تأثير الملوحة في اختلال توازن الشوارد وفي اصطناع الغلوكوزيدات المقوية للقلب في نبات الديجيتال الأرجواني *Digitalis purpuria*

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الملخص

خلفية وهدف البحث: تؤثر الملوحة في إنتاج الأرض وذلك من خلال التراكيز الموجودة فيها، ويعتقد أن الكالسيوم يؤثر في مستوى الشوارد المعدنية +Na بطريقة تنافسية. ويظهر هذا البحث تأثير الملوحة في اصطناع الغلوكوزيدات المقوية للقلب في نباتات الديجيتال الأرجواني.

المواد والطرائق: تمت زراعة نباتات الديجيتال الأرجواني *Digitalis purpuria* في ثلاثة تراكيز مختلفة من الملوحة 20 ، 40 ، 80 mM بإضافة محلول كلور الصوديوم Nacl خلال 20 أسبوعاً. و كان لأوراق النباتات المعرضة للملوحة الشديدة محتوى أعلى من شاردة البوتاسيوم K^+ ومحتوى أقل من Ca^{2+} مقارنةً بالأوراق لنباتات مجموعة الشاهد control.

النتائج: أصبحت النسبة الشاردية K^+/Na^+ لأوراق النباتات المعرضة للملوحة المركزة أقل من الواحد، عندما بدأت أعراض الشيخوخة بالظهور أصبح معدل النسبة الشاردية Na^+/K^+ أعلى كلما كانت شروط الملوحة أعلى، وكلما كان زمن التركيز أطول كان لدى جذور النباتات المعرضة لظروف الإجهاد الملحي محتوى أقل من K^+ كلما ازدادت الملوحة و كلما طال زمن التعرض لظروف الإجهاد الملحي.

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وقد كان تركيز شاردة Na^+ لجذور النباتات المعرضة للملوحة المركزة أعلى من تراكيز شوارد الصوديوم Na^+ في جذور النباتات في الشاهد، ولكنها تناقصت في نهاية التجربة بسبب التنخر وفقدان الأجزاء الفتية للجذور.

كلما ازداد تركيز الملوحة انخفضت تراكيز شوارد الكالسيوم Ca^{2+} بينما بقي معدل Na^+/K^+ في الجذور محافظاً على مستوى منخفض وذلك بسبب التنخر والتموت النسيجي وضياع الأجزاء الفتية للجذور.

الاستنتاج: لم تتداخل الملوحة وحالة عدم التوازن الشاردي بشكل ملحوظ مع الاستقلاب الحيوي..

الكلمات المفتاحية: الديجيتل الأرجواني، الملوحة، الغلوكوزيدات المقوية للقلب، اختلال توازن الشوارد.

Effect of Salinity on Ionic Disequilibrium and Cardinolids Synthesis in Digitalis Purpuria

Issam Al-Shma'a*

Abstract

Background and Objective: Salinity is involved in decline of plant production in arid and semi-arid lands. It is believed that Ca^{2+} maintains the structural integrity of the plasma membrane by cross linking the negatively charged head groups of the plasma membrane. In this paper we study the effect of salinity on cardenolide synthesis in *Digitalis purpuria* plants.

Material and Methods: Plants of *Digitalis purpuria* were grown in three salinity conditions: 20, 40 and 80 mM NaCl, during 20 weeks.

Results: Leaves of stressed plants had a higher K^+ and Na contents and a lower Ca content than leaves of control plants , k^+/Na^+ ratio of leaves of stressed plants become a lower than 1 when the senescence was started . Na^+/Ca^{2+} ratio became higher with higher salinity conditions and longer time of stress. The roots of stressed plants had lower K^+ content in higher salinity and longer time of stress. The Na^+ content of roots of stressed plants was higher than the Na^+ content in roots of control plants, but decreased at the end of experiment due to necrosis and loss of young parts of roots. Ca^{2+} content in roots was lower as the salinity stress was higher. The Na^+/Ca^{2+} ratio in roots was maintained low, due to necrosis and loss of young parts of roots. Salinity and ionic disequilibrium did not interfered significantly with cardenolids metabolism.

Conclusion: The content of cardenolides did not change significantly in salinity conditions until the death of the plants.

