequency was highest (65%) with 2mg/l IAA + 1mg/l 2ip concentrations. Shtereva *et al.* (1998), found that the effect of 2ip in combination with IAA on callus induction was greater than that of zeatin and IAA. A highly significant interaction between genotypes and growth regulators concentrations was observed on callus and somatic embryo frequencies. The callus and somatic embryo induction frequencies at the third trial were improved than the second and first trials. 2mg/l NAA + 1mg/l 2ip gave (25.24%) callus frequency at the third trial compared with (10.60%) at the second trial, as well as the embryo frequency also improved. Embryo was (10.25%) and (4.98%) at the third and second trials respectively. These results indicated the importance of selected genotypes and for optimum concentration of growth regulators for increasing callus and

Table (6): Analysis of variance of callus and somatic embryo frequency from anthers of three tomato genotypes (SSB, SM, CR) with three levels of growth regulators (2mg/l NAA + 1mg/l 2ip, 0.5mg/l NAA + 0.5mg/l 2ip, 1mg/l NAA + 0.5mg/l 2ip + 1.5mg/l kinetin) in a RCB design.

Source of variance	Degree of freedom	Mean square		Computed F	
		Callus (%)	Embryo (%)	Callus (%)	Embryo (%)
Replication	2.00	0.53	0.07	0.34^{ns}	0.16^{ns}
Treatment	8.00	366.03	46.56	231.33**	106.43**
Cultivar (A)	2.00	767.65	10.44	485.14**	23.86**
Growth regulator (B)	2.00	27.07	18.89	17.11**	66.05**
A xB	4.00	334.70	73.44	211.52**	167.90**
Errors	16.00	1.58	0.44		
Total	26.00				

** = significant at 1% level, * = significant at 5% level, ^{ns} = not significant.

Callus effect: $CV = 0.19198\ \%$, $h^2 = 0.9902$

Embryo effect: $CV = 0.2675\ \%$, $h^2 = 0.9754$

Table (7): Mean of callus and somatic embryo frequency from anthers of three tomato genotypes (SSB, SM, CR) with three different concentrations of growth regulators (2mg/l NAA + 1mg/l 2ip, 0.5mg/l NAA + 0.5mg/l 2ip, 1mg/l NAA + 0.5mg/l 2ip + 1.5mg/l kinetin).

Growth regulators (mg/l)	SSB		SM		CR		Mean of growth regulators	
	Callus (%)	Embryo(%)	Callus (%)	Embryo (%)	Callus (%)	Embryo (%)	callus (%)	embryo (%)
2 NAA +1 2ip	17.39	8.28	32.21	15.81	26.11	6.67	25.24	10.25
0.5 NAA +0.5 2ip	22.88	12.44	21.23	4.12	31.78	4.70	25.30	7.09
2 NAA +0.5 2ip +1.5 Kinetin	7.68	7.33	14.34	10.44	44.78	12.61	22.27	10.12
Mean of cultivars	15.98	9.35	22.59	10.12	34.23	8.00		
For cultivar (A) & growth regulator (B)			$LSD_{0.05}$				0.42	0.22
			$LSD_{0.01}$				0.58	0.30
For interaction between (A) x (B)			$LSD_{0.05}$				1.26	0.66
			$LSD_{0.01}$				1.73	0.91

somatic embryo induction ability in tomato anthers.

Moreover, the interaction between genotypes and growth regulators for androgenesis ability is considered as a fact, especially in tomato anthers. In the present study, high frequency of callus (44.78%) were recorded in the combination between (CR) cultivar and 2mg/l NAA + 0.5mg/l 2ip + 1.5 mg/l Kinetin concentrations, while the general optimum concentrations was 2 mg/l NAA + 1mg/l 2ip. More details of these findings were shown in (Figure 5, 6). In addition, highly heritability estimates in broad sense for callus and embryo frequencies were recorded (Table 6). These results indicated the importance of variation for improvement of androgenetic ability in tomato anthers. Therefore, for increasing of callus and somatic embryo induction ability, many trials should be done at the recent future includes many combinations between genotypes and different concentrations of growth regulators.

