ISSN 1110-0419

Annals Of Agric. Sc., Moshtohor,
Vol. 46(3): Ho. 385-392, (2008).

RESPONSES OF SOME *IN VITRO* THE PEACH ROOTSTOCKS TO SALINITY AND WATER STRESS

BY

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ABASTRACT

Rooted plantlets of both peach rootstocks (Bitter almond and Nemaguard) were cultured on the Murashige and Skoog medium supplemented with different concentrations of manitole (Causal agent for drought) or NaCl (causal agent for salinity) Induction of drought occurred through addition of 0.37.31, 74.34, and 111.92gm/L to increase the osmotic pressure to 0.10, 15, and 20Bar respectively in the cultured medium. However, salinity was induced through supplementation the culture medium with NaCl at levels 0,500, 1000,1500, 2000ppm. In- addition, acclimatization of In vitro plantlets after drought and salinity treatments was involved.

It is found that plantlets length and number of leaves parameters increased by adding manitole up to 37.31 (10 Bars) then decreased by continuous increase of manitole concentrations in both rootstocks under study. Simelarly, both parameters were positively responced with the first level of salinity (1000ppm NaCl) while sharply declined by continuous increase of NaCl concentrations and reached to the worest state at 2000 ppm. Meanwhile, leaf mineral contents were more or less affected by drought and salinity treatments. Furthermore, successful acclimatization was occurred in bitter almond rootstock by using a combination from peatmoss and sand at rate 3:1 (V/V).

INTRODUCTION

Water Stress and salinity are the limiting factors and major problems in some stone fruit trees grown in North Sinai. Under such conditions, the future of stone fruits plantings's threatened in being lower quantity and salinity hazard for these crops. Further-more, drought and salinity problems usually are confined to arid and semiarid regions where rainfall is not sufficient to leach salts from the plant zone.

The increasing demand and costs of production and management have caused intensified research on drought and salinity during the last three decades Epstein *et al.* (1980) believed that solving the salinity problems should include both the manipula-tion of the environment to benefit the plant – soil reclamation, drainage and water control and genetic manipulation. Therefore, salt stress changes the water relations of the most higher plants and salt tolerance often depends on drought tolerance (Greenway and Munrs,1980).

A rootstock's ability to confer salinity (Gorton and Cooper 1954) to trees in a com-plex process that undoubtedly involves physiological interaction between rootstocks and scion (Cooper and Gorton 1952). Never theless, rootstocks can be ran bed by their ability to avoid accumulation in leaves. Mass (1993) while maintaining growth and ade-quate nutrient uptake (Grattan and Grieve, 1992). Such characteristics are also apparent when rootstocks cultivars are grown as seedlings and challenged with salinity stress (Sykes, 1985; Syvertsen and Yeleioskey, 1988).

In vitro propagation method for drought and salt tolerance is an energy – efficient approach and should be combined with land management alternative.

Surveying of new peach rootstocks and selecting the best ones suitable for local conditions and tolerant to drought and salt stresses are the most recent, accurate and fast evaluation because large numbers can be evaluated in a limited amount of space and, once identified a variant can be rapidly micro propagated.

The objectives of this research were to:

(a) Examine the response of in *vitro* – propagated immature embryos, shoot tips and nodel explants of rootstocks for drought and salt tolerance.
(b) Determine the feasibility of screening peach rootstocks *in vitro* for drought and salt tolerance.

MATERIALS AND METHODS

This study was carried out in plant Tissue Culture Laboratory at Faculty of Environmental Agricultural Sciences (FEAS) El-Arish, North Sinai, Suez Canal University (SCU) during the period from 2004 to 2007 to establish a protocol for plantlets formation from peach rootstocks by using micro propagation techniques. Also, evaluation tolerance to the salinity.

Stress tolerance:-

3.1. Drought tolerance:-

The rooted plantlets of bitter almond and Nemaguard peach rootstocks were cultured on medium supplemented with the causal drought agent. Mannitol was tested at (0, 10, 15 and 20 Bar) to study the effect of osmotic stress on free water deficit as a casual agent for drought stress. The amount of mannitol needed to produce these osmatic potentials was 0, 37.31, 74.34 and 111.92 grams per liter (w/v), respectively. The concentrations of mannitol were calculated according to formula given by Helmerick and Pferifer (1954).

$$\text{where} \quad p = g \cdot \frac{RT}{Mv}$$

p = osmotic potential in atmosphere
g = gram of mannitol
m = molecular Wight of mannitol (182.17)
v= volume in liters (L)
R = 0.0825 liter atmospheres per degree per mole
T = absolute temperature

3.2. Salinity tolerance:-

The rooted plantlets of almond rootstocks were cultured on medium containing different concentrations of NaCl (0, 500, 1000, 1500 and 2000 ppm respectively) to study the effect of different concentrations NaCl on growth and development of *in vitro* Plantlets.