Keywords: Digitalis purpuria, salinity, cardinolids, ionic disequilibrium

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Introduction

Salinity is involved in decline of plant production in arid and semi-arid lands [1] . The activity of many enzymes is affected by addition of salts during the experience [2] . Some enzymes show marked inhibition by inorganic ion concentration above 100 mM [3] . It is believed that Ca^{2+} maintains the structural integrity of the plasme membrane by cross linking the negatively charged head groups of the plasme membrane [4]. At high concentration of Na+ this Ca^{2+} is displaced by Na+ causing an influx of Na+ . In high external salinity , Na+ may enter the cytoplasm passively across the plasma membrane because the cytoplasm is electrically negative with respect to the apoplast and the concentration of Na+ is probably higher outside the cytoplasm . In plants , a Na+ selective chanel has not been identified in the plasme membrane , therefore entry of Na+ into cytoplasm may be through a K+ channel with some permeability to Na+ [5] .In glycophytes, the membrane depolarizes in response to external NaCl . Despolarization will open the cation outward rectifier channels and Na+ could move into the cell at a rate governed by the electrochemical gradient for Na+ and the effective permeability of the chanels to Na+ [6] .

Summarising , salt stress reduced plant growth through water deficits , ion toxicity , ionic inbalance or a combination of any of these factors , and plays an important role on turgor,permeability, enzimatic activity and hormonal balance [7]. All these effects cause a primary metabolism fall , thus intermediary products could by used in secondary metabolite synthesis. In a previous work [8] we reported the effect of salinity in the nicotine production in *Nicotina rustica* plants . In this paper we study the effect of salinity on cardenolide synthesis in *Digitalis purpuria* plants .

Materials and methods

Culture conditions

Seeds of *Digitalis purpuria* were sown in equal sice flower pots containing a mixture of sand and vermiculite . Pots were moistened with the same amount of deionised water three times a week , when the germination began , the plantules were divided into four groups : the first was rinsed with Arnon and stoud solution and used as a control , the second was rinsed with the same solution , but 20 mM NaCl was added (20mM Nacl plants), the third with 40 mM NaCl added to it (40mM NaCl plants) and the fourth with 80 mM NaCl (80mM NaCl plants). The plants

were harvested after 14, 16, 18, 19, 20 , 21, 22, weeks of the treaments. In each sample three different plants were harvested and each plant was analyzed twice . Thus , the total number of analysis for each sample was six. Leaves and roots were sampled separately. The following measurements and analysis were carried out : measurement of growth (dry weight) , Na+ , K+ , Ca^{2+} , and total cardenolide contents .

Dry weight

Dry weight determinations were made by heating leaves and roots separately at 60°C for 48 hours and then to constant weight .

Ion determination

Na+ , k+ and Ca^{2+} determination were made by emission spectroscopy for plasma icp in a Mixter spectrometer multichannel sequenical pf plasma icpjy70 plus [9]

Cardenolide determination

The extraction and purification of cardenolide glycosides was carried out as described by Lught and Noordhock-Ananies (1974) [10] . Determination of cardenolide was made by means of colorimetric method based on a Baljet colour reaction , modified by Khafegy and Girgis 1974 [11] . Total cardenolide contents were measured as digoxin .

Results and Discussion

Dry weight

Plants grown under salinity conditions had lower dry weight (DW) values than control plants . This effect was more pronounced in plants grown on higher NaCl concentration , and was observed in both leaves and roots (Fig. 1) .

Univalent ions content

The content of K+ in roots and leaves of control plants remained relatively constant and in the interval considered as normal during the first 17 weeks of growth and went down at the end of the experiment (tab. 1and2) , probably due to inert substances accumulation in plants . Roots and leaves of control plants had a very low content of Na^+ and total M ($Na^+ + K^+$) contents were very similar to K^+ (tab. 1and2) .The roots of stressed plant had K^+ contents lower with higher salinity conditions of growth and longer time of stress . Na^+ contents of 20 mM plants roots increased during the experiment but 40 mM plants roots Na^+ contents increased only until the 18^{th} week of growth and then decreased , until the

end of the experiement; and finally Na^+ contents of 80 mM plants roots reached very high levels during the first 14 weeks of growth decreasing thereafter until the end of the experiment . A high content of Na^+ inhibited the root growth and led to necrosis and loss of young roots tissues in no-halophytic plants (Carmer et al., 1986) . Tot M contents in roots of stressed plants were similar to content of Na^+ .The results showed that in our experimental conditions , the limit for normal life of root of *Digitalis purpuri*a had a content of total M 2 times higher than the roots of control plants . Roots of 40 mM and 80 mM plants reached this limit after 18 weeks and 14 weeks of growth respectively , then they underwent necrosis and death . The content of K^+ in leaves of stressed plants were slightly higher than in leaves of control plants , specially in those of 40 mM plants . These results agree with Grenway et all (1966) [13] , who reported an increase in contents of K^+ and Na^+ in leaves of salt sensitive plants . Contents of Na+ and Tot M ions were notably higher in the leaves of plants grown in salinity conditions than in the leaves of control plants and were higher the greater the salinity conditions than in the leaves of control plants and were higher the greater the salinity conditions and time of stress . Our results showed that , generally , when the Na+ contents in leaves reached 0.7 meq/g DW , their content of univalent ions was two times higher than in control plant leaves , this high content is the limit of viability on *Digitalis purpuri*a leave-cells .

