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FACTORS AFFECTING MICROTUBER FORMATION IN SOME POTATO GENOTYPES

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ABSTRACT

The effect of some factors, namely BA concentration (5 and 10 mg/l), sucrose concentration (8, 10, 12, 13, 14 and 15%) and presence of active charcoal in medium (0 and 5 g/l) on microtuber formation of six potato genotypes, four tetraploid, viz. Diamant, Picasso, Santa and Forella and two dihapliod, namely 805 and 808, was studied in two experiments. Potato genotypes responded differently to such factors, where Forella cv. showed the best performance regarding microtuber number per jar, average weight of microtuber, and microtuber yield per jar. Increasing sucrose concentration up to 12% and adding active charcoal at concentration of 5 g/l in medium having 10 mg/l BA caused a remarkable increase in microtuberization parameters in all cultivars. On the other hand, increasing sucrose concentration or adding 5 g/l active charcoal to MS medium having 5 mg BA /l promoted microtuber formation in some cultivars, and inhibited this process in others.

INTRODUCTION

The production of microtubers has got significant importance in many countries of the world for the purpose of the production of disease-free seed tubers, germplasm exchange, and conservation (Roca *et al.*1979; Ranalli *et al*. 1994). Mass production system of potato microtuber can revolutionize the world potato agriculture in the near future. Moreover, many laboratories are producing microtubers commercially for their own national programs as well as for export. In South Korea, they are producing millions of microtubers annually (Hyouk *et al*. 1994). In the Philippines, the national breeding project on conservation and multiplication has a strong microtuber production programme in order to produce millions of minitubers every year (Paet and Zamora 1994). Baoquing *et al.* (1991) harvested at least ten usable size microtubers per flask after 30 days of culture. They tried to give a picture of a large scale and economic production of microtubers for the national programme with a comprehensive research outline. In USA, some commercial laboratories are producing millions of microtubers, which are sold with 15 cent each, while in Indonesia and China it costs only 5 cent due to cheap labor cost.

In vitro microtuberization is a complex process regulated by different factors. However, work on micro tuberization in potato has mainly focused on

the use of growth regulators, such as benzyl amino purine (Wang and Hu 1982; Hussey and Stacey 1984; Abbott and Belchel 1986), 2-chloroethyl trimethyl ammonium chloride (CCC) (Tovar *et al*. 1985), coumarin (Dodds *et al*. 1988), and jasmonoid (Pelacho *et al*. 1993), etc. Cytokinins are commonly included in media for in vitro tuberization of potatoes (Wang and Hu 1982), but their role is still obscure (Ewing 1985). Doubts have been raised whether cytokinins are directly responsible for tuber initiation (Ewing 1987). Addition of cytokinin to the agar medium promoted tuberization of stolons subcultured in darkness (Palmer and Smith 1970) and cytokinin like activity was nearly twice as high in induced as in non-induced tissues (Forsline and Langille 1976; Mauk and Langille 1978). Generally, the response to growth regulators depended upon a rang of factors including sucrose concentration and cultivars (Hussey and Stacay 1984; Ortiz- Montiel and Lozoya-Saldana 1987; Garner and Blake 1989). Concerning the effect of sucrose cocentration, Yan-Zheng and Guo-Dezhang (2004) found that the best medium for inducing microtuber production consisted of MS, 8% sucrose, 0.1% Active carbon, 30 mg l^{-1} coumarin and 3 mg l^{-1} 6-BA. Also Gopal *et al*. (2004) reported that higher concentration of sucrose (60-80 g/liter) promoted biomass production, microtuber production and microtuber dry matter content. Tariq *et al.* (2004) reported that MS medium supplemented with higher concentration of sucrose (6%) and 1µ M of BA recorded the maximum number of micro tubers. Sidikou *et al*. (2003) studied the effect of sucrose level at 2-12% and BA at 0 - 5 mg/ liter on tuberization and found that tuberization increased with increasing concentration of BA and sucrose. Islam *et al*.(1999) investigated the effect of sucrose level (6, 8, 10 and 12%) and BA (4, 5 and 6 mg /liter) on the tuberization, and they observed that the highest number of tubers per flask and average tuber weight were upon treatment with 6% sucrose, 5 mg/liter BA. Alix *et al*. (2001) studied the effect of sucrose level (1, 2, 4 and 8%) on potato microtuber initiation, and found that microtuber initiation was 100% at 8% sucrose falling to 40-50% at 4% sucrose and was absent at 1 or 2% sucrose. Regarding cultivar response, Hossain and Sultana (1994) reported that the preliminary studies on microtuberisation showed that the potato cultivars differed widely in this respect, i.e. the cultivar Patrones produced about 11 tubers per plant as against one - two for Heera, Dheera or Chamak. Medium containing activated charcoal gave the highest rate of tuberization and the largest microtubers in potato. It thus played a role in optimizing conditions for rapid, mass tuberization of these cultivars, and produced large microtubers for field planting (Bizarri. *et al*. 1995; Wang *et al.* 2006). Bulblet growth was also improved in lilium by supplement medium of AC. The medium with 2 g/L AC was most effective for bulblet growth (Bong *et al*. 2005). As it was reported by Hossain (2005), most of the studies were based on a few genotypes only. Thus, the present experiment was aimed at investigating the effect of different factors, namely, BA concentration, sucrose concentration, and presence of active charcoal on microtuberization over a w

