Effect of Salt Stress on Leaf Proline Accumulation and Chlorophyll Content of Some Fruit Species

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ABSTRACT

Proline, chlorophyll- a, b and total chlorophyll contents in the leaves of one- year- old uniform seedlings of "Nibaly" olive, "Safida" guava, "Balady" citron, "Banzahir" lime and "Timour" mango were studied in glasshouse trial in 1998/ 1999 and 1999/ 2000 seasons at salinity of 0, 25, 50 and 100 m.mol NaCl in the irrigation water. It was noticed that the leaves of citron seedlings contained the highest level of proline (7.64 mg/ g) followed by lime (6.17 mg/ g), mango (4.6 mg/ g), guava (2.36 mg/ g) and olive seedlings which had the lowest level of leaf proline content (1.57 mg/ g). Compared with controls, increasing NaCl concentration in the irrigation water induced significantly higher increases in the leaf proline content of the different fruit species. Based on the results, it is evident that the changes in proline levels have an inverse relationship with the salt tolerance. Therefore, the relative salt tolerance may be in the following descending order: olive, guava, mango, lime and citron. Under salinization, the levels of chlorophyll-a, b and total chlorophyll in the different fruit species seedlings were progressively decreased with increasing NaCl concentration in the irrigation water as compared with the control. In addition, the leaf chlorophyll content was significantly differed between the experimental fruit species under salinity treatments.

ADDITIONAL INDEX WORDS: Salinity, Fruit species, proline, chlorophyll.

INTRODUCTION

Fruit trees are among the most sensitive horticultural crops to soil salinity (Mass, 1990). Fruit yields are affected at relatively low threshold soil salinity and decrease more rapidly than most crops as salt concentrations increase above the threshold. In mature trees, the wood can provide a substantial sink for the storage of salt. With the cumulative effects of salt buildup in the wood from one year to the next, Na^+ levels suddenly begin to increase in the leaves. This observation has been reported for *citrus* (Dasberg *et al.*, 1991) and different fruit trees (Boland *et al.*, 1997). This rapid transport of Na^+ (and accompanying Cl^-) to leaves usually resulted in toxic and lethal foliar concentrations that lead to the death of individual branches or even the whole tree. High salt concentrations in the soil solution and/or irrigation water create high osmotic pressure, reducing the availability of soil water to the plant and poses threat of osmotic withdrawal of water from plant tissues and specific ions such as sodium and chloride may prove toxic at high concentrations (El-Hag and Sidahmed, 1997).

The accumulation of free proline and certain other amino acids in plant tissues in response to a variety of environmental stresses has been reviewed recently (El-Sayed *et al.*, 1996 and Sabbah *et al.*, 1996). Proline was the most prevalent free amino acid in *citrus* tree tissues and large differences in concentration occurred between stressed and non-stressed tissues (Syvertsen and Smith, 1983). Proline accumulation in response to salinity has been extensively documented in higher plants (Stewart *et al.*, 1966; Brown and Hellebust, 1975; El-Hefnawy, 1986; Bondok *et al.*, 1995 and El-Hamady *et al.*, 1996). Barnett and Naylor (1966) suggested that proline may act as storage compound for energy and reduced nitrogen and carbon to be used post-stress metabolism. Also, Stewart and Lee (1974) reported that proline might act as cytoplasmic osmoticum counteracting the effect of salt accumulated in the vacuole. Whether or not the increased proline concentration in response to environmental stresses is adaptive or merely in adaptive of existing stress has been debated (Syvertsen and Smith, 1983).

Regarding to the chlorophyll content of the salinized plants, it is obvious that all the chlorophyll contents were reduced as a result of increasing salinity (Ashraf, 1994). Likewise, Attalla (1987), Bondok *et al.* (1995) and El-Sayed *et al.* (1996) suggested that the chlorophyll content depend on the biological processes and development stages of the plant and also on the type and concentration of the salts.

The purpose of the present study is to evaluate the leaf proline accumulation and chlorophyll content of seedlings of olive, guava, citron, lime and mango with respect to their response to salinity in the irrigation water.

