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Response of Grapevines to Irrigation with Multicomponent Electrolyte Solutions in Presence of Chloride Salinity

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Abstract: The response of Zini grapevine to chloride salinity of six electrolyte concentrations of the irrigation water was investigated on two years old transplants grown hydroponically for nine months. Irrigation water salinity was increased at 20 $mol_c\ m^{-3}$ increments to a maximum of 100 $mol_c\ m^{-3}$ at constant stoichiometric ratios of 3:1 for Cl: SO_4 and 1:1 for Ca: Mg and fixed sodium adsorption (SAR) of 5. The results revealed significant decrease in plant height, number of young shoots and leaves and dry weight of the plants. Negative salinity effects on vegetative growth of the plants were appeared at an electrolyte concentration of 60 $mol_c\ m^{-3}$ and more. Increasing salinity levels increased proline contents and decreased chlorophyll concentration of the plants. Leaf Na and Cl contents were considerably increased and K and Ca contents decreased by increasing electrolyte concentration of irrigation water, whereas N, P and Mg were practically unaffected. Selectivity ratio for Na and K transport to the leaf was affected by salinity treatments. The exclusion ability for Na was markedly higher than that for Cl especially at higher electrolyte concentrations.

Key words: *Vitis vinifera,* concentration, proline, chlorophyll, K/Na

INTRODUCTION

Grape (*Vitis vinifera*) is one of the leading fruit crops grown in Jordan. The area planted with grape is increasing year after year. Several cultivars are grown, Zini is one of the most common local cultivars. Salinity of soil and irrigation water is a major barrier for sustainable crop production in arid and semiarid regions. Thus, it is often an important concern for irrigated agriculture. The limited fresh water resources and the increasingly need for food products insure the need for improving water use efficiency and using alternative sources such as saline water. Saline and waste waters are going to be an important future source of irrigation in Jordan. However, the long-term impacts of irrigation with these sources have negative influences on plant, soil and ground water aquifers; since irrigation with such water is often associated with build up of salts in soil. In Jordan, the average amount of salts deposited in the soil profile by irrigation water was found to range from 3500 to 6000 kg for each ha per year[1]. In the last two decades, grape plantations extended almost to all parts of Jordan, particularly the eastern and southern regions where drought and irrigation water salinity are the main limiting factors for growth and production. Most of the agricultural projects in these areas depend on underground water. Most of this water is dominated by high and toxic content of chloride that is concentrated as water evaporates from soil surface. The increase in groundwater salinity manifested by increasing Cl^- salts of Na, Ca and Mg was attributed to the excessive pumpage[2]. Information on impact of chloride toxicity of irrigation water will be of increasing importance if the area planted with this crop extended to other regions of the country and the proper management systems are not considered.

It was stated, in many researches, that salinity has osmotic[3,4] and toxic[5] effects on the plants. Grape trees are classified as a moderately salt-sensitive crop[6]. However, the salt tolerence in grapevine is rootstock[7-9] and cultivar[10-13] dependent. Most of the research quantifying the salt tolerance of plant species has been based on field studies and conducted by treating plants with saline solution of diverse chemical characteristics and confined to grape cultivares not grown in Jordan, thus making interpretation of results difficult. Furthermore, the salt tolerance of grapevines has usually been evaluated by salinizing the irrigation water with a single salt, usually NaCl as the salinizing agent[3,9,11,12,14-16], which does not reflect the composition of ordinary irrigation water. Under this condition, salinity caused a decrease in grapevine growth. This decrease was a function of total electrolyte concentration and specific ion toxicity. On the other hand, certain ions, like Cl, excrete specific effects that decrease growth and yield independent of osmotic effects. As soluble concentrations of these ions exceed a certain dose in the soil or irrigation water, specific toxic effects can be expected to occur[5,6].

Osmoregulation (osmotic adjustment) is one of the most important adaptive responses to water and salt stresses[17-19]. The decline in osmotic potential in response to stresses is achieved by accumulation of solutes within the cell and leaving of water from the vacuole. Concomitantly, the stressed plants accumulate osmotically active compounds, like carbohydrates and proline. The latter is the most prevalent free amino acid and organic solute that have been accumulated in halophytes and glycophytes[17,20]. However, the significance of proline accumulation under stresses has been a matter of debate[21,22]. Several studies were made to use proline accumulation for evaluation of tolerance or sensitivity of plants to water and salt stresses[19,20]. It has been found that increasing electrolyte concentration of irrigation water resulted in decrease in an increase in proline[3] and a decrease in chlorophyll[3,23] contents of grapevines.

The aim of this investigation was to study the influence of osmotic stress and specific chloride toxicity of multicomponent treatment solutions (comprising Na, Ca, Mg, K, Cl, SO_4 and HCO_3) on vegetative growth, proline, chlorophyll and mineral composition of grapevines.

