

## IV. RESULTS AND DISCUSSION

### I. Surface Sterilization.

In this regard three concentrations of sodium hypochlorite (NaOCl) (1.0, 1.5 and 2-0%), each concentration was combined with three immersion time (10, 15 and 20 min) were investigated. Mercuric chloride (HgcL<sub>2</sub>) at 0.1% was also investigated for surface sterilization for 10 min.

Surface sterilization treatments were investigated regarding their effect on contamination, browning, survival and growth percentages of both shoot tip and stem node sections of the two investigated rootstocks (Hansen 536 peach and MAC 9 apple) under study.

Data obtained from the conducted four experiments are presented in Tables (1-5).

#### I. Hansen 536 rootstock:

## I.i. Shoot tip:

Regarding the contamination response to the various treatments of surface sterilization, it is quite clear as shown from Table (1) and fig(1) that the highest concentration of NaOCI (2.0%) revealed the lowest number of shoot tip contaminated explants, and decreased contamination percentage, while the lower concentration of NaOCI (1.0%) gave the highest number and subsequently highest contamination percentage. Concerning the immersion duration, it is evident from Table (1) and Fig. (1) that shoot tip contamination %. reduced gradually by increasing the immersion-duration. The superior immersion duration was 20 min. regardless the NaOCI

concentration. The interaction between NaOCl concentration and immersion duration, it is clear that shoot tips sterilized by using NaOCl at 2.0% for 20 min followed by dipping in ethanol for 5 min reduced significantly the number of shoot tip contaminated explants for both season of study.

Concerning the contamination percent, it is obvious from Table (1) and Fig. (1) that contamination percent reduced gradually with increasing both NaOCl concentration and immersion duratim the 9<sup>th</sup> treatment 2.0% (NaOCl followed by dipping in ethanol for 5 min) gave the lowest rate of contamination percent during both season of study. Meanwhile, the reverse was true with the 1<sup>st</sup> treatment (NaOCl 1.0%). Immersing shoot tip explants for 10 min increased significantly contamination percent as compared with the other treatments NaOCl at 2.0% and ethanol for 2 min. combined with immersion for 20 min in the commercial bleech was the best treatment in reducing the shoot tip contamination percent.

Data represented in Table (1, 2) and Fig. (1,2) showed that shoot tip explants of Hansen 536 peach rootstock were affected by all surface – sterilization treatments with regard to browning, survival and growth percentage. It can be concluded that the lowest rate of browning that combined with the highest growth level was induced by using NaOCl at 1.5% for 20 min.

Referring to the influence on survival percentage of shoot tip explants, the highest survival % was obtained by dipping the explants in 70% ethanol for 5 min followed by using NaOCl at 2.0% for 20 min for both seasons of study.

## I.ii. Stem node sections:

Concerning stem one - node cutting as affected by surface sterilization treatments, it is clear from Table (1,2) and Fig. (1,2),

that all investigated parameters were affected significantly but the response to various treatments was less than that obtained with shoot tip explants. It could be concluded that lowest rate of both contamination and browning that associated with the highest survival and growth levels were induced by using 70% ethanol for 5 min followed by dipping in 2.0% NaOCl for 20 min.

Mercuric chloride (HgcL<sub>2</sub>) at 0.1% was used as a surface sterilizer for both explants (shoot tips and stem node sections) of Hansen 536 peach rootstock. It was used to compare its efficiency with NaOcl<sub>2</sub>.

Data in Table (5) and Fig.(5) revealed that all investigated parameters including contamination, browning, survival and growth percentages did not improve by using HgcL<sub>2</sub> at 0.1% for 10 min.

On the contrast, it reduced both growth and survival percentages as compared with NaOCl for both two types of explants for both seasons of study .

NaOCl at 2% was more effective than the other concentrations (1.0 and 1.5%) in relation to enhance all the previous parameters.

## II. MAC 9 apple rootstock.

Data presented in Table (3, 4) and Fig. (3, 4) showed the effect of disinfection solution concentration and immersion duration se on contamination, browning, survival and growth percentages of two explant types (shoot tip and stem node section) of MAC 9 apple rootstock.

### II.i. Shoot tips

Concerning contamination and survival percentage, data showed that the lowest contamination and the highest survival percentage were achived with 2.0% NaOCl for 20 min followed by dipping in 70% ethanol for 2 min for both seasons. On the other hand, 2%

NaOCl for 2 min was the superior treatment for increasing growth percentage as well as reducing browning percentage for both seasons of study.

## II. ii. Stem one- node cutting:

It is quite evident from Tables (3, 4) and (Fig. 3,4) that treatment (9) (immersion in 2.0% NaOCl for 20 min followed by dipping in 70% ethanol for 5 min) was the most effective treatment in this respect as it resulted in reducing both contamination and browning but raised survival and growth percentages for both seasons. On the other hand, treatment 1 (immersion in 1.0% NaOCl for 20 min) gave the highest level of contamination and lowest level of survival percentage. Meanwhile the highest browning percentage and the lowest growth percentage were recorded with treatment 7 (immersion in 1.0% NaOCl for 20 min) for both seasons of study.

Date presented in Table (6) and Fig. (6) reflect the on relation between the investigated parameters (contamination, browning, survival, and growth %) of the two types of explants (shoot tip and stem one - node cutting ) of MAC9 apple rootstock and the two surface sterilizers (NaOCl and HgCL<sub>2</sub>).

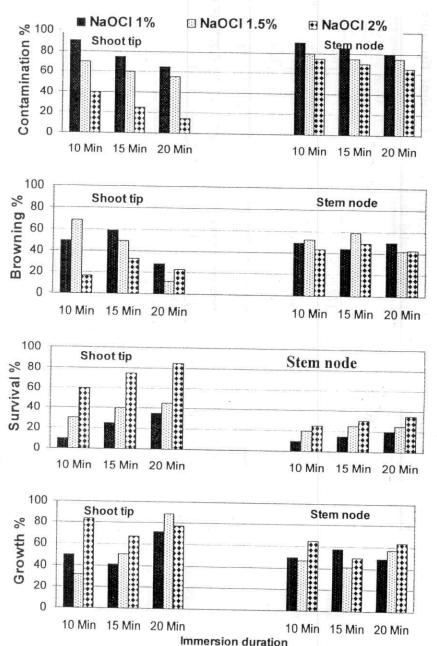
It is quit evident that 2.0% NaOCl encouraged a significant increase in shoot tip survival percentage while significantly reduced contamination for both seasons of study. The other two parameters (browning and growth) had insignificant difference regardless NaOCl concentration. Using mercuric chloride at 0.1% was not effective in this respect. The effect of the different treatments on stem one - node cutting parameters followed the same trend of shoot tip parameters.

immersion duration on and sodium hypochlorite concentration  $_{
m 0}$ Effect Table (1):

	contamination	tion, bro	contamination, browning, survival and growth percentages of shoot tip and stem node	vival	and	grow	th p	erce	ntages	of:	shoot	tip a	ınd st	еш 1	ode
	sections of	Hansen 5	sections of Hansen 536-peach rootstock during 2006 season.	ootsto	ck d	uring	7, 200	o se	son.						
Dress.	Parameters		Shoot tip	tip					100,000	Sten	Stem node sections	secti	suo		
	Tourstments	Cont.	Browning	Survival	val	Growth	vth	ت ا	Cont.	Bro	Browning %	Sur	Survival %	S.	Growth %
	ū	90.0 A		1 0.01		50.0		0.06		50.0	В	10.0	王	50.0	O
	1.0%+10 min NaOCl	70.0 AB	68.0 A	30.0 E	FG	31.9	ш	80.0	BC	52.8	В	20.0	C	47.2	C
	ii	40.0 E	16.6 DF	0.09	S	83.4	4	75.0	BCD	43.9	O.	25.0	BC	65.1	В
	2.0%+10 min NaOCl	75.0 BC	58.9 B	25.0	U	41.1	Δ	85.0	AB	44.4	O	15.0 D		57.6	В
	1.0%+15 min NaOCl	* 0.09	49.5 C	40.0	DE	50.5	U	75.0	BCD	58.9	¥	25.0	BC	41.1	D
	1.5%+15 min NaOCl	25.0 F	33.1 D	75.0	В	76.99	В	70.0	CD	50.0	В	30.0	O	50.0	O
	2.0%+15 min NaOCI	65.0 CD	28.4 DE	35.0	EF	9.17	В	80.0	BC	50.7	В	20.0	O	49.4	C
	1.0%+20 min NaOCI	55*>0 D	11.5 F	45.0	٥	88.5	A	75.0	ВСД	42.4	S	25.0	BC	57.1	В
	1.5%+20 min	15.0 F	23.2 E	85.0	<	76.8	В	65.0	О	42.9	U	35.0	Y	63.9	A
	2.0%+20 min													^	

Means followed by the same letter within each column for each category are not significantly different from each other at 1% level

Fig. (1): Effect of sodium hypochlorite concentration and immersion duration on contamination, browning, survival and growth percentages of shoot tip and stem node sections of Hansen 536-peach rootstock during 2006 season.

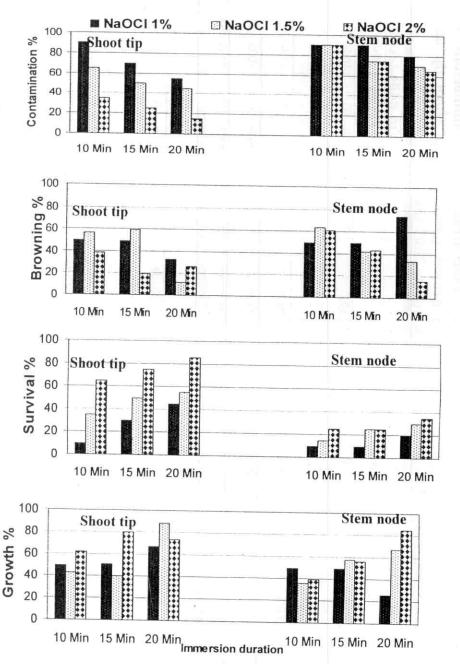


(2): Effect of sodium hypochlorite concentration and immersion duration on contamination, browning, survival and growth percentages of shoot tip and stem node sections of Hansen 536-peach rootstock during 2007 season. Table

Parameter		Shoot tip	t tip			Stem node sections	sections	
	Cont.	Browning	Survival	Growth	Cont.	Browning	Survival	Growth
Treatments	%	%	%	%	%	%	%	221
Na0Cl	90.0 A	50.0 B	10.0 G	50.0 F	90.0 A	50.0 C	10.0 E	50.0 E
1.0%+10 min							- 1	
NaOCI	65.0 B	56.6 AB	35.0 F	43.5 G	90.0 A	63.9 B	15.0 DE	36.1 F
1.5%+10 min							- 1	- 1
NaOCI	35.0 E	38.2 C	65.0 C	61.8 E	90.0 A	8 9.09	25.0 BC	39.4 F
2.0%+10 min								- 1
NaOC1	70.0 B	49.1 B	30.0 F	51.0 F	90.0 A	50.0 C	10.0 E	50.0 DE
1.0%+15 min					- 1			
NaOC1	50.0 CD	59.7 A	50.0 DE	40.3 G	75.0 BC	42.2 D	25.0 BC	ر د د
1.5%+15 min					- 1	- 1	- 1	
NaOCI	25.0 F	19.8 E	75.0 B	80.2 B	75.0 BC	43.2 D	25.0 BC	26.4 CD
2.0%+15 min						- 1	- 1	- 1
NaOCI	55.0 C	32.8 CD	45.0 E	67.2 D	80.0 B	73.9 A	20.0 CD	26.1 G
1.0%+20 min					- 1	- 1	- 1	
NaOCI	45.0 D	11.7 E	55.0 D	88.3 A	70.0 CD	33.3 A	30.0 AB	66.7 B
1.5%+20 min				- 1		- 1		- 1
NaOCI	15.0 G	26.3 D	85.0 A	73.1 C	65.0 D	15.1 F	35.0 AB	84.9 A
2.0%+20 min								

Means followed by the same letter within each column for each category are not significantly different from each other at 1% level.