Organogenesis and Plant Regeneration

All callus and somatic embryo were transferred into plant regeneration media with different concentrations of growth regulators. Slowly growth was occurred of callus and somatic embryo after planting, and finally died after 2-4 weeks (Figure 7). Previous researches indicated that recessive ms 10^{35} gene controlling male sterility in tomato was found to play an important role in the induction of callus and organogenesis in tomato anther culture of different cultivars (Zamir *et al.* 1980; Shtereva *et al.* 1998; and Zagorska *et al.* 1998). Lacking of (ms) gene produced small amount of callus and no regeneration occurred (Zamir *et al.* 1980). Anthers of sterile genotypes showed a better morphogenesis response than those from the fertile isogenies lines.

For improvement of organogenesis and plant regeneration of tomato anthers from adapted cultivars in Egypt, will be needed carrying out the hybridization with other cultivars, possess male sterile gene ms 10^{35} i.e. Roma, Pearson, San Marzano, Por, Sar, Vigapol, Day, David and Start (Zagorska *et al.* 1998). Other way, by hybridization with wild tomato species, which possess high regeneration ability, especially the genus *Lycopersicon* is composed of approximately 10 species, all of which have 12 pairs of chromosome. Therefore, the

Figure (5): Callus induction frequency of three tomato genotypes with different concentrations of growth regulators

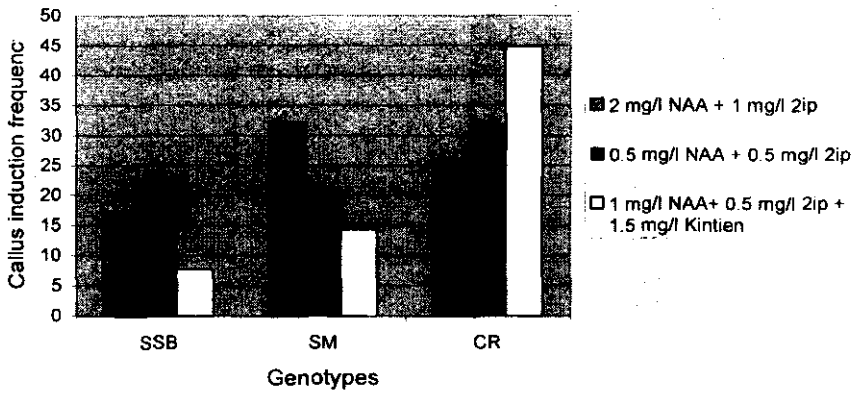
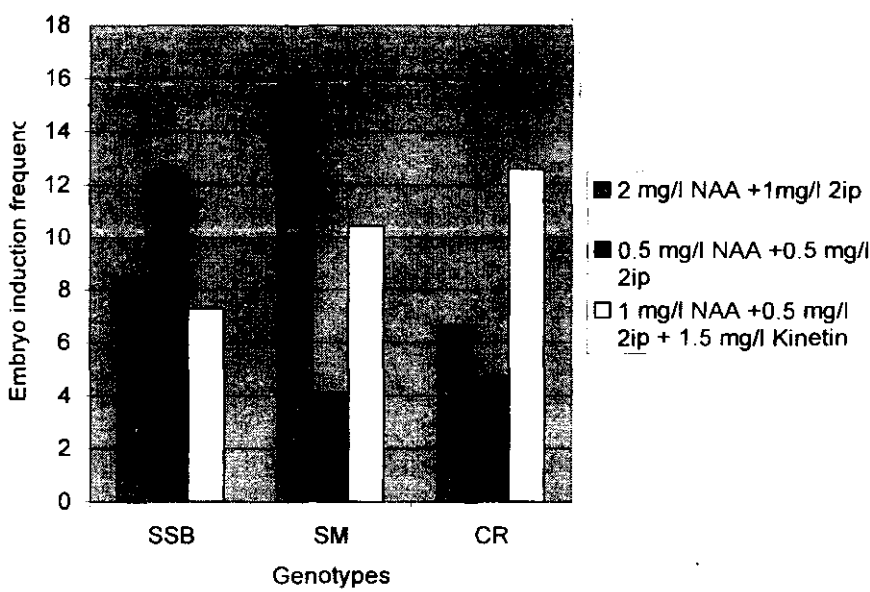
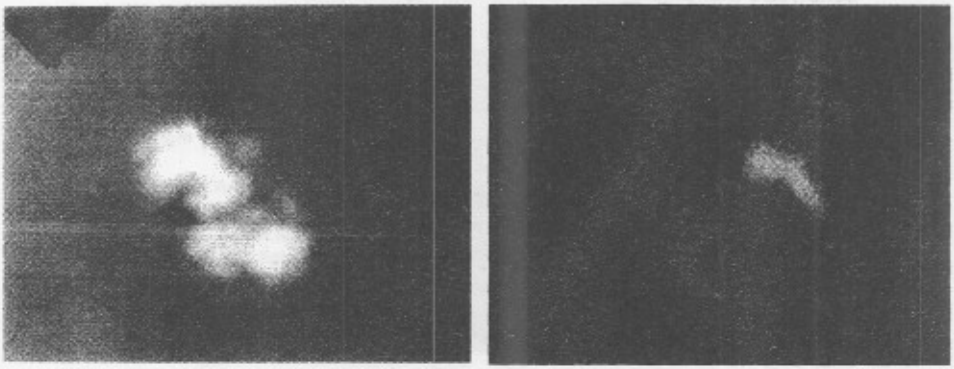