On the other hand Jeschke 1979 [14] showed that senescence is started when the K^+/Na^+ ratio is lower than 1(Table 3) . Our results showed that K^+/Na^+ ratio in leaves of 20 mM , 40 mM and 80 mM plants were lower than 1 at 19,18, 19 and 16 weeks respectively , and the roots of these plants at 18 and 16 respectively . We infer than these plants suffered stronger degrading processes the higher the salinity conditions were.

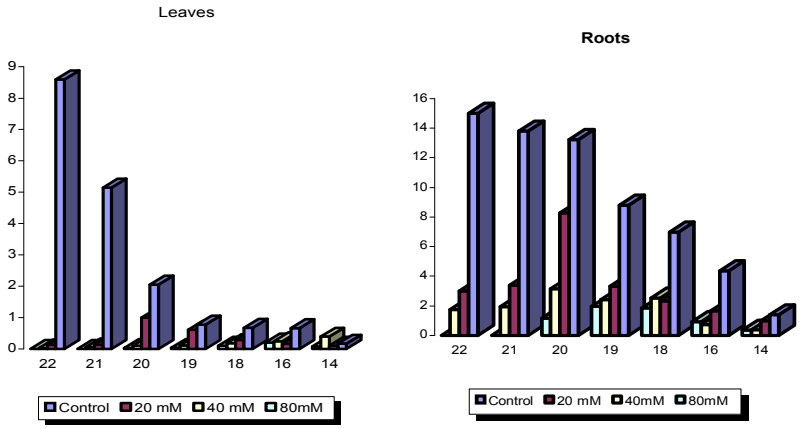
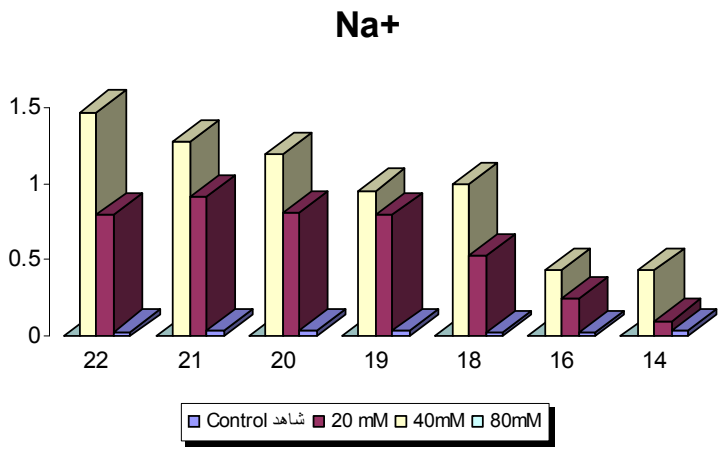


Figure 1. Dry weight (DW) expressed in g. Data are the average of six analyses. Weeks : weeks of treatment

Table 1.K+,Na+ and Tot M (Na^+,K^+) content in roots of treated and control plants, expressed in meq/g DW . Data are the average of six analyses. Weeks : weeks of treatment.

Weeks	Control	20mM	40mM	80mM
		Na^+		
14	0.033±0.001	0.098±0.004	0.435±0.001	0.594±0.010
16	0.019±0.000	0.249±0.020	0.431±0.017	1.113±0.087
18	0.025±0.007	0.527±0.031	1.000±0.070	2.006±0.205
19	0.032±0.015	0.799±0.062	0.956±0.085	2.000±0.101
20	0.032±0.030	0.808±0.082	1.196±0.099	2.008±0.112
21	0.031±0.016	0.911±0.077	1.275±0.073	-
22	0.029±0.033	0.800±0.068	1.471±0.065	-
		K^+		
14	0.753±0.054	0.751±0.049	0.842±0.060	0.728±0.044
16	0.765±0.039	1.055±0.095	1.049±0.084	1.000±0.080
18	0.793±0.063	0.933±0.065	0.838±.0.033	0.810±0.069
19	0.731±0.059	0.775±0.065	0.788±0.051	0.781±0.068
20	0.671±0.043	0.700±0.039	0.732±0.047	0.769±0.080
21	0.593±0.037	0.700±0.045	0.0768±0.057	-
22	0.599±0.039	0.681±0.050	0.715±0.040	-
		Na^+ + K^+		
14	0.680±0.054	0.740±0.073	1.077±0.060	1.222±0.095
16	0.690±0.039	1.230±0.104	1.480±0.084	1.000±0.273
18	0.800±0.063	1.320±0.127	1.779±.0.100	2.727±0.069
19	0.670±0.059	1.400±0.140	1.729±0.149	2.729±0.207
20	0.698±0.043	1.362±0.155	1.835±0.164	2.756±0.180
21	0.600±0.070	1.460±0.100	1.956±0.187	-
22	0.582±0.039	1.400±0.117	1.999±0.284	-