The following data were recorded on almond and Nemaguard rootstocks at the end of four weeks for drought and salinity. Data of plant tolerant was calculated visually as scores (according to Pottino, 1981).

1 = Negative results (dead) (-).
2= below average (+).
3= Average (++).
4= above Average (+++)
5=Excellent

Cultural conditions:

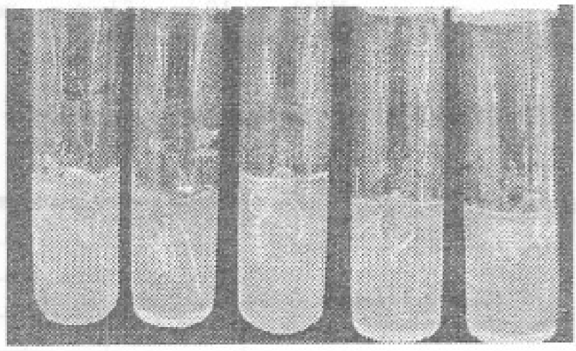
All cultures were randomly placed in an incubation room under suitable cultural conditions (the temperature was 25±2°C with light condition 16 hrs light and 8 hrs dark provided by fluorescent light and 2500 lux intensity. The cultures were subcultured monthly. Also, measuring light intensity by using traceable dual- range light meter.

Statistical analysis:

Data were Statistically analyzed by using Randomized Complete Block Design (RCBD) in factorial arrangement in four replicates (Snedecore and Cochran, 1990). Mean separations were done by using Mstat c computer program v.4 (1986).

RESULTS AND DISCUSSION

4.1. Stress tolerance:-



Bitter almond | Nemaguard peach

Photo. (1): Bitter almond and Nemaguard peach before salinity and drought stresses.

4.1. a. Drought stress:-

Data in Table (1) show that bitter almond Plantlets length and leaves number were decreased linearly with increasing mannitol in the medium .while root number and root length of almond rootstocks was not responded.

Table (1): Effect of different levels of water stress on growth of cultured bitter almond rootstock after four weeks of treatment.

Parameters / Mannitol (bar)	Plantlets length (cm)	Leaves Number	Roots Number	Roots length (cm)
Control	5.17 a	22.33 a	1.67 a	5.17 a
5	3.67 b	17.33 a	1.67 a	3.60 a
10	2.97 bc	9.00 b	1.67 a	2.73 a
15	2.60 c	1.67 c	1.00 a	3.00 a

- Means within each column followed by the same letter(s) are not significantly different at 5% level.

Data in Table (2) clear that increasing mannitol concentrations induced a significant decrease in Nemaguard peach plantlets length and leaves number. The least values were observed with 15 bar treatments. Whereas roots number of Nemaguard rootstock exhibit-ted there were not significant different. The effect of mannitol was nil on the culture Nemaguard rootstock during four weeks of treatment. On the other hand, the root length was significantly decreased with increasing mannitol concentration and the least values of root length was recorded by 15 bar treatment. These results are supported by the findings obtained by Escobar-Gutierrez *et al* (1998); Ledbetter *et al* (1998) and Rejshova *et al* (2007).

Table (2): Effect of different levels of water stress on growth of cultured Nemaguard peach rootstock after four weeks of treatment.

Parameters / Mannitol (bar)	Plantlets length (cm)	Leaves Number	Roots Number	Roots length (cm)
Control	5.33 a	19.67 a	1.67 a	3.60 a
5	3.53 ab	16.00 a	1.33 a	3.60 a
10	2.30 b	7.00 b	2.00 a	3.03 ab
15	2.90 b	1.33 c	1.33 a	2.13 b

- Means within each column followed by the same letter(s) are not significantly different at 5% level.

Data in Table (3) indicate that both rootstocks (bitter almond and Nemaguard peach) were took nearly the same trend at 5 bar of water stress. While, at 10 bar of water stress bitter almond rootstocks were greatly adapted than of Nemaguard due to increasing water stress. On the other hand bitter almond at 15 bar treatment surpassed Nemaguard peach in this concern.

Table (3): Effect of different levels of drought on growth of cultured bitter almond and Nemaguard peach rootstock after four weeks of treatment (according to Pottino, 1981).

Treatment	5 bar	10 bar	15 bar
Almond bitter	+++	+++	++
	++	+	-
	+++	++	+
Nemaguard peach	+++	++	+
	++	-	-
	+++	++	-

- Means within each column followed by the same letter(s) are not significantly different at 5% level.