MATERIAL AND METHODS

These experiments were carried out during the period from 2005-2006 in Plant Biotechnology Research Lab, Faculty of Agriculture, Cairo University, Giza, Egypt.

Six potato genotypes, namely Santa, Picasso, Diamant, Forella, 808, and 805 were used in the present experiments**.** The cultivars Santa, Picasso, and Diamant were received from Agriculture Research Center, Giza, Egypt, while Forella cv. and the dihaploid genotypes 805 and 808 were received from IPK Gene bank, Aussenstelle Nord, Gross Lusewitz, Germany.

Experiment treatments:

This work included two experiments as follows:

1- Effect of sucrose and active charcoal concentrations on microtubers production in six different genotypes.

This experiment consisted of 96 treatments, i.e., 8 sucrose concentrations (8, 9, 10, 11, 12, 13, 14 and 15%) X 2 active charcoal levels (0 and 5 g/l) X 6 potato genotypes (4 tertraploid, namely Diamant, Picasso, Santa, Forella, and 2 dihaploid, namely 808 and 805).

2- Effect of BA, sucrose concentration and active charcoal level on microtuber production in three different potato cultivars.

This experiment included 12 treatments which was a combination of 3 sucrose concentrations (8, 10 and 12%), 2 active charcoal levels (0 and 5 g/l) X 2 BA (benzyl adenine) concentrations (5 and 10 mg/l). This experiment was conducted separately on 3 different potato cultivars, namely Diamant, Picasso and Santa.

Plant material

Potato tubers (*Solanum tuberosum* L. cvs Diamant, Picasso, and Santa) were established from apical meristems of sprouted tubers on MS medium (Murashige and Skoog, 1962) with 3% sucrose, 100mgl^{-1} myo-inositol, 0.1mgl^{-1} BA and solidified with 0.7% agar. pH was adjusted to 5.8 before autoclaving at 121°C for 25 min. Sprouts were grown under 16h photoperiod at 2500 lux of white cool light and incubated at 24°C for 4 weeks in growth chambers. Forella, 805 and 808 genotypes were produced from Gen bank, Germany as plantlets in tubes. Thereafter, Shoot of all genotypes, i.e., Santa, Picasso, Diamant, Forella, 808 and 805 were subcultured every 28 days in baby gars (250 ml capacity) containing 40 ml of the same previously described culture medium via nodel cuttings as described by Espinoza *et al*. (1985). Subcultures were repeated for 4 times before cultured on tuberization medium.

Tuberization medium and incubation condition

Tuberization medium consisted of MS macro and micro elements salts, 0.7% agar and the additives mentioned in the experiments, i.e., 6 sucrose concentrations (8, 9, 10, 11, 12, 13, 14 and 15%) as well as two active charcoal levels (0 and 5 g/l) in the first experiment and two BA concentrations (5 and 10 mg/l) , 3 sucrose concentration (8, 10 and 12%) and two active charcoal levels (0 and 5 g/l) in the second experiment. pH was adjusted to 5.8 before autoclaving at 121°C for 25 min.

Three shoots, each shoot containing 6 nodel, were cultured on different media cultures which were kept at 17°C in continuous dark.

Recording data

Data were recorded after 3 months from culture in the two experiments as follows:

1- Number of microtubers per jar.
2- Average weight of microtuber.
3- Yield of microtubers per jar.

The experimental design

The experiments were set in a three factors completely randomized design (CRD) in four replicates, each replicate consisted of 3 jars, each jar contained 3 plants. The three factors of the first experiment were 6 cultivars X 8 sucrose concentrations X 2 active charcoal levels, while in the second experiment, they were 2 BA concentrations, 3 sucrose concentration X 2 active charcoal levels. The data on different parameters were collected and statically analyzed. Thereafter, the significant differences between means of treatments were recorded at 5% level using L.S.D as described by Steel and Torrie (1960).

RESULTS

Experiment 1: Effect of sucrose concentration and active charcoal on microtubers production in six potato genotypes.