Fig. (2):Effect of sodium hypochlorite concentration and immersion duration on contamination, browning, survival and growth percentages of shoot tip and stem node sections of Hansen 536-peach rootstock during 2007 season.

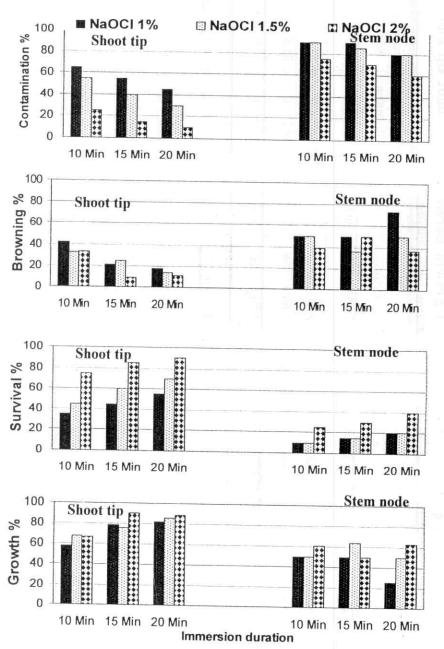


hypochlorite concentration and immersion duration on contamination, browning, survival and growth percentages of shoot tip and stem node of sodium Effect Table (3):

sections	sections of MAC 9 Apple rootstock during 2006 season.	9 Ap	ole roo	tstock	duri	ng 2(	)06 se	ason.					ı	I	
Parameter			Shoot tip	t tip			1			Stem	node	Stem node sections	suc	0	
/	Cont.	Bro	Browning	Survival	ival	Gro	Growth	Cont.	nt.	Browning %	ning	Survival	ival	Growth %	th Th
I reatments	% A 059	42.1	0/ V	35.0	H	57.9	ш	0.06	4	50.0	В	10.0	В	50.0	В
1.0%+10 min															
NaOCI	55.0 B	32.8	В	45.0	C	67.2	ш	0.06	A	50.0	М	10.0	ш	20.0	m
1.5%+10 min													(	t	
NaOCI	25.0 D	33.1	В	75.0	U	6.99	DF	75.0	BC	39.3	U	25.0	ပ	60.7	∢
2.0%+10 min				×											Ī
NaOC1	55.0 B	21.6	Э	45.0	D	78.4	BC	0.06	Ą	50.0	В	15.0	DE	50.0	m
1.0%+15 min									2			- 1		000	
Na0Cl	40.0 C	24.7	C	0.09	ш	75.4	CD	85.0	AB	36.1	υ	15.0	DE	63.9	₹
1.5%+15 min														0	0
NaOC1	15.0 E	9.3	Е	85.0	В	200.	K	70.0	Ω	50.0	В	30.0	n	20.0	n
2.0%+15 min												6	0		Ţ
NaOCI	45.0 C	17.5	D	55.0	Ľ,	82.5	82.5 ABC	80.0	BC	73.9	Ą	20.0	CD	70.1	ر
1.0%+20 min												0	0		
NaOC1	30.0 D	13.9	DE	70.0	В	86.1	AB	80.0	BC	20.0	m	70.0	2	20.0	n
1.5%+20 min								4			(	0 0 0		- 5	~
NaOC1	10.0 E	11.0	ш	0.06	A	89.0	A	0.09	ъ	36.9	ی	40.0	₹	03.1	ζ.
2.0%+20 min															

Means followed by the same letter within each column for each category are not significantly different from each other at 1% level

Fig. (3): Effect of sodium hypochlorite concentration and immersion duration on contamination, browning, survival and growth percentages of shoot tip and stem node sections of MAC 9 Apple rootstock during 2006 season.



survival and growth percentages of shoot tip and stem node concentration and immersion duration on hypochlorite of sodium Effect <u>4</u> Table

	contam	contamination, browning, survival and growth percentages of shoot up and stem more	, br	IUM0.	ng, su	rviva	and	gro	win p	ercen	lages	5	1001	The date	100		
	sections of MAC 9 Apple rootstock during 2007 season.	s of MA	6 J	App	le root	stock	duri	ng 20	07 sea	son.			ı				Ī
V	Parameter				Shoot tip	t tip						Stem	Stem node sections	section	suc	-	
		Cont.		Brov	Browning	Survival	ival	Growth	wth	Cont.	nt.	Brov	Browning o/	Survival	ival	Growth	vth
Ξ	Treatments	%		o`	%	%		,	%	0/			0	0/	,	7	0
	NaOCI	€0.09	A	36.8	A	40.0	ഥ	63.2	L.	0.06	K	50.0	m	10.0	ن ن	20.0	2
ij	1.0%+10 min									- 1		6	,		(		٥
	NaOCI	35.0 I	Ω	30.6	В	65.0	Ω	69.4	ш	0.06	¥	50.0	m	10.0	<u>ں</u>	20.0	<u>۔</u>
	1.5%+10 min														0	9	(
	NaOCI	25.0 E	Ш	26.5	В	75.0	ပ	73.6	Ω	85.0	AB	36.1	U	15.0	S S	63.9	ر
	2.0%+10 min		ļ			1		2									I
L	NaOCI	55.0 E	В	21.3	C	45.0	LT.	78.7	ပ	85.0	AB	50.0	В	15.0	BC	36.1	ਧ
	1.0%+15 min									- 1			4		,	0	0
	NaOCI	25.0 F	ш	26.5	В	75.0	U	73.6	Ω	80.0	m	20.0	n	70.0	n	20.0	٦
	1.5%+15 min									- 1	(		C	0	,	3 63	Ç
	NaOC1	15.0	Ö	5.9	ш	85.0	В	94.1	Ą	70.0	Ü	32.5	ر	30.0	₹	0.70	ر
	2.0%+15 min													0	,		1
	NaOCI	45.0 (	C	9.1	ш	55.0	ш	6.06	4	80.0	m	63.9	<	20.0	n	20.0	2
	1.0%+20 min											,	¢	0	٥	1	0
	NaOCI	20.0	ш	18.5	CD	80.0	BC	81.5	BC	80.0	n	79.1	n	70.0	מ	6.07	۵
	1.5%+20 min															0.40	Ţ
	NaOCI	5.0 F	Н	15.8	Ω	95.0	Ą	84.2	В	65.0	ာ	1.5.	K	33.0	∢	×4.7	ζ.
	2.0%+20 min																

Means followed by the same letter within each column for each category are not significantly different from each other at 1% level

Fig. (4): Effect of sodium hypochlorite concentration and immersion duration on contamination, browning, survival and growth percentages of shoot tip and stem node sections of MAC 9 Apple rootstock during 2007 season.

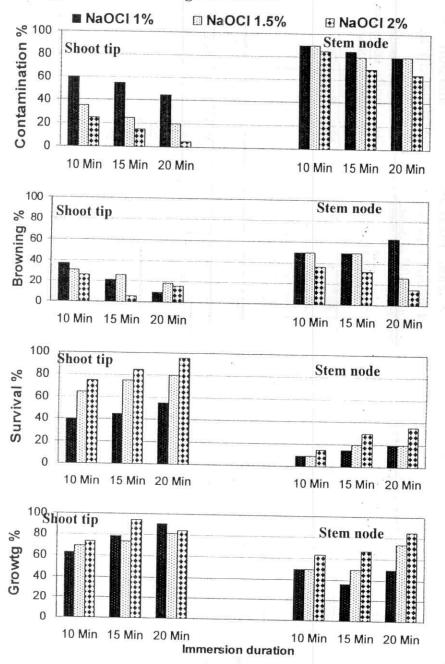


Fig. (5): Effect of some different concentration of sodium hypochlorite and Mercuric chloride on contamination, browning, survival and growth percentage of shoot tip and stem node sections of Hansen 536-peach rootstock during 2006 and 2007 seasons.

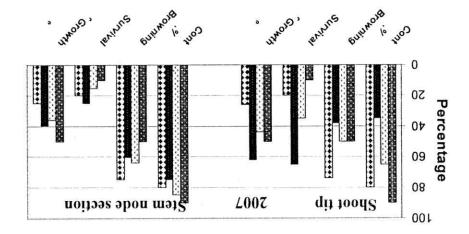


Table (5): Effect of some different concentration of sodium hypochlorite and Mercuric chloride sections of Hansen 536-peach rootstock during 2006 and 2007 seasons. on contamination, browning, survival and growth percentage of shoot tip and stem node

Parameter				Shoo	Shoot tip				1	E	Sten	node	Stem node sections	ons		
/	Cont		Brov	Browning	Survival	/ival	Gr	Growth	Co	Cont	Bro	Browning	Survival	ival	Growth	vth
/	%			%	%	o		%		%		%	%	0,	%	
Treatments								2006	)6							
4	90.0 A		50.0	A	0.01	0	50.0	В	90.0	A	50.0	Α	0.01	В	50.0	>
1% +10 min																200
NaOCI	70.0 B		68.1	A	30.0	₩	31.9	0	80.0	A	52.8	A	20.0	A	47.2	>
1.5%+10 min																
NaOCI	40.0 C		16.6	Α	60.0	A	83.4	Α	75.0	В	43.9	Α	25.0	A	56.1	<b>A</b>
2%+10 min							2								11	
Mercuric 0.1	85.0 A		36.1	Α	15.0	С	63.9	В	90.0	A	50.0	Α	0.01	В	50.0	Þ
mg/L +10 min																
								2007								e anne
NaOCI	90.0 A		50.0	B	10.0 D	J	50 0 A	Α	90 0 A	Α	50 0	Δ	0.01	ם ב	500	
1% +10 min			V - 20 - 20 - 20 - 20 - 20 - 20 - 20 - 2					,		1		:				,
NaOCI	65.0 B		50.0	В	35.0	B	43 5	A	850	B	639	Α	150	)	1 92	>
1.5%+10 min	1					1	į	;		ţ		:			00.1	2
NaOCI	35.0 C		38.2	C	65.0	Α	618	Α	750	J	60 6	Α	0 5 0	Δ	304	
2%+10 min	1		9					;		,	00.0	;	0.0			,
Mercuric 0.1	80.0 A		73.9	Α	20.0	C	26.1	Α	80.0	0	75.0	>	20.0	D	050	<b>&gt;</b>
mg/L +10 min	1				1					(		;	1		0	;
6 11		1				The second second	Name and Address of the Owner, where		The second							