MATERIALS AND METHODS

The present study was carried out during 1998/ 1999 and 1999/ 2000 seasons on five fruit species namely: olive (*Olea europea* L. cv. Nibaly), guava (*Psidium guajava* L. cv. Safida), citron (*Citrus medica* L. cv. Balady), lime (*Citrus aurantifolia* Swingle cv. Banzahir) and mango (*Mangifera indica* L. cv. Timour). Seeds of guava, citron, lime and mango and cuttings of olive were planted in the first week of September of both seasons in beds filled with 1:1 peat moss and sand in the glasshouse of Botany and Microbiology Department, College of Science (Women Students Section), King Saud University. At age of one - year-old, uniform plants of each species were transplanted into 10x10x30 cm polyethylene pots containing washed sand media. Each pot contained three seedlings and there were five pots for each of the four salt treatments. Thus, there were sixty plants for each of the five fruit species. Plants were irrigated with 300 ml of a complete nutrient solution (Hoagland and Arnon, 1953) three times a week until the end of the experiment. The saline treatments were 0 (control), 25, 50 and 100 m. mol sodium chloride added to the nutrient solution. Salt treatments were initiated one- month after transplanting. Controls received nutrient solution only.

Three leaf samples were collected from the seedlings of each fruit species of the different treatments for proline and chlorophyll determination. The first leaf samples were collected after one month of the saline treatments and then every four weeks until the end of the experiment after three months during both seasons. The leaves were washed several times with tap water and distilled water. In order to determine proline, leaf samples were dried in a forced-air oven at 70 °C for 25 h., micro milled and the free proline was extracted by shaking (0.5 g) of the samples with 10 ml 3% sulfosalicilic acid for 2 h. at 30 °C. Proline concentration (as mg/ g dry weight) was determined colorimetrically with glacial acetic acid and acidic ninhidrin by using UV/ Visible Spectrophotometer- LKB-Biochrom 4050 at 520 nm, according to Bates (1973) and Levy (1980) using L-proline as standard.

For chlorophyll determination, 1 g of the fresh leaf samples were dipped in 10 ml N, N- Dimethyl Formamide solution for 48 hrs. at 4 °C in the dark. Chlorophyll concentration (as mg/ 100 g fresh weight) was measured in the extraction colorimetrically by using UV/ Visible Spectrophotometer- LKB-Biochrom 4050 at 664 nm for chlorophyll-a and 645 nm for chlorophyll b (according to Moran and Porath, 1980). Total chlorophyll was calculated (as mg/ 100-g fresh weight) by using the following mathematic manipulations:

$$\text{Chlorophyll-a} = 9.78 \times D^{*}\,664 - 0.99 \times D\,645$$

$$\text{Chlorophyll-b} = 21.426 \times D\,664 - 4.65 \times D\,645$$

$$\text{Total chlorophyll} = 5.134 \times D\,664 + 20.436 \times D\,645$$

D^{*} = Optical density at the wave length.

The data obtained were subjected to the analysis of variance for split- plot design presented by Steel and Torrie (1980). Differences among leaf proline and chlorophyll contents of the fruit species were separated by Duncan's multiple range tests using the SAS statistics program package (SAS Institute Inc., 1995).

RESULTS AND DISCUSSION

I-Changes in the levels of proline in response to salinity levels:

Results in Table (1) and Fig.(1) showed that there were significant differences in the leaf proline content between the five fruit species seedlings during whole time of the experiment. It was also noticed that, as an average of salinity levels, the leaves of citron seedlings contained the highest level of proline (7.64 mg/ g dry weight) followed by lime (6.17 mg/ g), mango (4.6 mg/ g), guava (2.36 mg/ g) and the olive seedlings which had the lowest level of leaf proline content (1.57 mg/ g) as affected by sodium chloride treatments. In addition, results in Table (1) indicated that increasing NaCl concentration in the irrigation water induced significantly higher increases in the leaf proline content of the different fruit species. Results of the first leaf sample showed that the different NaCl salinity treatments caused a marked accumulation in the leaf proline as compared with that of the untreated ones. However, no significant differences were found between 25, 50 and 100 m.mol NaCl treatments. During the second and third leaf samples there were significant differences in the leaf proline content between NaCl treatments including the control (Table, 1).