Data in Table (4) show that increasing mannitol concentrations induced adverse effects on growth and development of Nemaguard and bitter almond .Also, increasing concentrations of mannitol caused a reduction in K^{+}, Na^{+}, Fe^{++} and Zn^{++} uptake for both rootstocks (Nemaguard peach and bitter almond). But bitter almond showed more tolerant to water stress than Nemaguard peach..

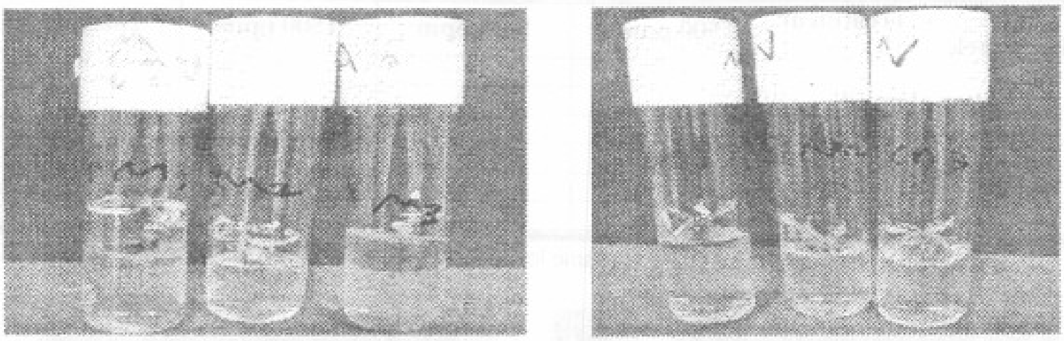
4.4. b. Salinity stress:

Data in Table (5) reflect the effect of salinity occurred by NaCl on almond rootstock after four weeks of treatments. It is appeared that the greatest plantlets length (5.33 cm) was noticed when 1000 ppm NaCl was added to the culture medium while the smallest value (4.00 cm) was recorded by 1500 and 2000 ppm treatments, However the other treatments came in between . As for, the leaves number of bitter almond rootstock exhibited a significant decrease with 2000 ppm. While the highest value was recorded by control and 500 ppm NaCl. Whereas the number of almond rootstocks roots were significantly decreased at 2000 ppm salinity followed by 1500 ppm and 1000 ppm .The roots length generally were ranged from (3.65) to (50.16) cm. The highest roots length noticed with the control .While the least values at 500 and 1500 ppm while the other treatments came in between.

Table (4): Effect of *in vitro* drought conditions on concentrations of nutrient elements contents (K, Na, Fe and Zn) for rootstocks Nemaguard peach and bitter almond .

Rootstock	Treatment (bar)	K (mgl^{-1})	Na (mgl^{-1})	Fe (mgl^{-1})	Zn (mgl^{-1})
Nemaguard peach	Control	40.50 ^{a}	50.40 ^{b}	5.80 ^{a}	1.28 ^{b}
	5 bar	24.00 ^{c}	23.00 ^{c}	4.00 ^{c}	1.60 ^{ab}
	10 bar	16.00 ^{e}	19.00 ^{d}	3.17 ^{f}	1.60 ^{ab}
	15 bar	7.00 ^{g}	12.00 ^{f}	2.30 ^{g}	0.50 ^{c}
Bitter almond	Control	32.40 ^{b}	51.30 ^{a}	4.10 ^{b}	2.20 ^{a}
	5 bar	14.00 ^{f}	18.00 ^{c}	3.70 ^{d}	1.70 ^{ab}
	10 bar	21.00 ^{d}	19.00 ^{d}	3.30 ^{e}	1.30 ^{b}
	15 bar	16.00 ^{e}	11.00 ^{i}	1.23 ^{h}	0.53 ^{c}

- Means within each column followed by the same letter(s) are not significantly different at 5% level



Bitter Almond **Nemaguard peach**

Photo (2): Effect of different levels of drought on growth of cultured bitter almond and Nemaguard peach rootstocks after four weeks of treatment.

Table (5): Effect of different levels of salinity (NaCl) on growth of cultured bitter almond rootstock after four weeks of treatment.