There were significant differences in microtuber number, microtuber average weight and yield per jar among the different genotypes. In this respect, Forella cultivar showed the best performance in all tuber characters and followed by genotype 808 in respect of microtuber number and followed by cv. Picasso regarding average weight of microtubers, and jar yield of microtubers. On contrast, genotype 805 showed the lowest value of microtuber number per jar, average microtuber weight and jar yield of microtubers, were recorded by genotypes Santa, 808 and 805, respectively. Increasing sucrose concentration from 80 to 120 g/l significantly increased number of microtubers, microtuber average weight and jar yield per jar. This was true in all genotypes (Table 1, 2 and 3). Further increase in sucrose concentration up to 13% led to death of all genotypes, except Forella cultivar (Data are not shown). Data presented in the same table reveal that adding active charcoal to the tuberization medium led to production of higher microtuber number per jar, and markedly increased both of average microtuber weight and microtuber yield per jar, as compared with medium without active charcoal. These results were true in all genotypes and at all sucrose concentrations of 80, 90, 100, 110 and 120 g/l.

The highest values of average microtuber weight (1.933 g) and microtuber yield per jar (18.027 g) were noticed in cv. Forella by using 12% sucrose in presence of active charcoal. Meanwhile, the highest number of microtubers per jar (11.3) was produced in genotype 808 in medium containing 12% sucrose + 5 g/l active charcoal.

Table. 1: Effect of concentrations of active charcoal (A.C), and sucrose on number of tuber / jar of some potato genotypes.

Genotypes	A.C (g /l)	Sucrose level (g/l)					Mean
		80	90	100	110	120	
Diamont	0	1.7	3	2	3.6	2.3	2.5
	5	3.3	4	5.3	5.6	7	5.1
Average		2.5	3.5	3.6	4.6	4.6	3.8
Picasso	0	2	2.7	2	2.6	3	2.5
	5	3.3	4	4	4	4.6	4
Average		2.7	3.3	3	3.3	3.8	3.2
Santa	0	1	1.7	1.3	2.3	2.3	1.7
	5	2.7	3	2.6	2.6	3.6	2.9
Average		1.8	2.3	2	2.5	3	2.3
Forella	0	3.3	3.7	5	6.3	7.3	5.1
	5	3.7	4	5.6	6.6	9.3	5.8
Average		3.5	3.4	5.3	6.5	8.3	5.5
805	0	٢	2.3	2.3	2.6	1.6	2.2
	5	2	2	2.6	3.3	5.6	3.1
Average		2	2.2	2.5	3	3.7	2.6
808	0	2.3	2	2.6	4	6	3.4
	5	2.3	2.3	4	4	11.3	4.8
Average		2.3	2.6	3.3	4	8.7	4.1
0		2.1	2.5	2.5	3.6	3.8	2.9
5		2.9	3.2	4.	4.4	٦,٩	4.4
Mean		2.5	2.9	3.31	4	5.4	

L.S.D. at 0.05 for Factor A (genotypes) 0.7
Factor B (A.C) = 0.4 Factor C (sucrose) = 0.7
A X B = 1.1 B X C = 0.9 A X C= 1.٧ A X B XC = 2.4

Table. 2: Effect of concentrations of active charcoal (A.C) and sucrose on tuber average weight (g) of some potato genotypes.