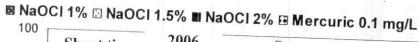
different from each other at 1% level. Means followed by the same letter within each column for each category are not significantly

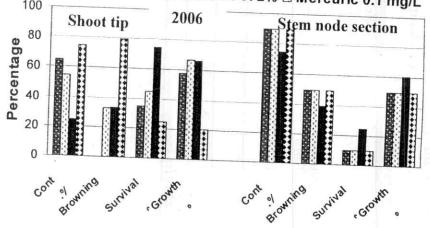
on contamination, browning, survival and growth percentages of shoot tip and stem node Table (6): Effect of some different concentrations of sodium hypochlorite and Mercuric chloride

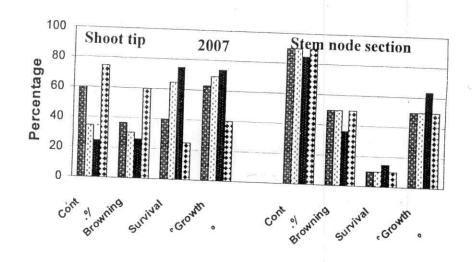
T J T T T T T T T T T T T T T T T T T T	olan A 3	MAC 9 rootstock during 2006 and 2007 seasons.	stock duri	ing 2006 an	d 2007 seas	ons.		
sections of Ap	ol Apple		+ + in			Stem node sections	sections	N ×
Parameter		Sucortup	dn	17	Cont	Browning	Survival	Growth
	Cont.	Browning %	Survival %	Growth %	% %	%	0%	%
<i></i>	./0	0/		2006	9(		- 1	
Treatments			0 0 50	579 A	90.0 A	50.0 A	10.0 B	50.0 A
NaOCI	65.0 AB	42.1 B	33.0				- 1	
1% +10 min	- 1	- 1	45.0 B	A 272 A	90.0 A	50.0 A	10.0 B	S0.0 A
NaOCI	55.0 B	32.8 B					- 1	- [
1.5%+10 min		-	1	66.0 A	75.0 B	39.3 A	25.0 A	60.7 A
NaOCI	25.0 C	33.1 B	A 0.67				•	- 1
2%+10 min		- 1		306 B	90 0 A	50.0 A	10.0 B	50.0 A
Mercuric 0.1	75.0 A	79.4 A	72.0 D	20.07				
mg/L +10 min				2007			. 7	
				- 1	0 00	SO 0 A	10.0 A	50.0 A
NaOCI	60.0 B	36.8 B	40.0 C	63.2 A	V 0.06			- 1
1% +10 min		- 1		V 103	A 0.06	50.0 A	10.0 A	50.0 A
NaOCI	35.0 C	30.6 B	65.0 B	+: 60				
1.5%+10 min		- 1	- 1	73.6 A	85.0 A	36.1 A	15.0 A	63.9 A
NaOCI	25.0 C	26.5 B	A 0.0/					
2%+10 min		- 1	0 0 0 0	40.0 B	90.0 A	50.0 A	10.0 A	50.0 A
Mercuric 0.1	75.0 A	60.0 A	7 0.62					
mg/L +10 min				-		L sotatory	are not si	uificantly
Mann follows		by the same letter within each column for each calcgoly are not of	etter within	n each colu	mn ior cac	II calcgory		
• Means Iollowed		10, 10, 10, 10, 10	levio					

Means followed by the same letter within cach different from each other at 1% level

Fig. (6): Effect of some different concentrations of sodium hypochlorite and Mercuric chloride on contamination, browning, survival and growth percentages of shoot tip and stem node sections of Apple MAC 9 rootstock during 2006 and 2007 seasons.







## П. Establishment stage:

- **II. I. Effect of BAP or 2ip solely:**
- П. II. Hansen 536 peach rootstock

#### II. IIa - shoot tip.

Data of Tables (7 and 8) and Fig. (7 and 8) reflected the effect of culturing shoot tips of Hansen peach rootstock on MS basel medium supplemented with either BAP or 2ip on some parameters which including (browning, survival, growth percentages and average shoot elongation). Each investigated cytokinin was added to the culture medium at three levels (0.5, 1.0 and 1.5 mg/L).

It is clear from Tables (7 and 8), Fig. (7) and Photo (1-A1) that BAP at 1.5 mg/L resulted in a significant reduction of browning while significantly maximized both survival and growth percentages as compared with the other BAP levels and control. Meanwhile BAP at 1.5 mg/L, showed significant increase in average shoot elongation as compared with the cytokinin omitted medium. Meanwhile significance was appeared between BAP at 0.5 and 1.0 mg/L from on side and BAP at 1.5 mg/L and cytokinin omitted medium from the other side. It could be concluded that the highest parameter values were associated with the highest BAP in vestigated level.

Concerning the effect of 2ip levels (0.5,1.0 and 1.5 mg/L) in culture medium on shoot tip parameters. Table (8), Fig. (8) and Photo (1-A2), clarify that culturing shoot tips on MS medium containing 1.5 mg/L 2ip encouraged significantly survival, growth percentages and average shoot elongation while it reduced

significantly browning percentage in relation to the other two 2ip levels.

#### Π. III. Stem node section:

Regarding the response of browning, survival, growth percentages and average shoot elongation developed from stem one - node cutting explants excised from Hansen 536 peach rootstock, to the type of (BAP or 2iP) and its levels (0,0.5,1.0 and 1.5 mg/L) of each investigated cytokinin, data obtained during 2006 and 2007 seasons are presented in Tables (7 and 8) and Figs. (7 and 8).

It is quite clear that all culturing media supplemented with the three levels (0.5, 1.0 and 1.5 mg/L) of BAP improved the three studied parameters, than the control.

Growth and survival percentages were increased statistically as affected by all BAP levels. However the increase was more pronounced with stem one - node cutting explants cultured on MS medium supplemented with 1.5 mg/L BAP. On the other hand, browning percentage was reduced significantly. As BAP level increase, the maximum reduction in explants browning increased specially with the highest BAP level (1.5 mg/L).

Regarding the response of average shoot elongation raised from stem one - node cutting explants, data in Table (7) revealed that the three levels of BAP which were added to culturing media as well as BAP omitted medium were statistically matched, but it could be noticed that 1.5 mg/L BAP was more effective (Photo 1-B1).

Moreover, 0.5 mg/L BAP added to MS basel medium came in the second rank in this respect as shown from Figure (7).

Dealing with the effect of 2ip levels supplied to culture media on stem one - node cutting parameters, it appears from Table (8), Fig. (8) and Photo (1-B2) that the addition of 2ip at 1.5 mg/L to culture medium induced the best result as compared with 2ip omitted medium (control) . It reduced browning percentage significantly, while significantly maximized survival and growth parameters as compared with the other two levels.

Concerning, the average shoot elongation parameter, developed from stem node section, data from Table (8) showed that there was no significant differences between the treatments. Meanwhile the all 2ip investigated concentrations improved average shoot elongation than the control on the other hand 0.5 mg/L 2ip added to culture medium was the best in this respect as shown in Fig. (8).

In general, the previous results reflected that , adding BAP at 1.5 mg/L to MS medium is recommended to achieve the highest survival, growth percentages derived from both shoot tip and stem node explants of Hansen 536 peach rootstock as well as it reduced browning percentage of both types of explants.

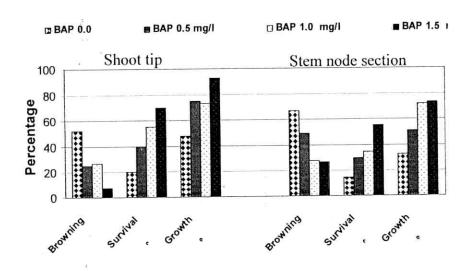
As for the average of shoot elongation parameter, 1.5 mg/L BAP is recommended for best result for both explants (shoot tip and stem node section).

Table (7): Effect of different BAP concentrations on shoot tip and stem node sections developed parameters of Hansen 536 peach rootstock during 2006.

Parameters  BAP  Concentrations  0.0  1.0	Browning (%) 52.2 A 25.3 B 27.2 B	ning (6) A A B B B	Survival (%) 20.0 D 40.0 C 55.0 B		Shoottip  (%) shootom  (%) shooting  (%) shooting  47.8 C 0.7 B  74.7 B 1.0 AB  72.8 B 1.0 AB	tip rowth (%) 8 C 7 B 3 B	el 6.7 1.0 1.0	ge of ot tion :)	Brov (° 66.7 66.7 49.1	0%) (%) 7 A 1 A 4 B	Stem node section   Stem node section     Browning   Survival   Growth   Average	ival C C B B B	Stem node section vival Growth (%)  C 33.3 B 1.  B 51.0 B 1.	vth (	eld eld 11.4	Average of shoot elongation (cm.) A A
1.5	7.17 C		0.07	<	70.0 A 92.8 A 1.6 A	<	1.6		27.1	В	27.1 B 55.0 A 72.9 A 1.6 A	4	72.9	- Y	9.1	
* Moone falleng 1		-	THE STATE OF THE PERSON NAMED IN	THE SHEET	The Persons Services	-		BAZETY SACRESCHES	No. of Concession, Name of Street, or other Persons and Street, or other P			1				

are not significantly Means followed by the same letter within each column for each category different from each other at 1% level

Fig. (7): Effect of different BAP concentrations on shoot tip and stem node sections developed parameters of Hansen 536 peach rootstock during 2006.



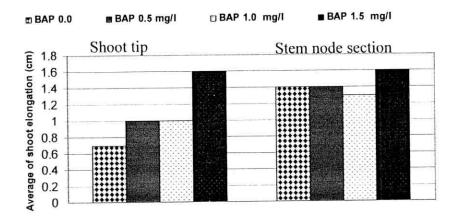
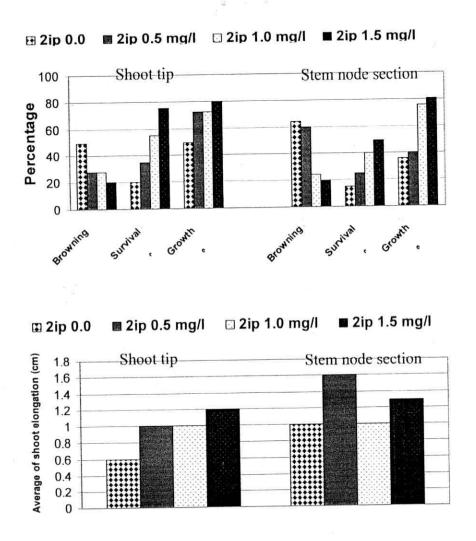


Table (8): Effect of different 2ip concentrations on shoot tip and stem node sections developed parameters of Hansen 536 peach rootstock during 2006.