The accumulation of proline is generally considered to be a response of the different fruit species to increasing salt stress. The results demonstrated a wide variation in response to salinity. This supports the idea that proline synthesized in the leaves and subsequently, is transported to roots and fruits as an adaptive mechanism to avoid any yield depression (Syvertsen and Smith, 1983 and Sabbah *et al*,. 1996). Results of the present study suggest that one should select those fruit species or cultivars with salinity adaptability under field conditions. This indicated that the ability of these fruit species to tolerate salinity may be depending on its leaf proline content. Based on the results, it is evident that the changes in proline levels have an inverse relationship with the salt tolerance. Therefore, the relative salt tolerance may be in the following descending order: olive, guava, mango, lime and citron.

Table 1. Effect of NaCl levels on the leaf proline content (mg/ g dry weight) of some fruit species during 1998/ 99 and 1999/ 2000 seasons.

Treatments	Leaf Samples						Average
	First		Second		Third		
	1998/ 99	99/2000	1998/99	99/2000	1998/99	99/2000	
Fruit species							
Olive	0.32 e	0.40 c	1.75 c	1.99 b	2.68 c	2.26 e	1.57 e
Guava	1.54 d	1.74 c	1.70 c	2.14 b	3.87 bc	3.19 d	2.36 d
Citron	7.23 a	7.49 a	7.00 a	7.16 a	8.98 a	8.00 a	7.64 a
Lime	5.17 b	5.57 b	6.31 a	6.97 a	7.03 a	6.01 b	6.17 b
Mango	3.29 c	3.49 bc	5.00 b	5.18 a	5.45 b	5.21 c	4.60 c
Salinity treatments							
0 (Control)	1.60 b	1.84 b	1.53 b	1.79 c	1.46 d	1.20 d	1.57 d
25 m. mol NaCl	3.84 a	3.90 a	4.61 a	5.29 b	4.96 c	4.66 c	4.54 c
50 m. mol NaCl	4.23 a	4.83 a	5.40 a	5.54 ab	6.52 b	6.12 b	5.44 b
100 m. mol NaCl	4.26 a	4.48 a	5.77 a	6.23 a	8.91 a	8.31 a	6.33 a

Means not sharing the same letter within columns are significantly different ($P < 0.05$), Duncan's multiple range tests.

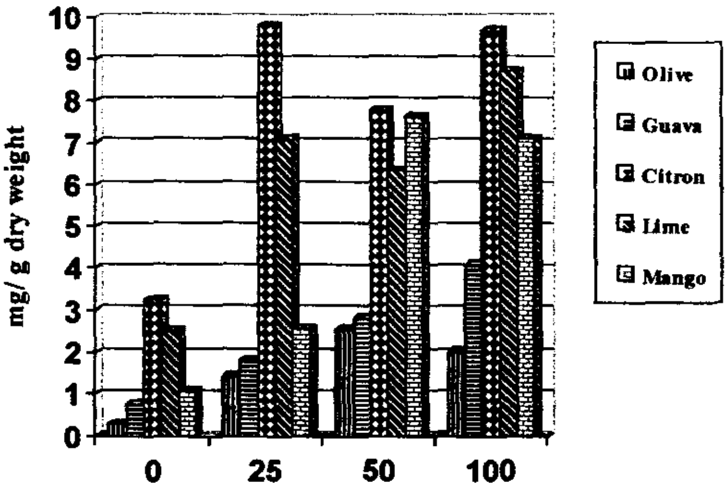


Fig.(1): Effect of NaCl levels on leaf proline content

These findings were in agreement with those obtained by many investigators such as Downton and loveys (1981) on grapevine, Kaul (1981) and El-Hefnawy (1986) working on guava seedlings, Bondok *et al.* (1995) on peach, El-Hamady *et al.* (1996) and Piqueras *et al.* (1996) on some *Citrus* cultivars and rootstocks and El-Sayed *et al.* (1996) working on 14 olive cultivars, found that leaf proline content increased under salinization. They also found that the less sensitive olive cultivars were ranked intermediate depending on their ability to exclude sodium and chloride from the leaves. Recently, Abdel-Kader (2002) working on certain fruit seedlings, found that olive appeared to be relatively salt tolerant specie as evidenced by survival of the plants with increasing NaCl salinity in the irrigation water. She also found that citron was extremely sensitive with salt concentration where its plants did not tolerate all NaCl treatments for a long time. She also revealed that guava, mango and lime seedlings appeared as medium tolerance to NaCl salinity stress.