Genotypes	A.C (g /l)	Sucrose level (g/l)					Mean
		80	90	100	110	120	
Diamont	0	٠,٠٥٦	٠,٠٥٩	٠,١٠٨	٠,٢٩٦	٠,١٢٣	٠,١٢٩
	5	٠,٢٦٢	٠,٢٨٢	٠,٤٩٧	٠,٦١٤	٠,٧٧٠	٠,٤٨٥
Average		٠,١٥٩	٠,١٧١	٠,٣٠٣	٠,٤٥٥	٠,٤٤٧	٠,٣٠٧
Picasso	0	٠,٣٠١	٠,١١٦	٠,١٦٤	٠,١٥١	٠,٣٥٩	٠,٢١٨
	5	٠,٦٩٠	٠,٦٨٢	٠,٤٨٧	٠,٤٠٧	٠,٨٠٦	٠,٦١٥
Average		٠,٤٩٦	٠,٣٩٩	٠,٣٢٥	٠,٢٧٩	٠,٥٨٣	٠,٤١٦
Santa	0	٠,١١٨	٠,١٥٢	٠,١٨٧	٠,٤٠٥	٠,٦٢٣	٠,٢٩٧
	5	٠,١٦٧	٠,١٩١	٠,٢١٥	٠,٤٥٧	٠,٧٥٥	٠,٣٥٧
Average		٠,١٤٣	٠,١٧٢	٠,٢٠١	٠,٤٣١	٠,٦٨٩	٠,٣٢٧
Forella	0	٠,١٨٤	٠,٢٣٢	٠,٤٢٠	٠,٥٤٤	٠,٨٥٧	٠,٤٤٧
	5	٠,٢٩٦	٠,٤٤٥	٠,٦٢٧	٠,٨٢٣	١,٩٣٣	٠,٨٢٥
Average		٠,٢٤٠	٠,٣٣٩	٠,٥٢٣	٠,٦٨٣	١,٣٩٥	٠,٦٣٦
805	0	٠,١٢٩	٠,١٣٧	٠,١٧٧	٠,١٧٢	٠,٢٢٨	٠,١٦٩
	5	٠,١٥٥	٠,١٥٨	٠,١٨١	٠,٢٤٥	٠,٤٧٦	٠,٢٤٣
X		٠,١٤٢	٠,١٤٨	٠,١٧٩	٠,٢٠٨	٠,٣٥٢	٠,٢٠٦
808	0	٠,٠٦٨	٠,٠٧٣	٠,١٢٢	٠,١٤٩	٠,١٦٧	٠,١١٦
	5	٠,١٤٠	٠,١٥٨	٠,١٧٥	٠,٢٥٥	٠,٦٨٦	٠,٢٨٣
Average		٠,١٠٤	٠,١١٦	٠,١٤٨	٠,٢٠٢	٠,٤٢٧	٠,١٩٩
٠		٠,١٤٣	٠,١٩٦	٠,٣٩٣	٠,٣١٩	٠,٤٦٧	٠,٢٢٩
٥		٠,١٢٩	٠,٢٨٦	٠,٢٨٥	٠,٣٦٤	٠,٩٠٤	٠,٤٦٨
Mean		٠,٢١٤	٠,٢٢٤	٠,٢٨٠	٠,٣٧٦	٠,٦٤٩	

L.S.D. at 0.05 for Factor A (genotypes) = 0.0٣٨
Factor B (A.C) = 0.224 Factor C (sucrose) = 0.035
A X B = 0.055 B X C = 0.050 A X C= 0.087 A X B XC = 0.123

Table. 3: Effect of concentrations of active charcoal (A.C), and sucrose on jar yield (g) of some potato genotypes.

Genotypes	A.C	Sucrose level (g/l)					Mean
		80	90	100	110	120	
Diamont	0	٠,١١٨	٠,٢٦٦	٠,١٤٣	١,٠٦٤	٠,٢٩٧	٠,٣٧٨
	5	٠,٨٥٦	٠,٧٨٦	٠,٧٠٥	٢	٣,٥٩٤	١,٥٨٩
Average		٠,٤٨٧	٠,٥٢٦	٠,٤٢٤	١,٥٣٢	١,٩٤٦	٠,٩٨٣
Picasso	0	٠,٦٠٣	٠,٣٠٩	٠,٢٦٥	٤	١,٠٥٠	٠,٥٢٥
	5	٢,٢٦٩	٢,٦٧٠	١,٨٢٠	١,٦٦٦	٣,٨٤٢	٢,٤٥٣
Average		١,٤٣٦	١,٤٨٩	١,٠٤٢	١,٠٣٣	٢,٤٤٦	١,٤٨٩
Santa	0	٠,١٠٩	٠,٢١٨	٠,٣٢٧	١,٠٢٥	١,٧٢٣	٠,٦٨٠
	5	٠,٤٢٣	٠,٥٠٤	٠,٥٨٥	١,٦٩٠	٢,٧٦١	١,١٩٣
Average		٠,٢٦٦	٠,٣٦١	٠,٤٥٦	١,٣٥٨	٢,٢٤٢	٠,٩٣٧
Forella	0	٠,٦٣٢	٠,٨٤٨	٢,٠٤٨	٣,٣٢٥	٥,٧٣٩	٢,٥١٨
	5	١,٠٦٦	١,٧٦٢	٣,٥٠٢	٥,٤١٦	١٨,٠٢٧	٥,٩٥٤
Average		٠,٨٤٩	١,٣٠٥	٢,٧٧٥	٤,٣٧٠	١١,٨٨٣	٤,٢٣٦
805	0	٠,٢١٣	٠,٣٢٦	٠,٣٦٧	٠,٤٦٥	٠,٤٥٦	٠,٣٦٥
	5	٠,٣٠٩	٠,٣٣٨	٠,٤٩٥	٠,٨٣٢	٢,٢٧٧	٠,٨٥٠
Average		٠,٢٦١	٠,٣٣٢	٠,٤٣١	٠,٦٤٨	١,٣٦٧	٠,٦٠٨
808	0	٠,١٦٥	٠,١٤٨	٠,٣٢٢	٠,٥٩٤	٠,٩٨٧	٠,٤٤٣
	5	٠,٣٣٦	٠,٣٧٢	٠,٧٧٦	٠,٩٩٢	٦,١٧١	١,٧٣٠
Average		٠,٢٥١	٠,٢٦٠	٠,٥٤٩	٠,٧٩٣	٣,٥٧٩	١,٠٨٦
0		٠,٣٠٧	٠,٣٥٣	٠,٥٧٩	١,١٤٥	١,٧٠٩	٠,٨١٨
5		٠,٨٧٧	٠,٨٧٧	١,٠٧٢	١,٣١٤	٦,١١٢	٢,٢٩٥
Mean		٠,٥٩٢	٠,٧١٢	٠,٩٤٦	١,٦٢٢	٣,٩١٠	