Parameters		Sh	Shoot tip			Stem noc	Stem node section	
2ip concentrations	Browning (%)	Survival (%)	Browning Survival Growth (%) (%) (%)	Average of shoot clongation (cm.)	Browning Survival Growth (%) (%)	Survival (%)	Growth (%)	Average of shoot elongation
0.0	50.0 A 20.0 C 50.0 B 0.6 B	20.0 C	50.0 B	0.6 B	63.9 A 15.0 B 36.1 B 1.0 A	15.0 B	36.1 B	1.0 A
0.5	27.8 B	35.0 C	35.0 C 72.2 A 1.0 A		60.0 A	25.0 B	40.0 B 1.6 A	1.6 A
1.0	27.7 B	55.0 B	72.3 A	27.7 B 55.0 B 72.3 A 1.0 AB	24.2 B 40.0 A 75.8 A 1.0 A	40.0 A	75.8 A	1.0 A
1.5	20.0 B	75.0 A	75.0 A 80.02 A 1.2 A	1.2 A	19.4 B	50.0 A 80.7 A 1.3 A	80.7 A	1.3 A

Means followed by the same letter within each column for each category are not significantly different from each other at 1% level.

Fig. (8): Effect of different 2ip concentrations on shoot tip and stem node sections developed parameters of Hansen 536 peach rootstock during 2006.



-54-

# II. 111. MAC 9 apple rootstock

# II. 111 a – shoot tip:

Data presented in Table (9) describe the effect of BAP levels in culturing media on shoot tip development parameters of MAC 9 apple rootstock. It is clear from Table (9) and Figure (9) that all BAP investigated levels were effective in reducing browning percentage significantly as compared with BAP omitted medium. Figure (9) showed that browning reduced gradually with increasing BAP level in culture medium and the maximum reduction of browning was achieved with the highest BAP level (1.5 mg/L). Meanwhile the other two investigated parameters, survival and growth were improved significantly when BAP was used in culture medium as compared with control. The improvement was associated with BAP level in culture medium i.e the two parameters increased as BAP level increased in culture medium. 1.5 mg/L BAP was the best treatment in this respect. However, average shoot elongation parameter was induced statistically with BAP levels as compared with control. Meanwhile, 0.5 mg/L BAP treatment was highly succeded for inducing significant difference with shoot elongation parameter, photo (2-a) when the other two levels (1.0 and 1-5 mg/L) of BAP were concerned.

Different oncentations of 2ip,0.5,1.0 and 1.5 mg/L added to MS culture media were also investigated in relation to shoot tip parameters. Data from Table (10) and Figure (10) revealed that all studied parameters were improved with the addition of 2ip to culture medium as compared with 2ip omitted medium (control). It is obvious clear that 1.5 mg/L 2ip reduced browning. On the other hand, it increased both survival and growth percentages.

Meanwhile, 2ip at 0.5 mg/L was the best level when average shoot elongation parameter was concerned (photo 2-b).

## **∏ 111. b. Stem node section:**

Data presented in Tables (9 and 10) and Figures (9 and 10) show clearly the beneficial effect of either BAP or 2ip each at three levels (0.5, 1.0 and 1.5 mg/L) which added to MS basal medium in increcising stem one - node cutting parameters of MAC 9 apple rootstock over cytokinin omitted medium. It is clear that addition of BAP or 2ip solely increased significantly survival and growth percentages over the control. On the contrast, they reduced significantly browning percentage. 1-5mg/L was the promising level for both cytokinins in this respect. The other two levels (1.0 and 0.5 mg/L) of both cytokinin (BAP or 2ip) came in the second and third rank respectively as shown from Figures (9 and 10).

Regarding average shoot elongation arised from stem node section, as affected by different levels of either BAP or 2ip. It is clear from Tables (9 and 10) that all BAP or 2ip levels added to culture media were statistically alike but there was an increament of shoot elongation combined with cytokinin level in culture medium,i-e- shoot elongation increased with increasing cytokinin level and reach the maximum elongation with the highest level (1-5 mg/L) of any used cytokinin as shown from Figures (9 and 10).

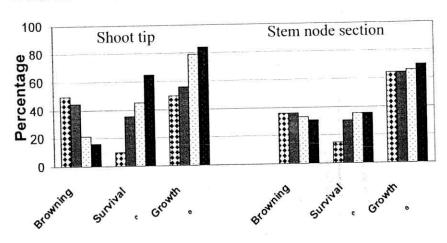
Table (9): Effect of different BAP concentrations on shoot tip and stem node sections developed parameters of MAC 9 Apple rootstock during 2006.

Parameters		Sho	Shoot tip	l l		Stem no	Stem node section	
2ip eoncentrations	Browning Survival Growth (%) (%) (%)	Survival (%)	Growth (%)	Average of shoot elongation (cm.)	Browning Survival Growth (%) (%) (%)	Survival (%)	Growth (%)	Average of shoot elongation
0.0	50.0 A	10 C	50.0 B	1.0 A	50.0 A 10 C 50.0 B 1.0 A 36.0 A	15.0 A 63.9 A 0.6 A	63.9 A	0.6 A
0.5	44.3 AB 35 B 55.8 B 1.8 A	35 B	55.8 B	1.8 A	35.9 A	30.0 AB 64.1 A 1.9 A	64.1 A	1.9 A
1.0	21.6 BC 45 B	45 B	78.4 A 1.2 A	1.2 A	33.2 A 35.0 A 66.1 A 1.7 A	35.0 A	66.1 A	1.7 A
1.5	16.2 C 65 A 83.8 A 1.2 A	65 A	83.8 A	1.2 A	30.4 A	35.0 A 69.6 A 1.4 A	A 9.69	1.4 A

\* Means followed by the same letter within each column for each category are not significantly different from each other at 1% level.

Fig. (9): Effect of different BAP concentrations on shoot tip and stem node sections developed parameters of MAC 9 Apple rootstock during 2006.





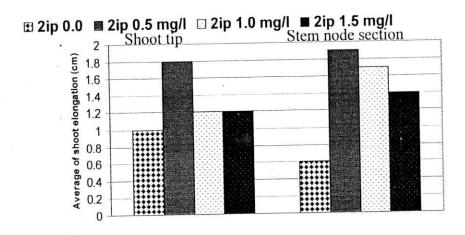
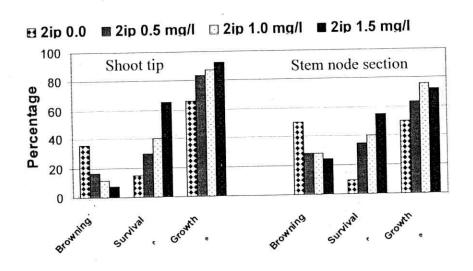


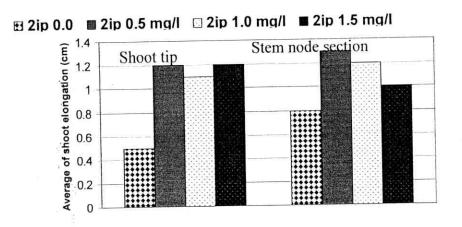
Table (10): Effect of different 2ip concentrations on shoot tip and stem node sections developed parameters of MAC 9 Apple rootstock during 2006.

Parameters		Sho	Shoot tip			Stem n	Stem node section	
2ip concentrations	Browning Survival	Survival (%)	Growth (%)	Average of shoot elongation (cm.)	Browni ng (%)	Survival (%)	Growth (%)	A)
0.0	36.0 A	15.0 C 65.9 B	65.9 B	0.5 B	50.0 A	50.0 A 10.0 C	50.0 B 0.8 B	(cm.)
0.5	17.0 B	30.0 B	30.0 B 83.2 A	1.2 A	28.4 B	28.4 B 35.0 B	63.0 A 1.3 A	1.3 A
1.0	12.2 B	40.0 B	40.0 B 87.1 A	1.1 A 24.3 B 40.0 B	24.3 B	40.0 B	75.7 A 1.2 AB	1.2 AB
1.5	7.7 B	65.0 A 92.3 A		1.2 A	28.5 B	55.0 A	28.5 B 55.0 A 71.5 A 1.0 AB	1.0 AB

\* Means followed by the same letter within each column for each category are not significantly different from each other at 1% level.

Fig. (10): Effect of different 2ip concentrations on shoot tip and stem node sections developed parameters of MAC 9 Apple rootstock during 2006.





II. ii - Effect of different combinations between BAP and 2ip

П iii: Hansen 536 peach rootstock:

П iii a- Shoot tip:

Depending on the data derived from using either BAP or 2ip solely, different combinations between BAP and 2ip were investigated concerning shoot tip parameters.

Data in Table (11) and Figure (11), indicate that all combinations between BAP and 2ip succeded in increasing significantly survival and growth percentages as compared with cytokinin omitted medium (control). The maximum inceament of both parameters was achived with the 3<sup>rd</sup> treatment (2.0 mg/L BAP + 2.0 mg/L 2ip) for both seasons of study. On the other hand, all combinations succeeded in reducing browning parameter significantly as compared with control the third treatment was superior in this respect.

Dealing with average shoot elongation parameter as affected by the different combinations between BAP and 2ip. It is clear from Table (11) that statistical differences were nil among the all studied combinations between BAP and 2ip when shoot elmgation parameter was concerned during the two seasons of study.