II- Changes in the leaf chlorophyll content in response to salinity levels:

1- Chlorophyll-a:

Results in Table (2) and Fig.(2) showed that there were significant differences in the leaf chlorophyll-a content between the five fruit species in the three leaf samples. As an average of salinity levels, the leaves of olive seedlings contained the highest concentration of chlorophyll-a (63.73 mg/ 100 g fresh weight) followed by guava (31.88 mg/ 100 g), citron (23.93 mg/ 100 g), mango

(16.42 mg/ 100 g) and lime seedlings which had the lowest concentration of leaf chlorophyll-a content (13.68 mg/ 100 g). However, no significant differences were found in the leaf chlorophyll-a content between lime and mango seedlings.

Results also indicated that NaCl salinity treatments reduced significantly the leaf chlorophyll-a content of the different studied fruit species as compared with the control. As an average of five fruit species, there were no significant differences in the leaf chlorophyll-a content between 25 and 50 m.mol and between 50 and 100 m.mol treatments (Table, 2).

2- Chlorophyll-b:

Results in Table (3) and Fig.(3) revealed that during the whole time of the experiment, there were significant differences in the leaf chlorophyll- b content between the fruit species. As an average of salinity levels, the leaves of olive seedlings had the highest level of chlorophyll- b (36.62 mg/ 100 g fresh weight) followed by guava (18.65 mg/ 100 g), citron (12.76 mg/ 100 g), mango (9.53 mg/ 100 g) and lime leaves which contained the lowest chlorophyll-b content (7.21 mg/ 100 g). However, no significant differences were found in the leaf chlorophyll- b content between lime and mango seedlings.

Table 2. Effect of NaCl levels on the leaf chlorophyll-a content (mg/ 100-g fresh weight) of some fruit species during 1998/ 99 and 1999/ 2000 seasons.

Treatments	Leaf Samples						Average
	First		Second		Third		
	1998/99	99/2000	1998/99	99/200(	1998/99	99/2000	
Fruit species							
Olive	60.09 a	72.69 a	66.90 a	62.50 a	58.08 a	62.12 a	63.73 a
Guava	51.23 b	55.63 b	30.89 b	24.83 b	13.24 b	15.44 b	31.88 b
Citron	30.38 c	32.60 c	29.62 b	21.18 c	11.90 b	17.94 b	23.93 c
Lime	11.77 d	16.17 d	21.51 c	16.11 e	7.17 c	9.37 e	13.68 d
Mango	8.11 d	12.33 e	29.16 b	25.00 b	9.84 bc	14.06 c	16.42 d
Salinity treatments							
0 (Control)	34.31 a	40.91 a	43.46 a	37.34 a	30.16 a	38.38 a	37.43 a
25 m. mol NaCl	30.12 b	34.42 b	35.00 b	30.90 b	21.38 b	25.78 b	29.60 b
50 m. mol NaCl	31.08 b	33.08 b	33.30 bc	29.22 b	14.10 bc	18.34 c	26.52 bc
100 m. mol NaCl	36.03 a	40.83 a	29.57 c	23.35 c	11.40 c	15.76 d	26.16 c

Means not sharing the same letter within columns are significantly different (P< 0.05), Duncan's multiple range tests.