Parameters / NaCl con. (ppm)	Plantlets length (cm)	Leaves Number	Roots Number	Roots length (cm)
Control	5.17^{ab}	22.33^{a}	1.67^{b}	5.17^{a}
500	5.00^{ab}	22.67^{a}	3.33^{a}	3.57^{b}
1000	5.33^{a}	11.33^{b}	1.33^{b}	4.57^{a}
1500	4.03^{b}	10.00^{b}	2.33^{ab}	3.57^{b}
2000	4.00^{b}	4.00^{c}	1.33^{b}	4.60^{a}

- Means within each column followed by the same letter(s) are not significantly different at 5% level

Data in Table (6) indicate the effect of salinity NaCl on Nemaguard peach rootstocks after four weeks of treatments. It is clear that the Plantlets length was significantly reduced after four weeks of treatment when 2000 ppm NaCl treatment was used. The other treatment gave statistically similar values in this concern as compared with control. Meanwhile, the number of Nemaguard peach leaves were induced high significant decrease by 2000 ppm salinity followed by 1500 ppm while the other treatments had similar effect as compared with the control. Concerning the number of Nemaguard peach roots, all the tested treatments did not significantly affected in this concern. Regarding, the roots length the most depressive effect was appeared with the using of 2000 ppm NaCl . However, the other treatments and control had similar statistically values. These results go in line with the findings reported by Sotiropoulos *et al* (2006); Harbe *et al* (2005) and Erturk *et al* (2007).

Table (7) declairs that Nemaguard was better performance than almond with all tested NaCl concentrations.

Table (6): Effect of different concentrations of NaCl on growth of cultured Nemaguard peach rootstock after four weeks of treatment.

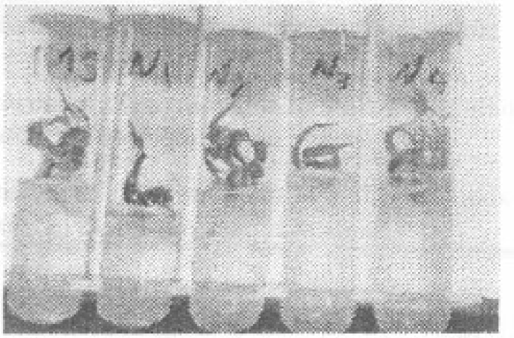
Parameters / NaCl con. (ppm)	Plantlets length (cm)	Leaves Number	Roots Number	Roots length (cm)
Control	5.33^{a}	19.67^{a}	1.67^{b}	3.60^{a}
500	4.73^{a}	19.67^{a}	3.00^{a}	4.17^{a}
1000	4.00^{a}	15.00^{b}	2.00^{ab}	3.97^{a}
1500	4.40^{a}	15.67^{ab}	2.00^{ab}	3.27^{a}
2000	2.47^{b}	8.00^{c}	1.67^{b}	2.23^{b}

- Means within each column followed by the same letter(s) are not significantly different at 5% level.

Table (7): Effect of different levels of salinity on growth of cultured almond and Nemaguard peach rootstock after four weeks of treatment (according to Pottino, 1981).

Rootstock \ Treatment	500 ppm	1000 ppm	1500 ppm	2000 ppm
Bitter almond	+++	++	+	+
	++	++	-	-
	+++	++	-	-
Nemaguard peach	+++	+++	+++	++
	+++	++	+	-
	+++	+	+	-

- Means within each column followed by the same letter(s) are not significantly different at 5% level.



Bitter almond **Nemaguard peach**

Photo (3): Effect of different levels of salinity on growth of cultured bitter almond and Nemaguard peach rootstocks after four weeks of treatment.

Data in Table (8) indicate that in both rootstocks K^{+}, Na^{+}, Fe^{++}, and Zn^{++} content were increased by increasing salinity (NaCl) levels. Moreover, the highest values of leaf K^{+} content was observed with bitter almond at 500 ppm while the least values was recorded by bitter almond at 1000 ppm salinity while the other interactions came in between .As for leaf Na^{+} content, Nemaguard peach rootstock at 2000 ppm salinity showed the highest values, while Nemaguard peach rootstock was the least values with control while the other interactions came in between . As for leaf Fe^{++} content, Nemaguard peach rootstock at 500 ppm had the highest level while the least values was recorded by control in bitter almond while the other interactions came in between concerning, leaf Zn^{++} content, bitter almond at 2000 ppm salinity gave the highest values while bitter almond with control induced had the least values while, the other interactions came in between. These results in agreements with the findings of croughan *et al* (1978) and Belal (1982).

Table (8): Concentration of elements contents K, Na, Fe and Zn for rootstocks Nemaguard peach and bitter almond under saline conditions *in vitro*.