However, the 4<sup>th</sup> treatment (2-5 mg/L BAP + 2.5 mg/L 2ip) was effective in improving shoot elongation photo (1-A3) followed by the 2<sup>nd</sup> treatment (1.5 mg/L BAP + 1.5 mg/L 2ip) for both seasons of study as shown from Figure. (11)

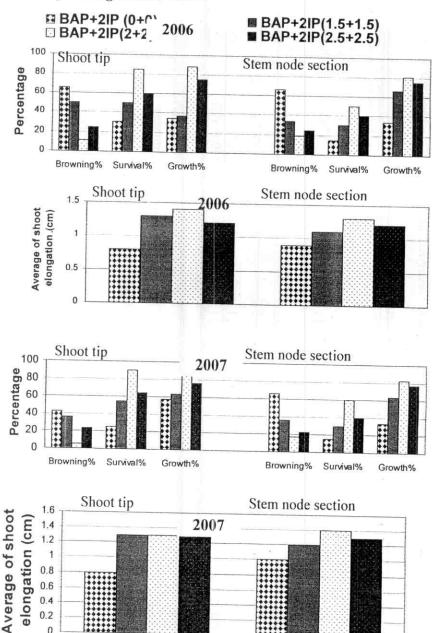
cutting explants of Hansen 536 peach rootstock (recorded after 4 weeks ) through establishment stage during 2006 and 2007. Table (11): Effect of different BA +2ip concentrations on some measurements of cultured shoot tip and one node

Evalente		Shoot tip	ot tip		e e	Stem node section	de section	
Shanda								
Parameters		Survival	Growth (%)	Browning Survival Growth Average of Browning Survival	Browning (%)	Survival (%)	Growth (%)	Average of shoot
BA + 2ip concentration		<u> </u>		elongation (cm.)	0			elongation (cm.)
(1/8m)		65.2 A 30.0 C	34.8 B 0.8	В	66.7 A 15.0 C 33.3 B	15.0 C	33.3 B	0.9 A
0.0 - 0.0	50.0 A	50.0 B	37.0 B	A	33.2 B 30.0 B	30.0 B	66.8 A	1.1 A
0.1 - 0.1		850 A	88.6 A	+	19.2 B	50.0 A	19.2 B 50.0 A 80.8 A	1.3 A
2.5 + 2.5	24.5 AB	24.5 AB 60.0 A		_	24.8 B	40.0 AB 75.3 A	75.3 A	1.2 A

						Ctom	ado coction	
Explants		Sh	Shoot tip			Stelli II	Stelli Hode section	
Parameters  BA + 2ip  concentration  (mo/l)	II.	Browning Survival	Growth (%)	Average of Browning Survival shoot (%) (%) (%) (%) (cm.)	Browning (%)	Survival	Growth (%)	Average of shoot elongation (cm.)
00+00	A C C P		25 C 578 B 0.8 B		66.7 A 15 D	15 D	33.3 B	1.0 A
0.0 + 0.0	36.2 A		63.8 B		35.9 B	30 C	64.1 AB	1.2 A
0.1 + 0.1	5.6 B	90 A		A	17.5 B	60 A	82.5 A	1.4 A
2.5 + 2.5	23.4 AB	65 B	3.4 AB 65 B 76.6 AB	1.5 A	22.9 B	40 B	77.1 A	1.3 A

Means followed by the same letter within each column for each category are not significantly different from each other at 1% level.

Fig. (11): Effect of different BA +2ip concentrations on some measurements of cultured shoot tip and one node cutting explants of Hansen 536 peach rootstock (recorded after 4 weeks ) through establishment stage during 2006 and 2007.



0.2 0

#### П ii b - Stem node section:

Dealing with the effect of different combinations between BAP and 2ip on stem node parameters. Data from Table (11) and Figure (11), reflected that responses of stem node explants parameters to different investigated cytokinin combinations were less than those noticed in shoot tip parameters.

Table (11) indicated that culturing stem node explants on culture medium supplemented with different combinations of BAP and 2ip improved studied parameters as compared with cytokinin omitted medium. Herin, The 3<sup>rd</sup> treatment (2.0 mg/L BAP + 2.0 mg/L 2ip) induced and caused significantly an improvement of survival, growth and average of shoot elongation parameters photo (1-B3) as well as it reduced browning percentage for both seasons of study .

## II 2 MAC 9 apple rootstock

## II2a - shoot tip:

Data of Table (12) show the effect of different BA + 2ip concentrations on shoot tip devloped parameters of MAC 9 apple rootstock. It is clear that all combinations improved significantly all investigated parameters as compared with control for both seasons as shown from Figure (12) and photo (2-C). The 4 Th treatments (2.5 mg/L BAP + 2.5 mg/L 2ip) was the superior one in this respect, where as it maximized growth and survival percentages as well as it reduced significantly the browning percentage. Concerning shoot elongation parameter it is clear from Table (12) that statistical differences were nil among the all studied treatments. However, the 4th treatment (2.0 mg/L BAP + 2.0 mg/L 2ip), improved shoot elongation parameter as compared with the other combinations for both seasons as shown from Figure (12).

#### Π 2b - Stem node section:

Data from Table (12) reflected that cytokinin combinations induced significant increase of survival parameter over the control for both seasons. Survival parameter was highly responded to the highest cytokinin combinations (2.5 mg/L BAP + 2.5 mg/L 2ip) as shown from Figure (12). Dealing with browning. Table (12) revealed that, the 3 rd treatment (2-0 mg/L BAP + 2-0 mg/L 2ip), caused a remarkable browning reduction for both seasons as shown from Figure (12). Growth parameter was highly affected by the 4th treatment (2.5 mg/L BAP + 2.5 mg/L 2ip). Concerning shoot elongation parameter, it is clear from Table (12), that all cytokinin combinations improved shoot elongation as compared with cytokinin omitted medium, the best result was obtained with the addition of 2.5 mg/L of both cytokinin to culture medium as shown from Figures (12). These results are in general agreement with the findings of Dantas et al., (2002) and El- Hammady et al., (2004) who found that the longest shoot during the establishment stage were recorded for Hansen cultivar cultured in MS medium with 2.0 mg/L BAP.

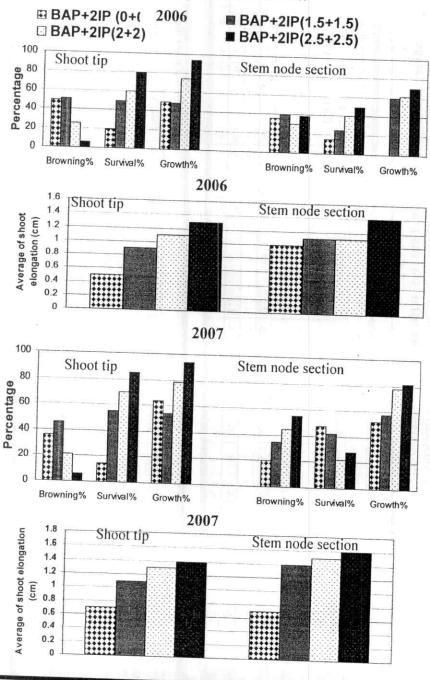
on some measurements of cultured shoot tip and one node cutting

explants of MAC 9 apple rootstock (recorded after 4 weeks ) through establishment stage during 2000 and 2007 experimental seasons.	9 apple re il seasons.	ootstocl	k (re	corded	after 4 weeks ) t 2006	hrougn esta	Dilsnmen	stage uni	ms 2007 SIII
Explants			Sh	Shoot tip			Stem no	Stem node section	
			-	-	L		Survival	Crowth	Designation Curvival Growth Average of
Parameters	Browning Survival Growth (%) (%)	Surviv:	[E.	Growth (%)			(%)	(%)	shoot
BA + 2ip					ealongation cm.				(cm.)
(1/200)									
(m/g) (m)	500 A 20 C 50 B 0.5	20	ľ	30 B	0.5 B	36.1 A	15 B	36.1 A 15 B 63.9 B 1.0	1.0 A
	20.00	010	) [		A 00	40 0 A	25 B	40 0 A 25 B 60.0 B 1.1	1.1 A
1.5 + 1.5	51.0 A 50 B 49.0 B 0.3	20	n	49.0 D		200	400	81 S B	1 1
2.0 + 2.0	26.1 B 60 B 73.9 A 1.1	09	В	73.9 A	1.1 A	30.0 B	40	30.0 B 40 A 61.3 B	
	6.3 C 80 A 93.7 A 1.3	80	A	93.7 A	1.3 A	38.5 A	50 A	38.5 A 50 A 69.4 A 11.4	1.4 A
6:1			ı		THE REPORT OF THE PERSON NAMED IN COLUMN 1				

Committee		Sho	Shoot tip			Stem 1	Stem node section	
							Atres C	A vorono of
	Browning (%)	Survival (%)	Growth (%)	Average of shoot elongation (cm.)	Browning Survi (%) val (%)	val (%)	(%)	shoot shoot elongation (cm.)
(mg/L)				1		000	d cra	A 70
-	36 I A	15 D	63.9 C 0.7	В	47.8 A 20 B 52.2	S 07	27.7C	_
	7			ν	A 3 Ch	35 A 574	57.4 B	4. A
15 + 15	46.0 A	55 C	24.0 C	F. I.1	47.0 V	5		
	T	a 07	78.4 B	1.3 A	22.3 B	45 A	77.7 A	I.5 A
$7.0 \pm 0.7$	d 0.1	0	2		205 A 55 A 218	V 22	818	1 6 A
25 + 25	5.9 C	85 A	94.1 A	I.4 A	28.3 B	7 CC	0.10	2.

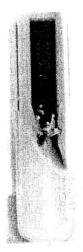
• Means followed by the same letter within each column for each category are not significantly different from each other at 1% level.

Fig. (12): Effect of BA +2ip concentrations on some measurements of cultured shoot tip and one node cutting explants of MAC 9 apple rootstock (recorded after 4 weeks ) through establishment stage during 2006 and 2007 experimental seasons.





1.5 mg/L BAP **A-1** 



1.5 mg/L 2ip **A-2** 



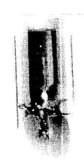
2.5 2ip+ 2.5 BAP mg/l A-3



1.5 mg/L BAP **B-1** 



1.5 mg/L 2ip B-2



2.0 2ip+ 2.0 BAP mg/l **B-3** 

Photo (1): Shoot elongation of Hansen 536 peach rootstock during establishment stage.

A. Shoot tip culture

B. Stem mode section.

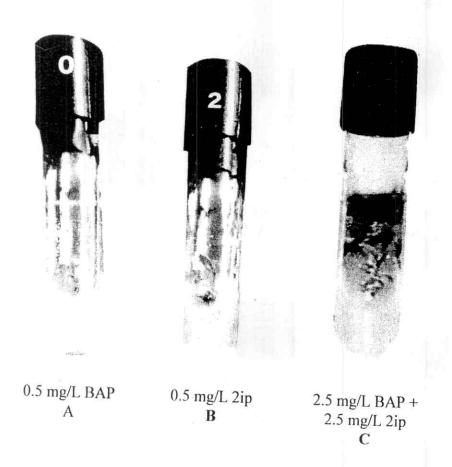


Photo (2): Shoot elongation of MAC 9 apple developed from shoot tip culture, during establishment stage.

#### Proliferation stage:

An experiment was carried out for each investigated rootstock (Hansen 536, peach rootstock, and MAC 9 apple rootstock), whereas three combinations between two concentrations of BAP (1.0 and 1.5 mg/L) and three concentrations of 2ip (0.5, 1.0 and 1.5 mg/L) were added to MS basal medium as well as MS cytoknin omitted medium (control) were used. The effect of the previous combinations on some multiplication parameters during proliferation stage was investigated. Obtained data were recorded in Tables (13, 14) and Fig. (13, 14) for Hansen 536, peach rootstock and MAC 9 apple rootstock respectively.

#### A. Hansen 536, Peach rootstock:

Data presented in Table (13) revealed clearly that MS basal medium supplemented with 1.0 mg/L BAP and 1.0 mg/L 2ip (treatment C) maximized most of the investigated parameters. It induced statistically the greatest values of average number of proliferated shoots, proliferated shoot percent and total length of proliferated shoots photo (3-A). The same trend was true during both seasons of study as compared with other treatments and control.