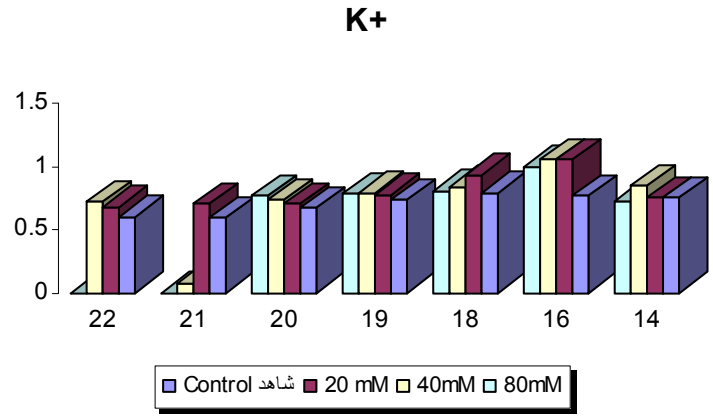


Figure 2. Na^+, K^+ content in roots of treated and control plants, expressed in meq/g DW. Data are the average of six analyses.
Weeks : weeks of treatment.

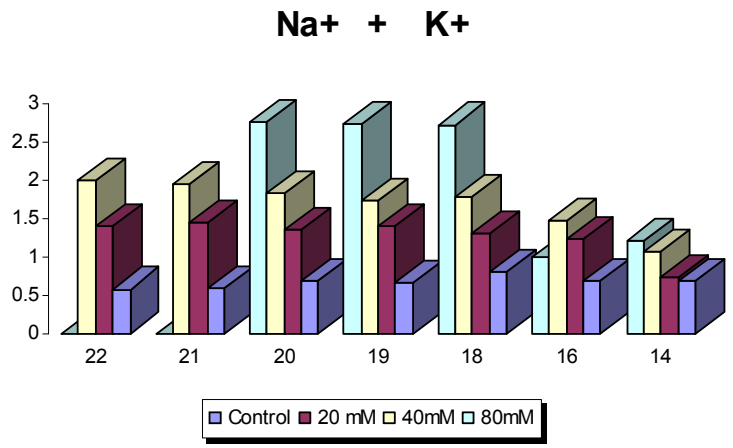


Figure 3. Tot M (Na^+ and K^+) content in roots of treated and control plants, expressed in meq/g DW. Data are the average of six analyses. Weeks : weeks of treatment

Ca2+ content

The Ca2+ content in roots of plants control increased until the 20th week and then decreased until the end of experiment , probably due to necrosis and loss of the youngparts of roots (Table 4) .The levels of Ca^{2+} in the roots of stressed plants of *Digitalis purpuria* increased during the course of the experiment , but they were lower than the levels in roots of control plants and were lower the higher the salinity conditions of grown , and the longer the time of stress . The Na^+/ Ca^{2+} ratio (Table 5) was low and relatively constant in the roots of control plants during the course of the experiment , but this ratio increased and then decreased in the roots of 20mM and 40 mM plants and decreased continuously in the roots of 80 mM plants Whittington and Smith 1992 [15] considered that high Na^+/ Ca^{2+} ratios damage semipermeability and permit Na+ and K+ uptake but lower

Na^+/Ca^{2+} ratio maintain semipermeability . We must not consider the decrease of Na^+/ Ca^{2+} ratio in the stressed roots as a protective mechanism , due to necrosis and loss of young parts of roots , because Ca^{2+} linked to the older part of plants . The levels of Ca^{2+} in the leaves

of *Digitalis purpuria* control plants were sufficient and increased slightly during the course of the experiment due to the increase in the number , of old leaves with respect to young ones . On the contrary , leaves of 20 mM and 40 mM plants increased their contents in Ca^{2+} for 18 weeks and then decreased due loss of old leaves rich in Ca^{2+} . The content of Ca^{2+} in leaves of 80 mM plants increased during the experiment because their leaves were always in senescence . The Na^{+}/Ca^{2+} ratio was low in the leaves of control plants , but very high in leaves of stressed plants , showing the damage in the latter .