Figure (6):- Somatic embryo induction frequency for three tomato genotypes with different concentrations of growth





(a) (b)

Figure (7): The difference between calluses in beginning of cell death at transferring into regeneration media (a) callus induction from Super Marmand cultivar (2mg/l NAA + 1mg/l 2ip concentrations in callus induction media), (b) callus induction from Super Marmand cultivar (1mg/l NAA + 0.5mg/l 2ip + 1.5mg/l Kinetin concentrations in callus induction media).

species have evolved not by variation in chromosome number or gross changes in chromosome structure, but rather by genetic anomalies which are manifested in some inter-specific hybrids (Rick 1976 b), as well as by using mutagens for induction of male sterile mutants in adapted cultivar (Shtereva and Atanassova 2001). Another way, by using nature extracts addition (Korkut *et al.* 2003).

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دراسات وراثية على زراعة المتوك فى الطماطم. العوامل المؤثرة على استحداث التكشف فى مزارع متوك الطماطم

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الهدف من هذه الدراسه هو، دراسة تأثير التركيب الوراثى وطول البرعم الزهرى ومنظمات النمو وكذلك تأثير المعاملة بالبروده قبل التحضين على استحداث الكالوس والاجنه الجسميه من مزارع المتوك للطماطم. استخدمت فى هذه الدراسه اربعة اصناف اجنبيه متأقلمه ومنزرعه بجمهورية مصر العربيه هم سوبر ستراين بى (SSB)، سوبر مارمند (SM)، كاسل روك (CR)، مونى ميكر (MM).