L.S.D. at 0.05 for Factor A (genotypes) = 0.580
Factor B (A.C) = 0.335 Factor C (sucrose) = 0.530
A X B = 0.821 B X C = 0.749
A X C= 1.298 A X B XC = 1.836

Experiment 2: Effect of BA concentration, sucrose concentration and active charcoal level on microtubers production in three different potato cultivars.

BA at 10 mg/l significantly increased number of microtubers per jar and microtubers yield per jar in cv. Picasso and Diamant as well as average microtubers weight in cv. Diamant as compared with the concentration of BA at 5 mg/l. On the contrary, in cv. Santa the reverse was true, where number of microtubers /jar, average microtubers weight and jar yield of microtubers were greater when BA was used at 5 mg/l, as compared with the concentration of 10 mg/l. Adding active charcoal to medium in presence of BA at 10 mg/l significantly increased number of microtubers /jar, average microtubers weight and jar yield of microtubers. This was true in all cultivars. In contrast, adding active charcoal to MS medium having 5 mg BA/l led to significant reduction in number of microtubers /jar in cv. Santa and Picasso and jar yield of microtubers in cv. Santa. Meanwhile, cv. Diamante responded differently to active charcoal in presence of BA at the concentration of 5 mg/l, where active charcoal promoted greater number of microtubers /jar, average microtubers weight and jar yield of microtubers. Increasing sucrose concentration significantly increased number of microtubers per jar in cv. Picasso at both concentrations of BA; i.e., 5 and 10 mg/l. Meanwhile, increasing sucrose concentration in the medium of the cultivars Diamante and Santa had a different effect, depending on BA concentration. Whereas

the increase in sucrose concentration led to an increase in the number of microtuber in presence of 10 mg BA/l, it caused a decrease in microtuber numbers in presence of 5 mg BA/l. Concerning effect of sucrose concentration on average microtubers weight and microtubers yield per jar, data in the same Tables reveal that both of average microtubers weight and microtubers yield per jar were promoted by raising sucrose concentration in presence of BA at 10 mg/l in medium. These results were true in all cultivars. On the other hand, in presence of BA at 5 mg/l medium increasing sucrose concentration from 80 to 120 g/l increased average tuber weight in the three cultivars. Meanwhile, such increase in sucrose concentration at the concentration of 5 mg/l BA led to increment, reduction and no effect in microtubers yield per jar in cv. Picasso, Santa and Diamant, respectively. The highest values of number of microtubers per jar, average microtubers weight and microtubers per jar were recorded by using 12% sucrose + 10 mg BA/ l in presence of active charcoal in cv. Diamant and Picasso.

On the other hand, in cv. Santa the highest values of microtubers number per jar, average microtubers weight and microtubers per jar were produced by the utilization of 5 mg BA/l +8% sucrose without active charcoal, 5 mg BA + 12% sucrose + 5 g active charcoal and 10 mg BA+ 12% sucrose + 5 g active charcoal, respectively.

Table. 4: Effect of concentrations of benzyl adenine (BA), active charcoal (A.C) and sucrose on tuber number per jar, average tuber weight (g) and jar yield (g) of Santa cultivar.