Cardenolide content

The content of Cardenolides in leaves and roots of control and stressed plants first increased and then decreased during the course of experiment (Fig.8). The salinity did not interfere in the Cardenolide accumulation since that Cardenolide in stressed plants was only slightly lower than in the control plants , and proportionally stressed plants had higher number of adult leaves . These facts suggest that ionic disequilibrium does not significantly interfere in the enzymatic systems of Cardenolide synthesis or degradation**.**

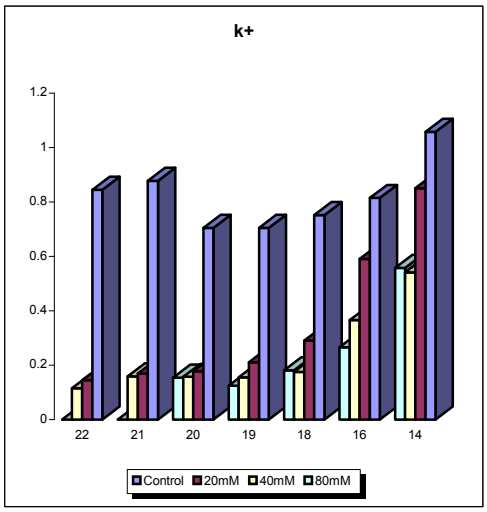
Conclusions

The plants of *Digitalis purpuria* stressed by salinity , did not have a suitable mechanism not to uptake Na^{+} , generally , had a high content in Tot M mainly in leaves . In roots the levels of total M , decreased in stressed plants in the course of the experiment due to necrosis and loss of young roots .The Na^{+}/ Ca^{2+} ratio was lower in leaves of control plants than in leaves of stressed plants , but during the course of the experiment this ratio decreased in leaves of stressed plants . This is due to loss of old leaves , but not to a protective mechanism .

The content of cardenolides did not change significantly in salinity conditions until the death of the plants . We must infer that there was no clear effect of ionic disequilibrium on enzymatic systems either of cardenolides synthesis or degradation .

Table 2. K^+,Na^+ and Tot M (Na^+,K^+) content in leaves of treated and control plants, expressed in meq/g DW . Data are the average of six analyses. Weeks : weeks of treatment.

Weeks	Control	20mM	40mM	80mM
	Na^+			
14	0.133±0.017	0.514±0.063	0.1.218±0.105	1.980±0.179
16	0.243±0.025	0.644±0.077	1.491±0.115	1.577±0.154
18	0.190±0.007	0.851±0.071	1.856±0.160	1.193±0.205
19	0.290±0.045	0.947±0.035	1.701±0.153	0.860±0.085
20	0.284±0.039	0.997±0.082	1.650±0.145	0.454±0.053
21	0.256±0.022	1.253±0.099	1.282±0.100	-
22	0.260±0.031	1.800±0.110	1.270±0.113	-
	K^+			
14	1.058±0.093	0.851±0.089	0.542±0.060	0.558±0.081
16	0.815±0.059	0.591±0.045	0.365±0.084	0.266±0.080
18	0.752±0.053	0.290±0.025	0.175±.0.033	0.181±0.019
19	0.705±0.069	0.210±0.017	0.155±0.051	0.125±0.008
20	0.705±0.073	0.178±0.033	0.157±0.047	0.154±0.080
21	0.878±0.077	0.169±0.031	0.159±0.057	-
22	0.845±0.089	0.145±0.011	0.115±0.040	-
	Na^+ + K+			
14	1.187±0.102	1.310±0.124	1.805±0.169	2.459±0.263
16	0.982±0.091	1.114±0.114	1.765±0.154	1.835±0.165
18	0.916±0.063	1.053±0.107	2.015±.0.180	1.244±0.146
19	0.860±0.059	1.035±0.095	1.789±0.159	0.857±0.096
20	0.801±0.073	1.098±0.088	1.715±0.160	0.569±0.064
21	0.927±0.079	1.311±0.104	1.351±0.121	-
22	1.014±0.100	1.319±0.117	1.275±0.110	-



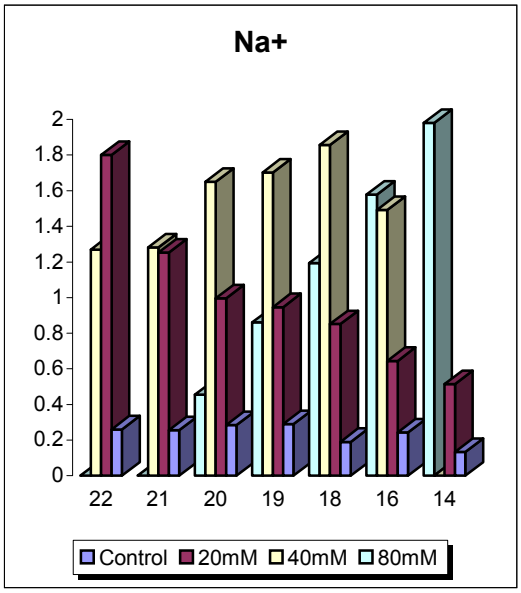


Figure 3. Na^+, K^+ content in leaves of treated and control plants, expressed in meq/g DW. Data are the average of six analyses. Weeks : weeks of treatment