On the contrast, when the higher level of BAP (1.5 mg/L) was combined with the higher level of 2ip( 1.5 m/gL) i.e., treatment (D), come in the second rank in this respect, followed by treatment (B) (1.0 mg/L BAP + 0.5 mg/L 2ip) in descending order.

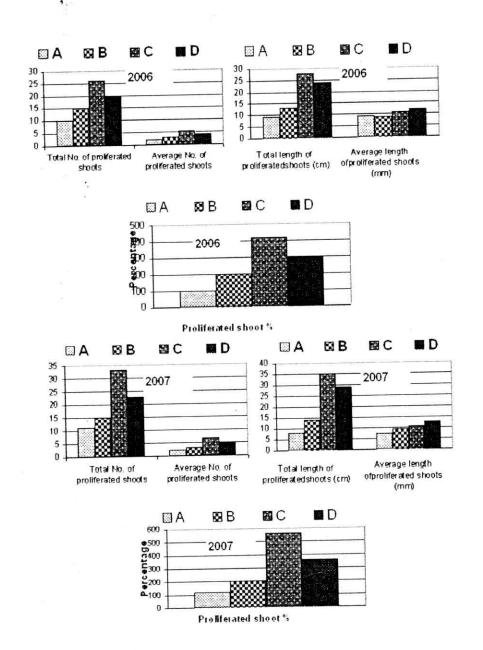
Regarding the fourth investigated parameter, average length of proliferated shoots as affected by the two different types of cytokinins (BAP and 2ip) which was added to MS basal medium,

Table (13): Effect of different combinations between BAP and 2ip on shoot tips proliferation parameters of Hansen 536, peach rootstock during 2006 and 2007 seasons.

Parameters		2006				2007					
Treatments	No	rage . of erated ots*	Prolife shoot		Average length of proliferated shoots* (mm)			Prolife shoot		Aver lengt prolife shoo (mr	h of rated ts*
Α	2.0	D	100	D	9.0 C	2.2	D	120	D	7.2	D
В	3.0	С	200	С	8.7 D	3.0	С	200	С	9.3	С
С	5.2	A	420	A	10.7 B	6.6	A	560	A	10.6	В
D	4.0	В	300	В	12.0 A	4.6	В	360	В	12.6	A

- A MS cytokinion omitted medium (Control)
- B MS + 1.0 mg/L BAP + 0.5 mg/L 2ip
- C MS + 1.0 mg/L BAP + 0.1 mg/L 2ip
- D MS + 1.5 mg/L BAP + 1.5 mg/L 2ip
- MS Murashige and Skoog medium supplemented with 30g/L sucrose and 7.0 g/L agar

Fig. (13): Effect of different combinations between BAP and 2ip on shoot tips proliferation parameters of Hansen 536, peach rootstock during 2006 and 2007 seasons.



date tabulated in Table (13) and Fig. (13) show clearly that adding both BAP and 2ip each at 1.5mg/L (treatment D) gave the greatest value of average length of proliferated shoots. Such trend was true during both seasons of study. Cytokinins omitted medium (control) revealed the lowest values of all investigated parameters during both seasons of study. These results are in harmony with **Bayomi** (1998) who recommended BAP than kinetin for the best proliferation of Communism pear.

### MAC 9 apple rootstock:

Regarding the effect of different combinations between BAP and 2ip added to MS basal culture medium on some multiplication parameter during proliferation stage, data presented in Table (14) and Fig. (14) reflect that adding BAP and 2ip to MS basal medium each at the higher level (1.5 mg/L) was the most effective treatment in enhancing and maximizing the values of all investigated parameters as compared with the other treatments. Such trend was true during both seasons of study. Whereas, treatment (C) came after the superior one in this respect.

However, the reverse was true with three investigated parameters (average No. of proliferated shoots, proliferated shoot percent and total length of proliferated shoots) when the shoots were cultured on cytokinin omitted medium (control). whereas the fourth investigated parameter (average length of proliferated shoots) reflected the least value when shoot culture medium was supplemented with BAP and 2ip each at 1.0 mg/L treatment (C)

It could be concluded from the previous results, that all investigated proliferation parameters of cultured shoots during multiplication stage was greatly responded to the addition of both cytokinins (BAP and 2ip ) to shoot culture medium. However, each rootstock followed its own trend, but it could be emphasized that the combination between BAP and 2ip each at 1.0 mg/L was the superior treatment with Hansen 536 peach rootstock. In addition the combination between the higher level (1-5 mg/L) of both cyloknins (BAP and 2ip ) was the most preferable treatment for MAC 9 apple rootstock photo (3-B) .

These results emphasized that shoot tip cultured of both studied rootstocks (Hansen 536 and Mac9) in medium containing BAP and 2ip together—enhanced shoot proliferation since both cytokinins maximized proliferation parameters proliferation rates were 4.9 and 5.0 fold increase for Hansen 536 and Mac9 rootstocks respectively, depending on cytokinin concentrations. These results are in agreement with the findings of **James and Thurbon (1981)**. Who reported that multiplication rate of M9 shoot culture vried between 2 and 4.5 fold depending on auxin: cytokinin levels. 1-2 mg/L BAP and 0.1 – 0.5 mg/L IBA were most fervouable although the auxin could be omitted without adversely affecting the multiplication rate. **Baruah** *et al.*, (1996) and Cos *et al.*, (2004), recommended 2.0 mg/L BAP for the best proliferation of apple and peach rootstocks.

## Effect of subculture number on shoot proliferation:

Shoots of both investigated rootstocks which were developed from shoot tip cultures through establishment stage were exiced, transferred and cultured in order to start this experiment. The best medium for each rootstock which improved all shoot parameters during establishment stage was selected for this experiment. Shoots of Hansen 536 peach rootstock were cultured

on MS medium supplemented with BAP and 2ip each at 1.0 mg/L, whereas shoots of MAC 9 apple rootstock wee cultured on MS medium supplemented with BAP and 2ip each at 1.5 mg/L. The shoots were subcultured on the same starting culture media every 4 weeks. The data on the average number, total and average length of proliferated shoots and percent of proliferated shoots during four subcultures were recorded in Tables (15, 16) and Fig. (15, 16)

It is clear from Tables (15, 16) and Fig. (15, 16) that all the investigated parameters increased by increasing re-subculture. The fourth subculture was superior in this respect whereas it maximized the all studied parameters for both rootstocks. Such trued was true with Hansen 536 and MAC 9 rootstocks photo (4-A and B).

These results are in line with those reported by Sun et al., (2000); Martins and pedratti (2001) and Cos et al., (2004) who found that regeneration rate of apple M.7, M.9 and peach- almond hybrid could reach 5 fold in MS medium supplemented with 1.5 mg/L BAP.

Table (14): Effect of different combination between BAp and 2ip on shoot tips roliferation parameters of Apple MAC 9 rootstock during 2006 and 2007 seasons.

Parameters			2006	í		2007				
Treatments	Averag of prolife shoo	rated	Prolifera	2000	Average length of proliferated shoots* (mm)	Avera No. prolifer	of rated	Proliferated shoot (%)	Average length of proliferated shoots (mm)	
A	1.4	D	40	D	11.4 B	1.4	D	40 D	13.4 B	
В	3.6	С	260	С	11.0 C	4.0	C .	300 C	11.9 C	
С	4.6	В	360	В	9.2 D	4.8	В	380 B	11.4 D	
D	5.8	A	480	A	13.2 Λ	6.2	A	520 A	16.0 A	

A MS cytokinion omitted medium (Control)

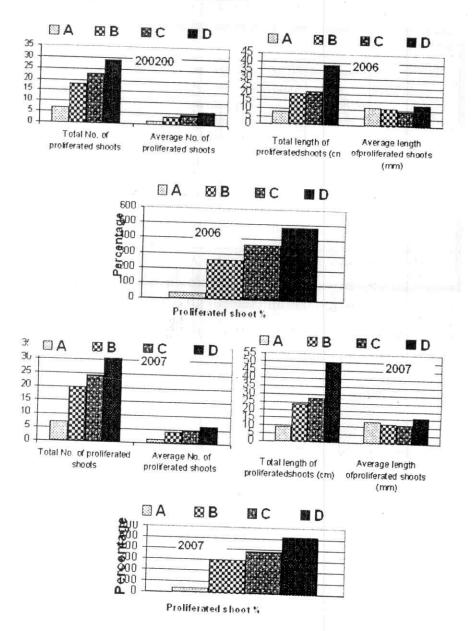
B MS + 1.0 mg/L BAP + 0.5 mg/L 2ip

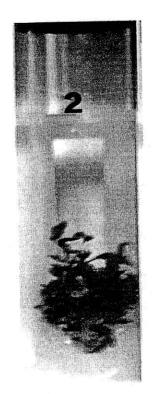
C MS + 1.0 mg/L BAP + 0.1 mg/L 2ip

D MS + 1.5 mg/L BAP + 1.5 mg/L 2ip

MS Murashige and Skoog medium supplemented with 30 g/L sucrose and 7.0 g/L agar

Fig. (14): Effect of different combination between BAp and 2ip on shoot tips roliferation parameters of Apple MAC 9 rootstock during 2006 and 2007 seasons.



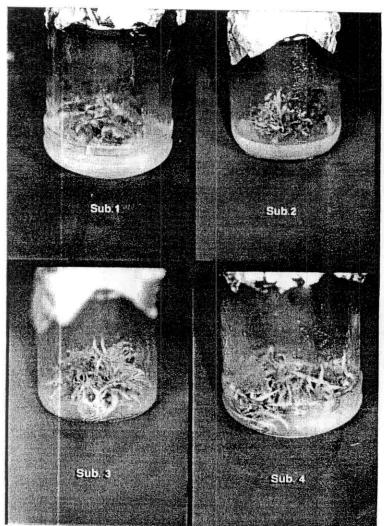


A- Hansen 536 MS + 1.0 mg/L BAP + 1.0 mg/L 2ip **A** 



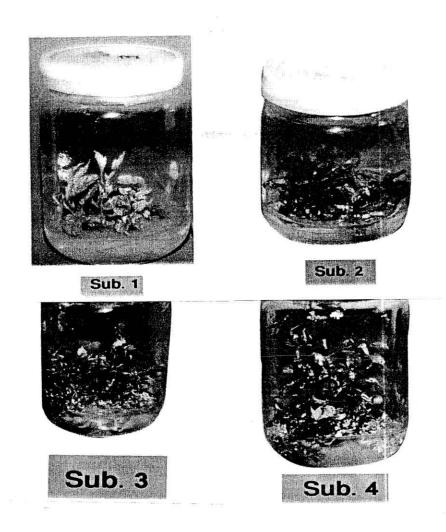
B- Mac9 MS + 1.5 mg/L BAP + 1.5 mg/L 2ip B

Photo (3): Shoot proliferation of shoot tip culture of Hansen 536 and Mac 9 rootstock



(A): Shoot proliferation of Hansen 536 peach rootstock, subcultured in : MS medium + 1.0 mg/L BAp + 1.0 mg/L 2ip.