BA (mg / l)		Tuber number/ jar	Average tuber weight (g)	Jar yield (g)
10		2.4	٠,٢٠٣	٠,٥٣٢
5		٣,٠	٠,٢٧١	٠,٧٢٩
L.S.D		٠,٤	٠,٠٢٦	٠,٠٧٩
A.C (g / l)				
٠		٣,٧	٠,١٨٢	٠,٥١٢
٥		٢,٧	٠,٢٩٢	٠,٧٤٨
L.S.D at 0.05		٠,٤	٠,٠٤٦	٠,٠٧٩
Sucrose (g / l)				
٨٠		٣,١	٠,١٧٤	٠,٥٦٠
١٠٠		٢,٦	٠,٢٠٦	٠,٥٥١
١٢٠		٢,٣	٠,٣٣٠	٠,٧٧٩
L.S.D at 0.05		٠,٥	٠,٠٣٢	٠,٩٧٨
BA X AC				
10	0	١,٨	٠,١٢٠	٠,١٩٣
	5	٣,٠	٠,٢٨٦	٠,٨٧١
5	0	٣,٧	٠,٢٥٤	٠,٨٣٢
	5	٢,٣	٠,٢٩٨	٠,٦٧٥
L.S.D at 0.05		٠,٦	٠,٣٦٧	٠,١١٣
BA X Sucrose				
١٠	٨٠	٢,١	٠,١٣٦	٠,٢٧١
	١٠٠	٢,٣	٠,٢٠٦	٠,٤٨٢
	١٢٠	٢,٨	٠,٢٦٨	٠,٨٤١
	٨٠	٤,١	٠,٢١٤	٠,٨٤٩
	١٠٠	٣,٠	٠,٢٠٦	٠,٦٢٠

Table. 5: Effect of concentration of benzyl adenine (BA), active charcoal (A.C) and sucrose on tuber number per jar, average tuber weight (g) and jar yield (g) of Diamant cultivar.

BA (mg / l)	Tuber number/ jar	Average tuber weight (g)	Jar yield (g)
10	4.8	٠,٣٦٣	١,٩١٤
5	٣,٤	٠,٢٠٢	٠,٧٣١
L.S.D	٠,٥	٠,٠٦١	٠,١٣٨
A.C (g / l)			
٠	٣,٠	٠,١٤٦	٠,٣٦٥
٥	٥,٢	٠,٤١٥	٢,٢٨٠
L.S.D at 0.05	٠,٥	٠,٠٦١	٠,١٣٨
Sucrose (g / l)			
٨٠	٤,٣	٠,٢٦٣	١,١١٠
١٠٠	٣,٩	٠,٢٦٦	١,٢٥٩
١٢٠	٤,١	٠,٣١٩	١,٦٠٠
L.S.D at 0.05	٠,٦	٠,٦٠١	٠,١٦٩
BA X AC			
10 0	٣,٦	٠,١٧٩	٠,٤٧١
5	٦,١	٠,٥٤٧	٣,٣٥٨
0	٣,٠	٠,١١٣	٠,٢٥٩
5	٥,٢	٠,٢٨٧	١,٢٠٣
L.S.D at 0.05	٠,٧	٠,٦٩٤	٠,١٩٥
BA X Sucrose			
١٠ ٨٠	٤,٥	٠,٣٧٣	١,٥١٦

	١٠٠	٤,٤	٠,٣٤٣	١,٧٣٢
	١٢٠	٥,٦	٠,٣٧٥	٢,٤٩٥
	٨٠	٤,١	٠,١٥٣	٠,٧٠٣
	١٠٠	٣,٥	٠,١٨٩	٠,٧٨٥
	١٢٠	٢,٥	٠,٧٦٣	٠,٧٠٦
L.S.D at 0.05		٠,٩	٠,١٠٦	٠,٢٣٨
A.C X Sucrose				
٠	٨٠	٣,٤	٠,١٦٥	٠,٣٠٥
	١٠٠	٢,٦	٠,١٣٩	٠,٤٠٠
	١٢٠	٣,٠	٠,١٣٤	٠,٣٩٠
	٨٠	٥,٣	٠,٣٦	١,٩١٤
	١٠٠	٥,٣	٠,٣٩٣	٢,١١٧
	١٢٠	٥,١	٠,٥٠٤	٢,٨١٠
L.S.D at 0.05		٠,٩	٠,١٠٦	٠,٢٣٨
BAX AC X Sucrose				
10	0 80	٣,٥	٠,٢٤١	٠,٣٠٢
	100	٣,٣	٠,١٧٦	٠,٦٠٦
	120	٤,٠	٠,١٢١	٠,٥٠٦
	5 80	٥,٥	٠,٥٠٤	٢,٧٣١
	100	٥,٥	٠,٥١٠	٢,٨٥٩
	120	٧,٣	٠,٦٢٨	٤,٤٨٤
5	0 80	٣,٣	٠,٠٨٩	٠,٣٠٨

Table. 6: Effect of concentration of benzyl adenine (BA), active charcoal (A.C) and sucrose on tuber number per jar, average tuber weight (g) and jar yield (g) of Picasso cultivar