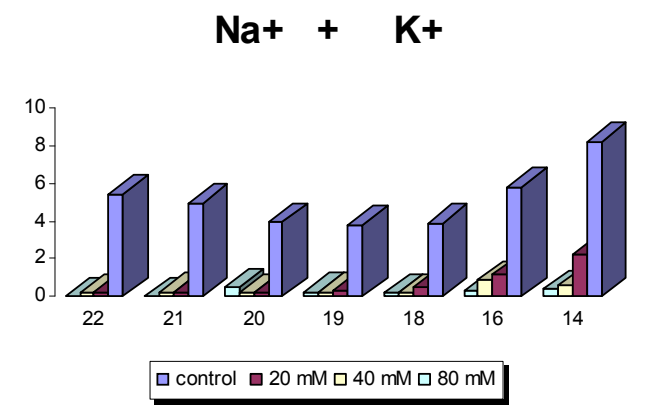
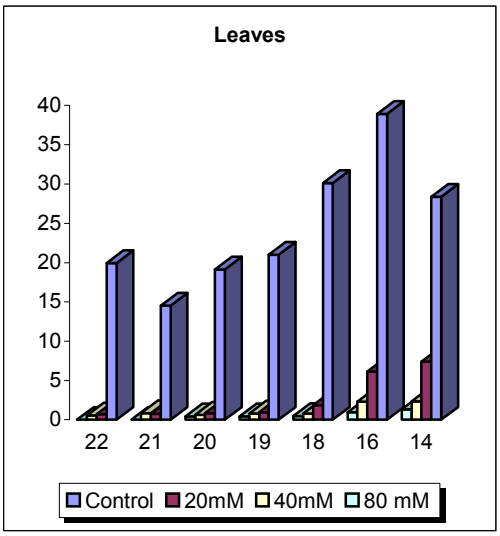
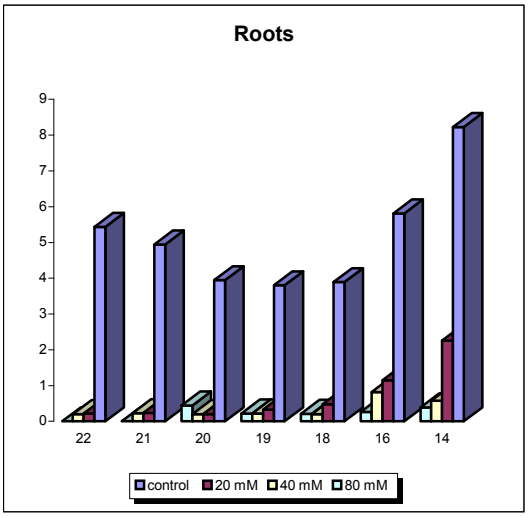


Figure 4. Tot M (Na^+ and K^+) content in leaves of treated and control plants, expressed in meq/g DW. Data are the average of six analyses. Weeks : weeks of treatment

Table 3. K^+/Na^+ contents ratio in leaves and roots

Weeks	Leaves			
	Control	20mM	40mM	80mM
14	28.39	7.38	2.33	1.33
16	38.92	6.11	2.30	0.96
18	30.13	1.85	0.80	0.46
19	21.03	0.96	0.80	0.44
20	19.13	0.85	0.65	0.42
21	14.50	0.74	0.85	-
22	19.96	0.72	0.56	-
Roots				
14	8.23	2.26	0.58	0.39
16	5.81	1.15	0.82	0.26
18	3.89	0.48	0.20	0.21
19	3.80	0.33	0.22	0.22
20	3.95	0.20	0.20	0.44
21	4.95	0.24	0.23	-
22	5.44	0.22	0.20	-