Rootstock	salinity (ppm)	K (mgl^{-1})	Na (mgl^{-1})	Fe (mgl^{-1})	Zn (mgl^{-1})
Nemaguard peach	Control	40.50 ^{g}	50.40 ^{j}	3.17 ^{f}	1.28 ^{cde}
	500	84.60 ^{d}	58.50 ^{g}	4.82 ^{a}	2.26 ^{ab}
	1000	52.20 ^{f}	64.80 ^{e}	4.23 ^{c}	1.34 ^{cd}
	1500	36.90 ^{h}	65.70 ^{d}	3.52 ^{e}	1.47 ^{cd}
	2000	91.80 ^{b}	84.60 ^{a}	4.40 b	2.50 ^{a}
Bitter almond	Control	32.40 ^{i}	51.30 ^{i}	1.23 j	0.49 ^{f}
	500	108.0 ^{a}	55.80 ^{h}	2.41 h	0.840 ^{def}
	1000	24.30 ^{j}	63.00 ^{f}	1.52 i	0.63 ^{ef}
	1500	61.20 ^{e}	67.50 ^{c}	2.58 g	1.63 ^{bc}
	2000	88.20 ^{c}	74.70 b	3.76 ^{d}	1.13 ^{cdef}

- Means within each column followed by the same letter(s) are not significantly different at 5% level

4.5. Acclimatization:

It appears from photo (12) that bitter almond rootstock (*Prunus amygdalus)* was successfully acclimatized by using combination of peatmoss and sand at rate 3:1 (v/v).

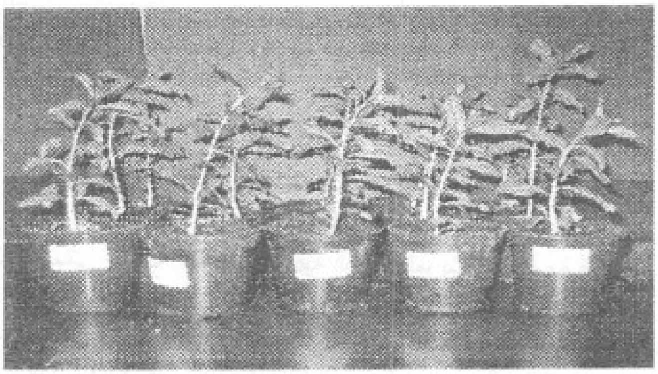


Photo (4): The acclimatization of bitter almond rootstock plantlets.

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استجابة بعض أصول الخوخ للملوحة والإجهاد المائي داخل الأنابيب

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أجريت هذه الدراسة فى معمل زراعه الانسجه النباتية ، كليه العلوم الزراعية البيئية بالعريش ،جامعة قناة السويس ، خلال الفترة من ٢٠٠٤ حتى ٢٠٠٧م، وتهدف هذه الدراسة إلى إكثار بعض اصول الخوخ باستخدام تقنيات زراعة الانسجه ودراسة مدى تحمل النباتات للملوحة

والإجهاد المائي داخل الأنابيب. حيث تم تقييم كل من (*Prunus persica*) واللوز المر (*Prunus amygdales*) من أصول الخوخ تحت مستويات مختلفة من الإجهاد حيث تم استخدام كلوريد الصوديوم بتركيزات صفر، ٥٠٠، ١٠٠٠، ٢٠٠٠ جزء فى المليون كمصدر للملوحة بينما تم استخدام المانيتول بتركيزات صفر ، ٣٧,٣١ ، ٧٤,٣٤ ، ١١١,٩٢ جرام / لتر . كمعامل لحدوث الجفاف ليصل الجفاف الى صفر ، ١٠ ، ١٥ ، ٢٠ بار وفى نهاية التجربة تم حساب القياسات المختلفة (طول النبات ، عدد الأوراق وعدد وطول الجذر).

أوضحت النتائج أن طول النباتات وعدد الأوراق كان الأفضل تحت التركيزات المنخفضة من المانيتول (المستوى الأول) بينما تأثرت تأثرا سيئا بزيادة التركيز فى كل من الأصلين تحت الدراسة (اللوز المر والنيماجارد) بينما تحسنت هذه القراءات فى حاله اضافه ١٠٠٠ جزء فى المليون من كلوريد الصوديوم إلى البيئة وبزيادة تركيزات الصوديوم فى البيئة حدث تدهور واضح وسريع فى هذه الصفات ووصل هذا التدهور اقصاه عند أضافه ٢٠٠٠ جزء فى المليون وتباينت محتويات الاوراق من العناصر المعدنية بالزيادة أو النقصان نتيجة معاملات الملوحة والجفاف وأوضحت النتائج نجاح الاقلمه لنباتات زراعه الانسجه باستخدام مخلوط من البيئات الزراعية تحتوى على البيتموس والرمل بنسبه ٣: ١ (حجما).