BA (mg / l)	Tuber number/ jar	Average tuber weight (g)	Jar yield (g)
10	٣,٣	٠,٣٥٣	١,٣٢٨
5	١,٨	0.367.	٠,٦٨٢
L.S.D	٠,٤	٠,٠٣٥	٠,١٢٣
A.C (g / l)			
٠	٢,٤	٠,٢٩٢	٠,٦٨٧
٥	٢,٦	٠,٤٣٧	١,٣٢٣
L.S.D at 0.05	٠,٤	٠,٠٣٥	٠,١٢٣
Sucrose (g / l)			
٨٠	٢,٣	٠,٢٢٩	٠,٥٤٢
١٠٠	٢,٣	٠,٢٩٤	٠,٧٠٠
١٢٠	٣,٠	٠,٥٧٠	١,٧٧٣
L.S.D at 0.05	٠,٤	٠,٠٤٢	٠,٢١٣
BA X AC			
10 0	٢,٧	٠,١٨٧	٠,٤٨٧

	5	٣,٩	٠,٥٢٠	٢,١٧٠
5	0	٢,٢	٠,٣٩٧	٠,٦٨٧
	5	١,٣	٠,٣٥٤	١,٣٢٣
L.S.D at 0.05		٠,٥	٠,٠٤٩	٠,١٧٤
BA X Sucrose				
١٠	٨٠	٢,٩	٠,٢٦٩	٠,٧٦٢
	١٠٠	٢,٣	٠,٢٧١	٠,٨٨٣
	١٢٠	٣,٠	٠,٥٢١	٢,٣٤٠
	٨٠	٣,٠	٠,١٩٠	٠,٣٢٣
	١٠٠	٣,٨	٠,٣١٨	٠,٥١٧
	١٢٠	٥,٠	٠,٦١٩	١,٢٠٦
L.S.D at 0.05		٠,٦	٠,٠٦٠	٠,٢١٣
A.C X Sucrose				
٠	٨٠	٢,٤	٠,١٨٩	٠,٤٣٢
	١٠٠	٢,١	٠,٢٦٧	٠,٥٤٩
	١٢٠	٢,٨	٠,٤٢١	١,٠٧٩
٥	٨٠	٢,٣	٠,٢٦٩	٠,٦٥٢
	١٠٠	٢,٤	٠,٣٢١	٠,٨٥١
	١٢٠	٣,٣	٠,٧١٩	٢,٤٦٧
L.S.D at 0.05		٠,٦	٠,٠٦٠	٠,٢١٣
BAX AC X Sucrose				
10	0	80	٢,٨	٠,١٨١	٠,٤٧١
		100	٢,٣	٠,١٤٧	٠,٣٢٤
		120	٣	٠,٢٣٣	٠,٦٦٥
	5	80	٣	٠,٣٥٦	١,٠٥٣
		100	٣,٨	٠,٣٩٥	١,٤٤١
		120	٥	٠,٨٠٩	٤,٠١٥
5	0	80	٢	٠,١٩٧	٠,٣٩٤

DISCUSSION

In the present investigation, there were significant differences among the different genotypes in the number of microtubers per jar, average microtubers weight and microtubers yield per jar (Table 1, 2 and 3). The average microtubers number per jar ranged from 1 in cv. Santa to 11.3 in the genotype 808. Meanwhile, the average microtubers weight ranged from 0.056g in cv. Diamant to 1.933 g in cv. Forella. On the other hand, microtubers yield per jar ranged from 0.109 g in cv. Santa to 18.027 g in cv. Forella. Such levels were produced from culturing 3 shoots per jar. The previous studies revealed that Zakaria (2003) harvested 8.12 microtubers per Erlenmeyer flask of cv. Diamant. Hossain (2005) Obtained from 0.5 to 4 microtubers per test tube having three micro plants and found that the

average microtubers weight ranged from 0.066 g to 0.105 g and the yield of each tube ranged from 0.103 g to 0.407 g, depending on genotype. The differences in genotype response are due to the varietal differences and their interaction to the different environmental condition. The highest values of the number of microtubers per jar, average microtubers weight and microtubers yield per jar in the present work was obtained in all cultivars, except in cv. Santa, in medium contented 10 mg BA / l + 12% sucrose and 5g/l active charcoal. Hossain (2005) used in his study MS medium containing 5 mg BA/l + 500 mg CCC/l + 8% sucrose in absence of active charcoal. The result in respective of average microtubers weight indicated obtaining high value up to 1.933 g (in cv. Forella). Simko (1990), Hossain and Sultan (1998), Zakaria (2003) and Yassmin (2005) obtained a mean microtubers weight of about 100 mg from culturing their cultivars in the dark. In contrast, Randhawa and Chamder (1990) obtained much higher microtubers weight ranging from 0.850 to 2.060 g in MS +10 mg BA/l + 8% sucrose in six Indian potato cultivars, which is contradictory to the findings of the present investigation. Probably, varietal differences and their response are main reasons for such a good performance for some genotypes (Tovar *et al.* 1985).