Photo (4): Effect of number of subcultures on shoot culture proliferation of Hansen 536 rootstock



(B): Shoot proliferation of Mac9 apple rootstock
Subcutlured in : MS medium +1.5 mg/L BAP + 1.5 mg/L 2ip.

Photo (4): Effect of number of subcultures on shoot culture proliferation of Mac9 rootstock

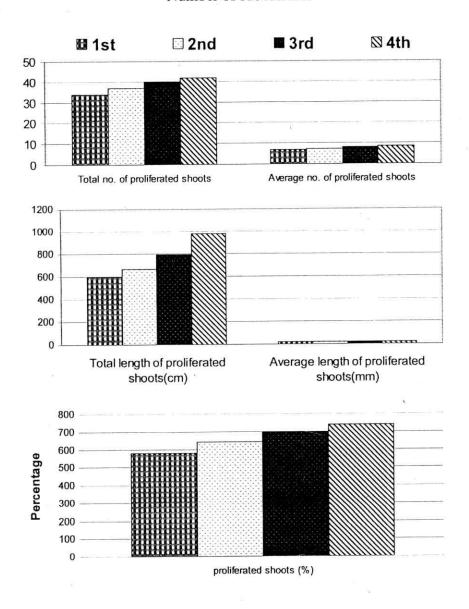
Table (15): Some proliferation measurements of Hansen 536, peach rootstock as influenced by number of subcultures (4 weeks intervals).

Subculture no.	no. of sho proliferated* (% shoots		Average length of proliferated* shoots(mm)		
1 <sup>st</sup>	6.2 B	520 D	17.9 D		
2 <sup>nd</sup>	6.8 B	580 C	22.0 C		
3 <sup>rd</sup>	9.0 A	800 B	24.4 B		
4 <sup>th</sup>	9.4 A	850 A	25.5 A		

- Initial shoot number was 5 shoots.
- Sub-culture was carried out 4 weeks intervals.
- $\bullet$  MS medium +1.0 mg/L BAP +1.0 mg/L 2ip was used for all subcultures.
- \* An average of two seasons.

Fig. (16): Some proliferation measurements of MAC 9 apple rootstock as influenced by number of subcultures (4 weeks intervals).

Number of subcultures



#### Rooting stage:

#### Shoot peach rootstock

#### a. Effect of combination between IBA and NAA on Hansen 536 rooting.

Four combinations between two kinds of auxins (IBA and NAA) each was added to half strength MS medium at four levels (0.5, 1.0, 1.5 and 2.0 mg/L) as well as auxnin omitted half strength MS medium as control, were investigated regarding their effect on some rooting parameters (rooting % average root number and average root length) of Hansen 536 cultured shoots.

The obtained data during both 2006 and 2007 seasons are presented in Table (17) and Fig. (17).

Data revealed that the three investigated rooting parameters were in positive relation ship. All investigated rooting media which supplied with different levels of IBA + NAA enhanced clearly all the rooting parameters than the control during both seasons of study. The combination between IBA + NAA each at 1.5 mg/L treatment (D) was the superior one in this respect, photo (5- A) as it increased significantly the all rooting parameters. Whereas, the lower concentration of both auxins (1.0 mg/L) came in the second rank. On the contrary the higher level of both auxins (2.0 mg/L) was less effective.

Table (17): Effect of BAP and NAA concentrations on rooting parameters after four weeks from culturing multiplied shoots of Hansen 536 peach rootstock during 2006 and 2007.

Parameters/		2006		,	2007			
Treatments	Rooting %	Average root number	Average root length (mm)	Rooting %	Average root number	Average root length (mm)		
A	30.0 D	1.2 D	80.0 D	25.0D	1.3D	80.0D		
В	60.0B	2.2 C	126.0 B	60.0B	2.5C	120.0C		
C	60.0B	3.0 B	136.0 A	60.0B	3.0B	122.0C		
D	80.0A	3.3 A	138.0 A	80.0A	3.3A	130.0A		
E	40.0C	2.5 C	120.0 C	40.0C	2.7C	125.0B		

<sup>1/2</sup> MS half strength of murashige and skoog medium.

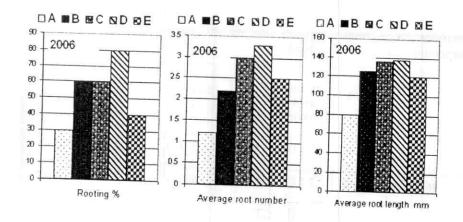
B 
$$\frac{1}{2}$$
 MS + 0.5 mg/L IBA + 0.5 mg/L NAA

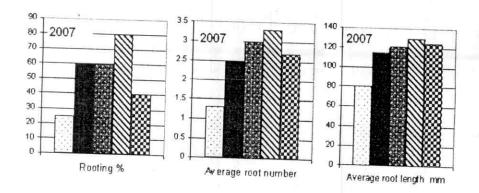
D 
$$\frac{1}{2}$$
 MS + 1.5 mg/L IBA + 1.5 mg/L NAA

E 
$$\frac{1}{2}$$
MS + 2.0 mg/L IBA + 2.0 mg/L NAA

Rooting parameters were recorded 4 weeks after shoot cultures.

Fig. (17): Effect of BAP and NAA concentrations on rooting parameters after four weeks from culturing multiplied shoots of Hansen 536 peach rootstock during 2006 and 2007.





# b. Effect of combination between IBA and PG on shoot rooting.

Four treatments represented different combinations between IBA which added to rooting media at four levels (0.5, 1.0, 1.5 and 2.0 mg/L) and PG at one level 100 mg/L as well as auxin omitted half strength MS medium as control, were investigated in response to rotting parameters. Data tabulated in Table (18) and Fig. (18) clear that culturing shoots of Hansen 536 peach rootstock on half strength MS medium supplemented with IBA and PG improved all the rooting parameters over the control, during both reasons of study. Moreover, ½ slenght MS medium + 1.0 mg/L IBA + 100 mg/LPG gave the highest values of all investigated rooting parameters photo (5-B) followed by treatment (D) (½ MS + 1.5 mg/L + 100 mg/L PG). On the other hand, auxin omitted medium (control) had the lowest values.

#### MAC 9 apple rootstock:

# a. Effect of combination between IBA and NAA on shoot rooting:

Different combinations between IBA + NAA each at 4 level (0.5, 1.0, 1.5 and 2.0 mg/L) were added to half strength MS medium in order to investigate their effect on rooting parameters of cultured shoots of MAC apple rootstock. Obtained data recorded in Table (19) and Fig. (19).

It is quite evidence from data that, the most promoting response of both rooting % and average root number was achieved when rooting medium contained 1.5 mg/L of each IBA and NAA. (treatment D) for both seasons of study photo (5-C). On the contrary, it treatment (D) gave the lowest value of average root

Table (18): Effect of IBA and PG concentrations on rooting parameters of cultured shoots of Hansen 536 peach rootstock after four weeks from culturing during 2006 and 2007.

Parameters		2006			2007		
Treatments	Rooting %	Average root number	Average root length (mm)	Rooting %	Average root number	Average root length (mm)	
A	40.0C	1.9 D	70.0 D	40.0 D	1.8 D	83.0 D	
В	60.0B	3.6 C	100.0 C	80.0 B	3.6 C	100.0 C	
С	80.0A	6.5 A	149.0 A	100.0A	6.4 A	148.0 A	
D	60.0B	4.0 B	122.0 B	100.0A	4.5 C	125.0 B	
E	60.0B	4.0 C	109.0 C	60.0 C	4.6 B	110.0 C	

1/2 MS half strength of murashige and skoog medium.

B 
$$\frac{1}{2}$$
 MS + 0.5 mg/L IBA + 100 mg/L PG

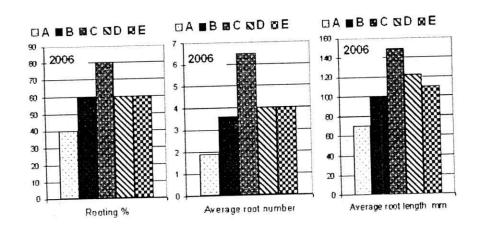
C 
$$\frac{1}{2}$$
 MS + 1.0 mg/L IBA + 100 mg/L PG

D 
$$\frac{1}{2}$$
 MS + 1.5 mg/L IBA + 100 mg/L PG

E 
$$\frac{1}{2}$$
MS + 2.0 mg/L IBA + 100 mg/L PG

Rooting parameters were recorded 4 weeks after shoot cultures.

Fig (18): Effect of IBA and PG concentrations on rooting parameters of cultured shoots of Hansen 536 peach rootstock after four weeks from culturing during 2006 and 2007.



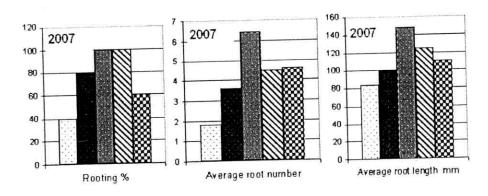


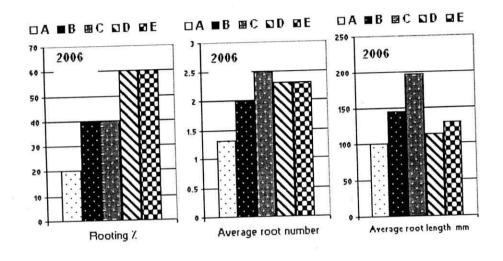
Table (19): Effect of BAP and NAA concentrations on rooting parameters after four weeks from culturing multiplied shoots of MAC 9 apple rootstock during 2006 and 2007 seasons.

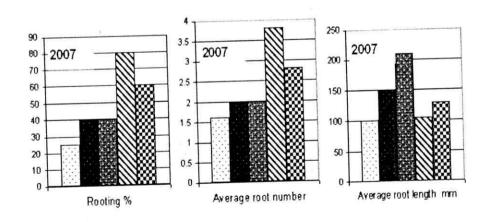
Parameters/		2006			2007	2007		
Treatments	Rooting %	Average root number	Average root length (mm)	Rooting	Average root number	Average root length (mm)		
A	20.0 C	1.3 D	100.0 D	25.0 D	1.6 D	100.0 D		
В	40.0 B	2.0 C	145.0 B	40.0 C	2.0 C	150.0 B		
C	40.0 B	2.5 B	196.0 A	40.0 C	2.0 C	210.0 A		
D	60.0 A	2.3 A	114.0 D	80.0 A	3.8 A	105.0 D		
E	60.0 A	2.3 B	130.0 C	60.0 B	2.8 B	129.0 C		

<sup>1/2</sup> MS half strength of murashige and skoog medium.