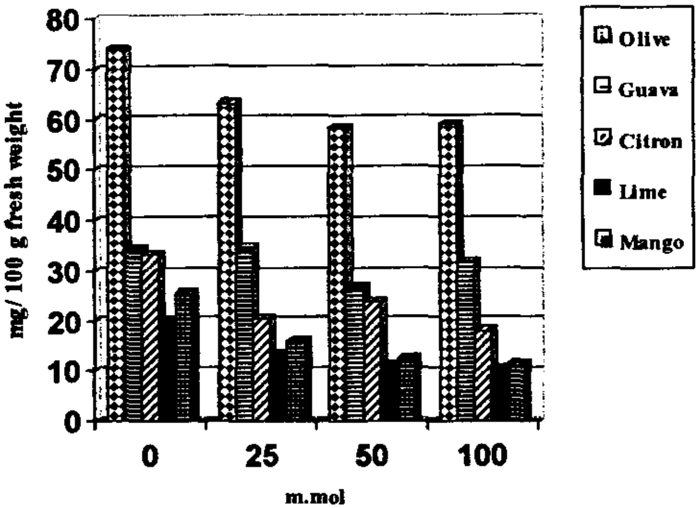


Fig.(2): Effect of NaCl levels on leaf chlorophyll-a content

As for the influence of salinization on the different fruit species, data showed that, as an average of five fruit species, all NaCl concentrations in the irrigation water caused significant decreases in the leaf chlorophyll- b content as compared with the control. In contrast, no significant differences were noted in the leaf chlorophyll-b content between treatments (Table, 3).

3- Total chlorophyll:

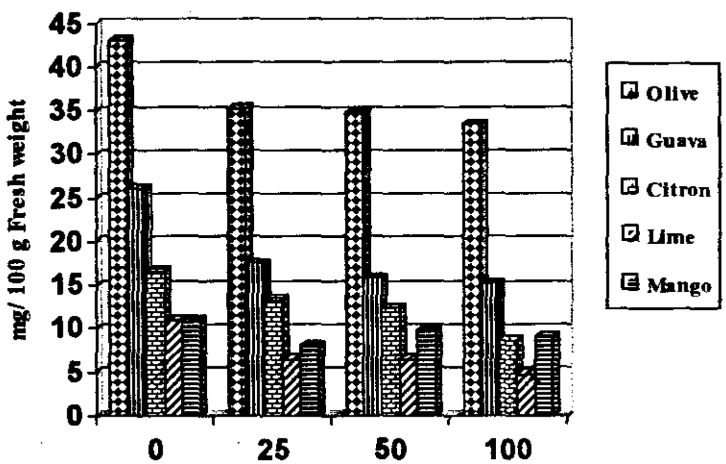
Results in Table (4) and Fig. (4) indicated that there were obvious differences in the leaf total chlorophyll content between the experimental fruit species. It was also noted that the leaves of olive seedlings contained the highest level of total chlorophyll (100.36 mg/ 100 g fresh weight) followed by guava (50.85 mg/ 100 g), citron (37.75 mg/ 100 g), mango (25.94 mg/ 100 g) and the leaves of lime seedlings which had the lowest concentration of total chlorophyll (20.91 mg/ 100 g).

Results also revealed that the levels of leaf total chlorophyll in the fruit species significantly decreased with increasing NaCl concentration in the irrigation water as compared with the control. It was also noted that, as an average of five fruit species, there were no significant differences in the leaf total chlorophyll content between 25 and 50 m.mol and between 50 and 100 m.mol NaCl salinity treatments, however.

Table 3. Effect of NaCl levels on the leaf chlorophyll-b content (mg/ 100 g fresh weight) of some fruit species during 1998/ 1999 and 1999/ 2000 seasons.

Treatments	Leaf Samples						Average
	First		Second		Third		
	1998/99	99/2000	1998/99	99/2000	1998/99	99/2000	
Fruit species							
Olive	47.75 a	43.65 a	29.10 a	25.00 a	35.01 a	39.21 a	36.62 a
Guava	25.93 b	21.13 b	24.76 b	20.36 b	9.82 b	9.92 b	18.65 b
Citron	17.12 c	12.92 c	15.76 c	11.96 d	9.35 b	9.45 b	12.76 c
Lime	11.12 d	7.06 d	10.45 d	8.25 e	3.11 d	3.31 c	7.21 d
Mango	5.90 e	5.74 d	16.27 c	14.07 c	6.55 c	8.65 b	9.53 d
Salinity treatments							
0 (Control)	23.89 a	19.67 a	25.56 a	21.40 a	18.24 a	20.68 a	21.57 a
25 m. mol NaCl	20.90 b	16.68 b	17.36 b	15.16 b	11.35 b	15.55 b	16.17 b
50 m. mol NaCl	21.10 b	17.00 ab	18.44 b	14.24 b	10.35 bc	12.45 b	15.59 b
100 m. mol NaCl	22.75 a	16.65 b	15.52 c	13.12 b	9.32 c	9.56 c	14.48 b