قسمت الدراسة الى عدة محاولات، المحاوله الاولى عام ٢٠٠١ لدراسة تأثير كل من طول البرعم الزهرى وتركيزات مختلفه من منظمات النمو للصنفين سوبر ستران بى (SSB) وسوبر مارمند (SM) على تكرار حدوث الكالوس والاجنه الجسميه فى بيئات زراعة المتوك فى الطماطم. فى المحاوله الثانيه عام ٢٠٠٢، كانت بهدف دراسة تأثير المعمله بالبروده قبل التحضين و تركيزات اخرى من منظمات النمو للاصناف سوبر ستران بى (SSB)، سوبر مارمند (SM)، كاسل روك (CR)، ومانى ماكير (MM). فى المحاوله الثالثه (تجربه عامليه)، اشتملت على ثلاث تراكيب وراثيه هى سوبر ستران بى (SSB)، سوبر مارمند (SM)، كاسل روك (CR)، وثلاث بيئات محتويه على تركيزات هرمونيه مختلفه هى (2mg/l NAA + 1mg/l 2ip، 0.5mg/l NAA + 0.5mg/l 2ip، 1mg/l NAA + 0.5mg/l 2ip + 1.5mg/l Kinetin).

ويمكن تلخيص نتائج هذا البحث فيما يلى :

فى المحاوله الاولى

كان تكرار تكوين الكالوس منخفض فى جميع المعاملات فيما عدا المعاملة الخاصه بدون اضافة منظمات نمو(٢٠,٣٧)، بينما تكرار تكوين اجنه جسميه كان اعلى عند استخدام تركيز (2mg/l NAA + 1mg/l 2ip) (٦,١١). أفضل طول للبرعم الزهرى

المستخدم لفصل المتك منه كان ٤ ملل لكل من القدره على انتاج كالوس وتكوين اجنه جسميه على السواء، يليه طول ٦ ملل، بينما طول ٢ملل كان غير مناسب.

فى المحاوله الثانيه

وجد ان افضل التراكيب الوراثيه (CR) فى القدره على تكوين كالوس واجنه جسميه، يليه الصنفان سوبر مارماند، وسوبر سترات بى، بينما الصنف مونى ميكر امتلك قدره ضعيفه على الاستجابه. فيما يتعلق بتأثير معاملة البروده قبل التحضين، كان التأثير غير معنوى بين كل من المعامل وغير المعامل. بينما العلاقه بين المعامله بالبروده قبل التحضين والاصناف فى تأثيرها على الاستجابه لاستحداث الكالوس والاجنه الخضريه كان معنويا. استجابه عاليه للصنف سوبر مارمند للبروده، وعلى العكس كانت استجابه الصنف سوبر سترات بى عاليه لعدم المعامله بالبروده والتحضين فورا بعد الزراعه، بينما الصنف كاسيل روك اظهر عدم استجابه للمعامله بالبروده. يعتبر التركيز الهرمونى (2mg/l NAA + 1mg/l 2ip) اعلى التركيزات (١٠,٦)، ويليه التركيز (0.5mg/l NAA + 0.5mg/l 2ip) والذى اعطى (٩,٤٨). استحداث الاجنه الجسميه كان اقل تكرارا من استحداث الكالوس.

فى المحاوله الثالثه

سجلت اختلافات معنويه عاليه من تأثير التراكيب الوراثيه ومنظمات النمو كما تم تسجيل وجود تفاعل بينهما على كل من القدره على تكوين كالوس واجنه جسميه. التفاعل بين التركيب الوراثى والتركيز الهرمونى كان شديد التأثير على الاستجابه لتكوين الكالوس والاجنه الجسميه، فقد وجد ان الصنف كاسيل روك مع التركيز (1mg/l NAA + 0.5mg/l 2ip + 1.5mg/l Kinetin) امتلك اعلى تكرار للكالوس (٤٤,٧٨%)، بينما الصنف سوبر مارماند مع التركيز الهرمونى (2mg/l NAA + 1mg/l 2ip) امتلك اعلى تكرار لتكوين الاجنه الجسديه (١٥,٨١%). كانت انسب التركيزات بشكل عام هو (2mg/l NAA + 1mg/l 2ip) لاستحداث الكالوس وتكوين الاجنه الجسميه. امتلك كل من استحداث الكالوس وتكوين الاجنه الجسميه كفاءة توريث عاليه فى هذه الدراسه.