- A ½ MS + auxin omitted medium (Control)
- B  $\frac{1}{2}$  MS + 0.5 mg/L IBA + 0.5 mg/L NAA
- C  $\frac{1}{2}$  MS + 1.0 mg/L IBA + 1.0 mg/L NAA
- D  $\frac{1}{2}$  MS + 1.5 mg/L IBA + 1.5 mg/L NAA
- E  $\frac{1}{2}$ MS + 2.0 mg/L IBA + 2.0 mg/L NAA

Fig. (19): Effect of BAP and NAA concentrations on rooting parameters after four weeks from culturing multiplied shoots of MAC 9 apple rootstock during 2006 and 2007 seasons.





length. Meanwhile average root length was maximized with reducing auxin level in rooting media to 1.0 mg/L for both IBA and NAA. Such trend was true during both seasons of study. The rooting media which contained either the lowest level (0.5 mg/L) or the highest level (2.0 mg/L) of IBA + NAA came in the second rank regarding the improvement of rooting parameters. Auxin omitted medium (control) was the least effective one in response to rooting parameter.

# b. Effect of combination between IBA and PG on shoot rooting:

Four rates of IBA (0.5, 1.0, 1.5 and 2.0 mg/L) as well as one rate of PG (100 mg/L) were combined and added to ½ strength MS medium to investigated their effect on some rooting parameters. Obtained data tabulated in Table (20) and Fig. (20). Data show that the highest level of IBA (2.0 mg/L) associated with 100 mg/L PG (treatment E) statistically maximized the all investigated rooting parameters during both seasons of study photo (5-D), whereas the lowest level of IBA (0.5 mg/L) combined with 100 mg/L PG statistically minimized all rooting parameters as well as it was closed relatively to auxin omitted medium (control). The above results verify that culturing shoot (developed from shoot lip) cultured of Hansen 536 and Mac9 rootstocks in half strength MS rooting medium was recommended in increasing all studied rooting parameters. Moreover, the addition of 100 mg/L PG to 1.0 or 1.5 mg/L IBA gave the highest (100 and 80) rooting percentages for Hansen 536 and Mac9 rootstocks, respectively.

These results are in general agreement with the findings of Gamage et al, (2000) and Jun et al., (2000). They recommended

half strength MS medium in increasing rooting parameters. Meanwhile, Manta and Sharma (2000) recorded 80% rooting of M .106 and cleared that the addition of PG to rooting medium was not beneficial . On the contrary, a synergistic effect of IBA and PG on M.9 rooting was observed invespective of whether the transfer to hormone- free medium was made (James and Thurbon, 1979). In another study, the reported that PG at 162 mg/l had no effect on the shoot multiplication rate of M.9, but shoot cultures grown in the presence of PG gave higher rooting percentages than cultures grown in its absence. PG acted as an auxin synorgist in root initation (James and Thurbon, 1981).

### Acclimatization stage:

The plantlets acclimatization period extended for 2 months, where. Hansen 536 peach and MAC apple plantlets were transferred greenhouse. Plantlets were transferred individually, each in plastic pot (25 x 25 cm dimensions) filled with one of the four investigated growing media mixtures. Each plantlet was completely covered with while transparent plastic bag for three weeks.

Table (20): Effect of IBA and PG concentrations on rooting parameters of cultured shoots of MAC 9 apple rootstock after four weeks from culturing during 2006 and 2007.

Parameters		2006		2007			
Vreatments	Rooting	Average root number	Average root length (mm)	Rooting	Average root number	Average root length (mm)	
A	40.0 D	1.9 C	100.0 D	45.0 D	2.3 D		
В	60.0 C	2.5 B	128.0 C			110.0 D	
С	(0.0.0		120.00	60.0 C	2.9 D	125.0 D	
C	60.0 C	2.8 B	178.0 B	80.0 B	3.0 C	172.0 C	
D	80.0 B	3.2 A	178.0 B	80.0 B			
E	100.0		2.0.0 B	60.0 B	3.6 B	194.0 B	
I.	100.0 A	3.2 A	182.0 A	100.0A	3.8 A	203.0 A	

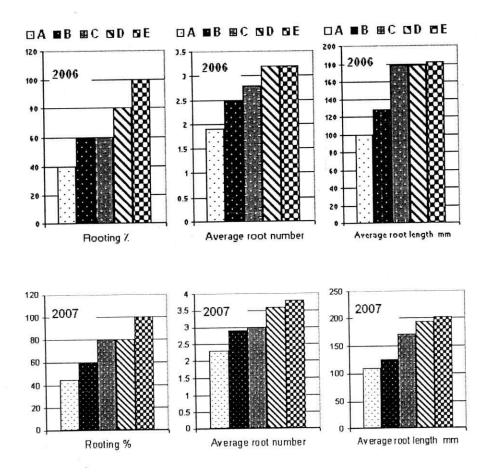
1/2 MS half strength of murashige and skoog medium.

B 
$$\frac{1}{2}$$
 MS + 0.5 mg/L IBA + 100 mg/L PG

D 
$$\frac{1}{2}$$
 MS + 1.5 mg/L IBA + 100 mg/L PG

E 
$$\frac{1}{2}$$
MS + 2.0 mg/L IBA + 100 mg/L PG

Fig. (20): Effect of IBA and PG concentrations on rooting parameters of cultured shoots of MAC 9 apple rootstock after four weeks from culturing during 2006 and 2007.





A. 1.5 mg/L IBA 1.5 mg/L NAA



B. 1.0 mg/L IBA 100 mg/L PG

Shoot rooting of Hansen 536 with cultured in  $\frac{1}{2}$  strength MS medium supplemented:



C. 1.5 mg/L IBA 1.5 mg/L NAA



Hoging Stage

D. 2.0 mg/L IBA 100 mg/L PG

Shoot rooting of MAC9 with cultured in ½ strength MS medium supplemented:

Photo (5): Shoot rooting originated from shoot tip culture of Hansen 536 and Mac9 rootstocks.

Taking into consideration, that the plantlets were irrigated and fertilized once a week with crystalon compound fertilizer (commercial name) which consisting of 19%N, 19% P and 19% K at the rate of 2.0 g/L. Survival percentage and average stem length of both transferred investigated rootstock plantlets to green house in relation to growing media (4 mixtures investigated of sand, peatmoss and vermiculite) were recorded after eight weeks obtained data during two successive months of the acclimatization were tabulated in Table (21) and Fig. (21) and illustrated by photo (6).

Regarding the influence of different mixtures of sand, peat moss and vermiculite substrates as growing media on survival percentage %, it was quite clear that a significant differences were clearly detected during two months of acclimatization for both rootstock plantlets.

Herein, the mixtures (growing medium) of Sand + peat moss + vermiculite at 1:1: 1 ratios exhibited statistically the highest survival percentage (80%) for both rootstock plantlets. On the contrary, both mixtures of peat moss + vermiculite (at 1: 1 ratio) and sand + peat moss (at 1: 1 ratio) resulted significantly in the least survival percentage (40% and 40%) for MAC 9 apple rootstock and Hansen 536 peach rootstock, respectively. In addition, sand + peat moss (at 1: 1 ratio) and sand + vermiculite (at 1: 1 ratio) were statistically in between (60%) when survival % of MAC 9 apple plantlets was concerned, whereas vermiculite + either sand or peat moss at equal proportion as growing media were statistically in between (60%) when survival % of Hansen 536 peach rootstock plantlets was concerned.

Concerning the average of stem length as influenced by different mixtures of growing media. Data presented in Table (21) and Fig. (21) shows that planting media comprised of sand + peat moss + vermiculite at equal proportion by volume was the superior one in relation to average of stem length of both investigated rootstock plantlets. The reverse was true when sand + peat moss at equal proportion was used as substrate of growing medium, where it showed the lowest average of stem length for both rootstock plantlets.

Generally it could be concluded that survival % and average of stem length of both investigated rootstock plantlets through the acclimatization stage was obviously influenced by the different substrate mixture used as growing media. In this respect, mixture of sand + peat moss + vermiculite at equal proportion was statistically the superior growing medium since it exhibited the maximum survival % (80%) for both rootstock plantlets as well as it maximized the average of stem length of both studied rootstocks photo (6).

These results are in general with the findings of Le and Collet (1991) and Nunes et al., (1991) on Mac9 and M.26 apple rootstocks. They reported that plantlets of Mac9 and M.26 were transferred to vermiculite or commercial substrate under greenhouse conditions with survival rates of 90% and 70% respectively, after 30 days. Moreover, Nesiem et al., (2006) and El-Bohr et al., (2007) found that transplanting date palm plantlets in soils mixture of sand, peat moss, vermiculite and perilite (1: 1: 1: 1) gave the highest survival percentage.

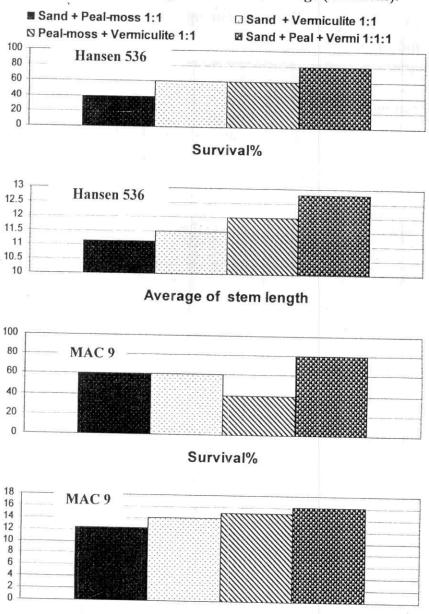
Table (21): Survival percentage and average of stem length of Hansen 536 peach and MAC 9 apple rootstock plantlets (in vitro propagated by shoot tip culture) as influenced by planting media used during acclimatization stage (2 months).

	Hanse	n 536	MAC 9			
Planting media mixtures ratios by volume	Survival* (%)	Average of stem* length	Survival* (%)	Average of stem* length		
Sand + Peat- moss 1 : 1	40.00 C	11.15 D	60.00 B	12.30 D		
Sand + Vermiculite 1 : 1	60.00 B	11.50 C	60.00 B	14.10 C		
Peal-moss + Vermiculite 1 : 1	60.00 B	12.00 B	40.00 C	15.0 B		
Sand – Peal + Vermi 1 : 1 : 1	80.00 A	12.80 A	80.00 A	16.15 A		

<sup>-</sup> Means with in column followed by the seam letters are not significantly different at 5% level.

<sup>\*</sup> An average of two seasons.

Fig. (21): Survival percentage and average of stem length of Hansen 536 peach and MAC 9 apple rootstock plantlets (in vitro propagated by shoot tip culture) as influenced by planting media used during acclimatization stage (2 months).



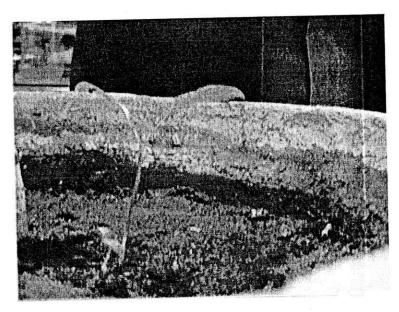




Photo (6): Platelets of Hansen 536 and Mac9 rootstock during acclimatization stage.