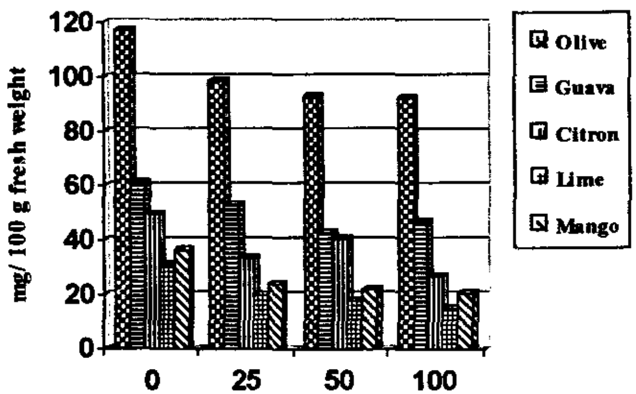
Means not sharing the same letter within columns are significantly different ($P < 0.05$), Duncan's multiple range tests.



m.mol
Fig(3):Effect of NaCl levels on chlorophyll-b content

Table 4. Effect of NaCl levels on the leaf total chlorophyll content (mg/100 g fresh weight) of some fruit species during 1998/ 99 and 1999/ 2000 seasons.

Treatments	Leaf Samples						Average
	First		Second		Third		
	1998/99	99/2000	1998/99	99/200(	1998/99	99/2000	
Fruit species							
Olive	113.19 a	110.99 a	90.70 a	92.80 a	96.13 a	98.35 a	100.36 a
Guava	78.00 b	75.92 b	50.17 b	52.57 b	23.12 b	25.32 b	50.85 b
Citron	48.53 c	44.47 c	41.25 c	43.65 c	22.20 b	26.42 b	37.79 c
Lime	24.17 d	21.95 d	26.10 d	30.20 d	10.41 c	12.63 d	20.91 e
Mango	18.26 e	13.82 e	40.14 c	44.36 c	18.43 b	20.67 c	25.94 d
Salinity treatments							
0 (Control)	61.59 a	57.19 a	62.13 a	66.35 a	52.53 a	54.93 a	59.12 a
25 m. mol NaCl	54.26 b	47.86 b	47.50 b	51.72 b	35.04 b	39.13 b	45.92 b
50 m. mol NaCl	53.15 b	49.11 b	48.10 b	52.20 b	25.42 c	29.82 c	42.97 bc
100 m. mol NaCl	59.44 a	56.84 a	40.65 c	40.89 c	22.00 d	24.04 d	40.64 c



Fig(4):Effect of NaCl levels on leaf total chlorophyll content

In general, the levels of leaf chlorophyll- a, b and total chlorophyll in the experimental fruit seedlings markedly decreased with increasing NaCl concentration in the irrigation water. In addition, the leaf chlorophyll content was significantly differed between the experimental fruit species as affected by NaCl salinity. High salt concentration in the soil solution create high osmotic pressure, reducing the availability of soil water to the plant and impeding nutrients uptake, thus, resulting in slowing of all biochemical processes including chlorophyll biosynthesis. It has been reported (Carter and Myers, 1963) that NaCl, $CaCl_2$ and Na_2SO_4 inhibited chlorophyll and carotene production in grapefruit leaves.

Nieves *et al.* (1991) reported marked reduction in lemon leaf chlorophyll by salt treatments. Also, Ezz and Nawar (1994) found that irrigation one- year- old sour orange seedlings with saline water (up to 4500 p.p.m. NaCl + $CaCl_2$) reduced leaf total chlorophyll and chlorophyll-a contents, but did not affect chlorophyll-b concentration as compared with the control. Bondok *et al.* (1995) found that the peach leaf chlorophyll content decreased with increasing salinity. Similarly, El-Hag and Sidahmed (1997) found that the leaf chlorophyll content of lime seedlings was progressively reduced by high salinity. El- Dawwey (1998) reported that with regard to the type of salt, NaCl, Na_2SO_4 and $CaCl_2$ caused adverse effects in decreasing order on leaf chlorophyll content of Balady mandarin and Manfalouty pomegranate seedlings.