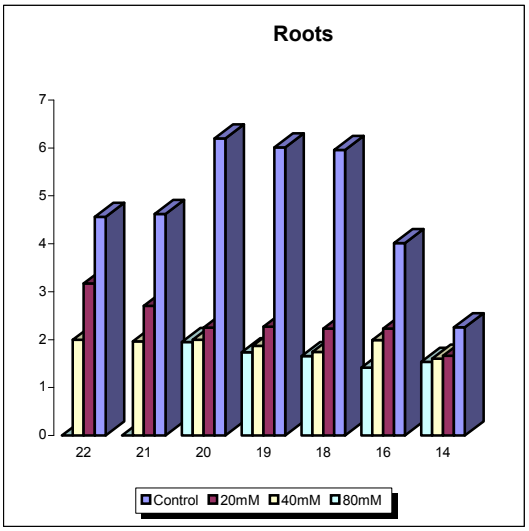


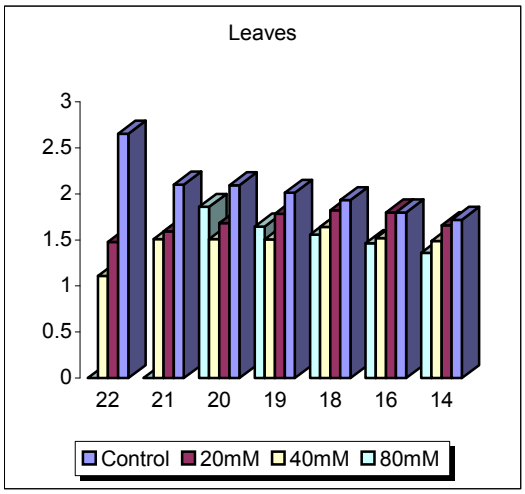


Fifgure 5. K^+/Na^+ contents ratio in leaves and roots

Table 4. Ca^{2+} values in leaves and roots expressed in meq/g DW. Data are the average of six analysis. Weeks: weeks of treatment .

Weeks	Leaves			
	Control	20mM	40mM	80mM
14	1.719±0.143	1.662±0.150	1.490±0.127	1.364±0.114
16	1.8011±0.158	1.801±0.144	1.521±0.142	1.468±0.162
18	1.934±0.166	1.826±0.169	1.644±0.190	1.560±0.157
19	2.014±0.191	1.786±0.138	1.510±0.128	1.647±0.166
20	2.094±0.183	1.684±0.162	1.513±0.161	1.861±0.171
21	2.101±0.204	1.597±0.128	1.513±0.129	-
22	2.655±0.217	1.481±0.133	1.109±0.150	-
Roots				
14	2.255±0.238	1.662±0.089	1.606±0.153	1.537±0.122
16	4.014±0.310	2.229±0.132	1.992±0.167	1.413±0.130
18	5.958±0.447	2.230±0.137	1.744±.0.143	1.656±0.164
19	6.010±0.384	2.273±0.129	1.870±0.129	1.737±0.164
20	6.198±0.362	2.248±0.200	1.998±0.166	1.951±0.129
21	4.619±0.299	2.705±0.211	1.966±0.169	-
22	4.563±0.273	3.171±0.197	1.997±0.174	-

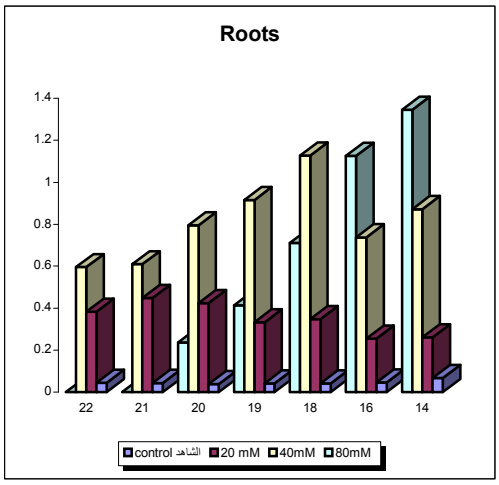




Fifgure 6. Ca^{2+} values in leaves and roots expressed in meq/g DW.

Table 5. Na^{+}/ Ca^{2+} contents ratio in leaves and roots

Weeks	Leaves			
	Control	20mM	40 mM	80mM
14	0.012	0.052	0.229	0.372
16	0.008	0.091	0.283	0.699
18	0.011	0.234	0.585	1.240
19	0.014	0.348	0.579	1.198
20	0.015	0.432	0.745	1.071
21	0.016	0.511	0.832	-
22	0.008	0.553	1.233	-
Roots				
14	0.068	0.261	0.872	1.345
16	0.046	0.255	0.738	1.126
18	0.042	0.348	1.127	0.712
19	0.042	0.332	0.915	0.414
20	0.039	0.424	0.795	0.237
21	0.044	0.448	0.610	-
22	0.045	0.383	0.596	-