The present study proved that increasing sucrose concentration up to 12% in medium containing BA at concentration of 10 mg/l led to improving of microtuberization. This concentration of sucrose caused a significant increase in number of microtubers per jar, microtubers average weight and microtubers yield per jar. These results were achieved in all tested genotypes in the two conducted experiments (Table1-6). On the other hand, in medium containing 5 mg BA/l, increasing sucrose concentration increased number of microtubers in cv. Picasso, whereas this concentration of sucrose decreased number of microtubers in cv. Diamant and Santa. The previous study indicated that the highest microtubers per flask were obtained by using 6% sucrose in presence of 5 mg BA/l as compared with sucrose level at 8, 10 and 12 % (Islam *et al*. 1999). In other studies, the best medium for inducing microtubers production contained 8% sucrose + 3 mg BA/l (Yan-Zhong and Guo –Dezhang, 2004; Gopal *et al*. 2004), 6% sucrose + 1 µM BA (Tariq *et al*. 2004). Also, Sidikuo *et al*. (2003) studied the effect of sucrose level at 2 -12% and BA at 0-5 mg /l on tuberization and found that tuberization increased with increasing concentration of BA and sucrose. The results indicated the importance of the interaction between sucrose level and BA concentration. The present investigation also indicated that BA at 10 mg/l increased microtuberization in cv. Picasso and Diamant, as compared with BA at 5 mg/l, whereas the reverse was achieved in cv. Santa. As it was mentioned before addition of cytokinin to agar medium promoted tuberization in the darkness (Palmer and Swith 1970). The optimum concentration of BA for microtuberization was 10 mg for some cultivers (Randhawa and Chamder 1990) and 5 mg/ l for other (Randhawa and Chamder 1990; Sidikuo *et al.* 2003). This clearly indicates that different genotypes are mainly responsible for such a wide variation. Adding active charcoal to medium in presence of BA at 10 mg/l significantly increased number of microtubers /jar, average tuber weight and jar yield of microtubers per jar. This was true in all cultivars. In contrast, active charcoal adding to MS medium having 5 mg BA/l led to

significant reduction of number of microtubers /jar and jar yield of microtubers in cv. Santa and Picasso. Meanwhile, cv. Diamante responded differently to active charcoal in presence of BA at the concentration of 5 mg/l, where active charcoal promoted greater number of microtubers /jar, average microtubers weight and jar yield of microtubers. The previous investigations indicated that medium containing activated charcoal gave the highest rate of tuberisation and the largest microtubers in potato. It thus played a role in optimizing conditions for rapid, mass tuberization of potato cultivars, and produced large microtubers for field planting (Bizarri. *et al*. 1995; Wang *et al*. 2006). Generally, it was previously proved that media with charcoal had substantially lower levels of phenylacetic and O-OH benzoic acids, compounds that may inhibit tuberization (Fridborg *et al*. 1978). Activated charcoal also has been shown to absorb 5- hydroxymethyl furfural, an inhibitor formed by sucrose degradation during autoclaving, as well as substantial amounts of auxin and cytokinins. Thus it may absorb inhibitors that would prevent growth as well as reduce the level of growth promoters that would cause continued proliferation.

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بَعْض العوامل المؤثرة علي عملية تكوين الدرينات في البطاطس

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اجريت تجربتين لدراسة تأثير بَعْض العوامل المؤثرة علي عملية تكوين الدرينات في البطاطس مثل استخدام البنزايل ادنين بتركيز (10,5 mg /l)، السكروز بتركيز (٨, ١٠, ١٢, ١٣, ١٤ و١٥ %) واضافة الفحم النشطِ في بيئة الزراعة بتركيز (صفر, ٥ g/l) و ذلك باستخدام ٦ من

انـــواع و اصـــناف البطـــاطس، أربعـــة tetraploid هـــم : Diamant، Picasso، Santa وForella وإثنان dihapliod: ٨٠٥ و٨٠٨. انواع و اصناف البطاطس كان لها رد فعل مختلف لكلا من هذه العواملِ، الصنفForella . اظهر أفضل أداءٍ من ناحية عددِ الدرينات لكلّ برطمان،و متوسط وزن الدرينات لكل برطمان، ومحصول البرطمان من الدرينات. زيادة تركيز السكروز إلى ١٢ % واضافة الفحم النشط بتركيزِ g /l ٥ الي بيئة الزراعة المحتوية علي mg /l ١٠ بنزايل ادنين ادي الي حدوث زيادة ملحوظة في اوزان واعدادِ الدرينات في كُلّ الاصناف. من ناحية اخرى، زيادةُ تركيزَ السكروز أو اضافة g /l ٥ من الفحم نشط إلى بيئة MS المحتوية علي mg /l 5 بنزايل ادنين حفز عملية تكوين الدرينات في بعض الاصناف وثبطها في البعض الاخر