CONCLUSION

It can be concluded from the above mentioned results that one should select those fruit species or cultivars with salinity adaptability under field conditions. In addition, the accumulation of proline and the reduction of chlorophyll contents are generally considered to be responses of the different fruit species to increasing salinity stress.

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الملخص العربي

تأثير الإجهاد الملحي على محتوى الأوراق من البرولين والكلوروفيل لبعض أنواع الفاكهة

حياة مصطفى عبد القادر، صباح محمود صباح و أحلام عبد المنعم السيد

محطة بحوث البساتين بالصبحية- معهد بحوث البساتين- مركز البحوث الزراعية.

أجري هذا البحث خلال موسمي ١٩٩٨/ ١٩٩٩و ١٩٩٩/ ٢٠٠٠م بغرض التعرف على تأثير الملوحة بكلوريد الصوديوم في ماء الري (بتركيزات صفر، ٢٥، ٥٠، ١٠٠ ملليمول) على محتوى الأوراق من الحمض الأميني برولين وكل من كلوروفيل- أ ، ب ، الكلوروفيل الكلي في شتلات متماثلة بعمر سنة، نامية بالصوبة الزجاجية لقسم النبات والأحياء الدقيقة – كلية العلوم – جامعة الملك سعود وهي: زيتون (صنف نيبالي)، جوافة (صنف سافيدا)، ترنج (صنف بلدي)، ليمون بلدي مالح (ليمون بنزهير) والمانجو(صنف تيمور). أظهرت النتائج بوجه عام أنه تحت ظروف الإجهاد الملحي إحتوت أوراق شتلات الترنج على أعلى نسبة من حمض البرولين (٧,٦٤مجم/ جم وزن جاف) يليها الليمون البنزهير (٦,١٧مجم/ جم)، المانجو (٤,٦ مجم/ جم)، الجوافة (٢,٣٦مجم/ جم) وأخيرا شتلات الزيتون التي احتوت أوراقها على أقل نسبة من البرولين (١,٥٧مجم/ جم). كما أظهرت النتائج أن زيادة تركيز ملح كلوريد الصوديوم في ماء الري قد أدى إلى حدوث زيادة مؤكدة إحصائيا في محتوى أوراق شتلات الفاكهة المختلفة من البرولين مقارنة بالكنترول. وبالنظر إلى النتائج التي تم التحصل عليها من التجربة، وجد أن التغير في مستوى البرولين بالأوراق له علاقة عكسية بتحمل الإجهاد الملحي. وبناءا على ذلك، فإنه يمكن اعتبار أن تحمل أنواع الفاكهة المختلفة لملوحة كلوريد الصوديوم في ماء الري ربما يتبع الترتيب التنازلي الآتي: الزيتون ، وهو الأكثر تحملا للملوحة ثم الجوافة فالمانجو والليمون البنزهير وأخيرا الترنج، وهو الأقل تحملا للإجهاد الملحي. كما أظهرت النتائج أيضا أن زيادة تركيز ملح كلوريد الصوديوم في ماء الري (حتى ١٠٠ملليمول) قد أدى إلى حدوث إختزال ونقص في محتوى أوراق شتلات الفاكهة المختلفة من كلوروفيل أ، ب و الكلوروفيل الكلي وذلك بالمقارنة بالكنترول. بالاضافة إلى ذلك، فقد اختلف محتوى الأوراق من الكلوروفيل بين شتلات الفاكهة وبعضها البعض تحت ظروف المعاملات الملحية بكلوريد الصوديوم. وعموما، يمكن اعتبار أن تراكم حمض البرولين ونقص محتوى الأوراق من الكلوروفيل أنه يشكل استجابة لشتلات الفاكهة المختلفة للزيادة في الإجهاد الملحي في بيئة النمو.