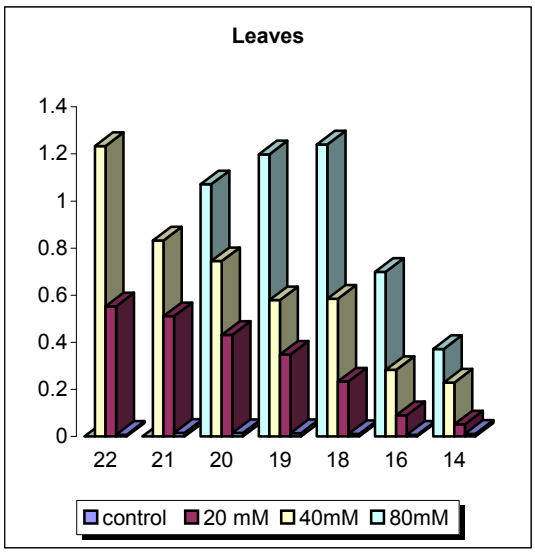
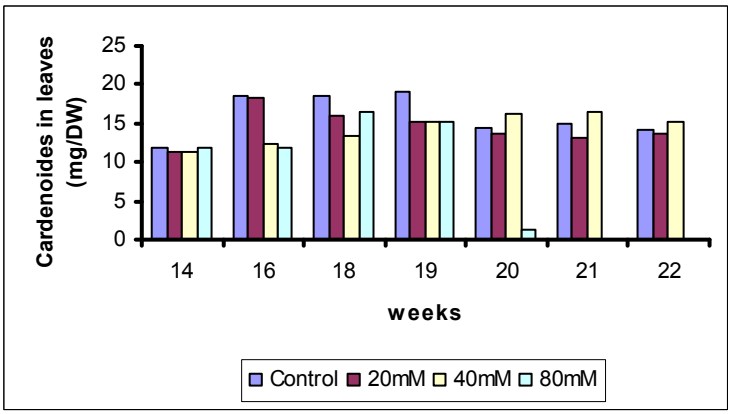
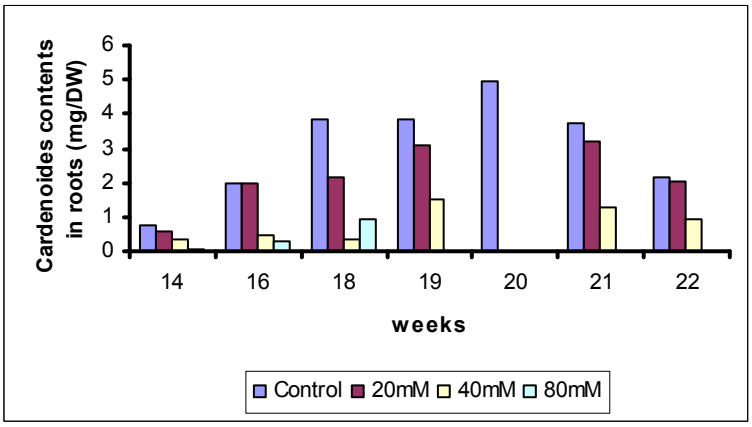


Figure 5. K^+/Na^+ contents ratio in leaves and roots

Table 6. Cardenoides contents in leaves and roots expressed in mg/DW

Weeks	Control	20mM	40mM	80mM
leaves				
14	11.73± 0.03	11.33± 0.05	11.33± 0.11	11.73± 0.07
16	18.49± 0.02	18.18± 0.07	12.49± 0.07	11.92± 0.03
18	18.50± 0.05	15.95± 0.08	13.29± 0.02	16.37± 0.04
19	19.08± 0.01	15.29± 0.02	15.09± 0.01	15.27± 0.01
20	14.52± 0.12	13.78± 0.01	16.15± 0.07	1.18± 0.02
21	14.9±0.01	13.09±0.03	16.42±0.06	--
22	14.29±0.03	13.59±0.10	15.32±0.01	
Roots				
14	0.73±0.005	0.59±0.003	0.37±0.004	0.05±0.001
16	1.98±0.020	1.96±0.003	0.44±0.005	0.31±0.005
18	3.87±0.020	2.13±0.011	0.33±0.002	0.91±0.003
19	3.86±0.008	3.10±0.004	1.54±0.011	0.01±0.00
20	4.96±0.002	3.91±0.003	1.23±0.010	0.00
21	3.75±0.010	3.23±0.011	1.26±0.09	0
22	2.13±0.005	2.01±0.20	0.93±0.002	0




Cardenoides contents in roots (mg/DW)
6
5
4
3
2
1
0
14
16
18
19
20
21
22
weeks
Control
20mM
40mM
80mM

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تاريخ ورود البحث إلى مجلة جامعة دمشق: 2007/2/25.
تاريخ قبوله للنشر:2008/5/20.