MATERIALS AND METHODS

The experiment was conducted in a partially controlled glasshouse located at the Agricultural Research Station, University of Mutah during the period March, 2003-November, 2003. Uniform two years old Zini grapevine transplants grown on their own roots were used in these experiments. The transplants were planted in plastic bags (28 cm in diameter and 28 cm in height), containing soil. One transplant was planted in each bag. At the bottom of each pot, there were many holes to facilitate collecting drainage water. Physical and chemical properties of the soil were determined according to the standard procedures of the United States Salinity Laboratory Staff (USSL)[24] as presented in Table 1. The data indicate that soil structure was clay loamy, soil reaction was basic, organic matter content was low, Exchangeable cations (N, P, K, Ca, Mg and Na) were enough, electrical conductivity was 1.0 dS m^{-1}. There was no salinity problem in the study soil at the beginning of the research. The transplants were trained to 3-4 shoots per plant two weeks after planting.

The saline irrigation water was prepared by mixing chloride, sulfate and bicarbonate salts of sodium, calcium and magnesium with distilled and/or tap water in order to obtain the desired multicomponent electrolyte solution, typical of some saline irrigation water compositions of some areas in Jordan. Trace elements were also added at the 0.5 strength Hoagland nutrient concentration[25]. Tap water supplemented with half strength Hoagland solution was used as control. Under all treatments, potassium and bicarbonate were kept at 5 and 10%, respectively. Nitrogen and phosphorus were each added at a rate of 10 mg L^{-1}. In addition to the control treatment,

Table 1: Physical and chemical properties of the soil used in the experiment

Properties	Measurement
pH	8.0
EC (dS/m)	1.0
N (%)	0.085
P (ppm)	37.2
K (ppm)	307.0
Ca (meq/L)	6.7
Mg (meq/L)	7.3
Na (meq/L)	1.47
SAR	0.64
Mechanical analysis	
Sand (%)	26.0
Silt (%)	39.0
Clay (%)	35.0
Texture	Clay loam

Table 2: Analytical concentration (mol$_c$ m^{-3}) of the chemical species employed in preparation of the irrigation water

Electrolyte concentration (mol$_c$ m^{-3})	EC (dS m^{-1})	mol$_c$ m^{-3} $[Ca^{++}]_T$	$[Mg^{++}]_T$	$[Na^+]_T$	$[K^+]_T$	$[HCO_3^-]_T$	$[Cl^-]_T$	$[SO_4^-]_T$
Control	0.8	3.4	2.2	2.2	0.1	3.8	2.4	1.6
20	1.9	4.3	4.3	10.4	1.0	2.0	13.5	4.5
40	3.8	10.8	10.8	16.4	2.0	4.0	27.0	9.0
60	5.5	17.9	17.9	21.2	3.0	6.0	40.5	13.5
80	7.1	25.4	25.4	25.2	4.0	8.0	54.0	18.0
100	8.5	33.1	33.1	28.8	5.0	10.0	67.5	22.5

Table 3: Single ion activities of the major species employed in the irrigation water as obtained from WATEQ program

Electrolyte concentration (mol$_c$ m^{-3})	(Ca^{++})	(Mg^{++})	(Na^+)	(K^+)	(HCO_3)	(Cl^-)	(SO_4^-)	I*
Control	2.06	1.36	1.97	0.09	3.35	2.15	0.86	0.011
20	2.14	2.20	8.92	0.85	1.67	11.57	1.98	0.025
40	4.40	4.59	13.42	1.61	3.11	22.00	2.91	0.050
60	6.43	6.76	16.82	2.34	4.42	31.91	3.57	0.075
80	8.33	8.82	19.56	3.03	5.63	41.47	4.10	0.100
100	10.10	10.77	21.97	3.70	6.77	50.77	4.55	0.120

*Ionic strength

the irrigation water treatments included 6 combinations of saline water compositions having:

A: Cl:SO_4 stoichiometric ratio of 3:1
B: Constant stoichiometric ratio of 1 for Ca:Mg.
C: Constant level of SAR=5
D: Total electrolyte concentrations of 20, 40, 60, 80 and 100 $mol_c\ m^{-3}$, in addition to control.

Calculations were done using a computer program especially developed to facilitate the job. Table 2 shows the chemical composition of the irrigation water used throughout the research. Ionic specification of the above solutions was performed using WATEQ program[26] at pH 7 to keep track of precipitation of gypsum and calcite. Single ion activities of the major species in treatment solutions are shown in Table 3.

The main stem height was measured from the soil surface to its apex. Number of young shoots/plant and leaves/young shoot were counted at the end of the investigation. Dry weights of the plants were determined using an oven at 68°C to a constant weight. Proline content was colorimetrically estimated in fresh leaf samples extracted with sulphosalicylic acid and the extracts reacted with acid-ninhydrin, ten months after beginning of the investigation, according to the method of Bates *et al.*[27] Chlorophyll content was estimated by homogenization of one-gram fresh leaf sample, usually the 7th leaves from the shoot apex, in 20 mL of 80% acetone. After filtration, the extraction was repeated with another 20 mL of 80% acetone. The combined filtrates were made up to 50 mL with acetone[28]. Chlorophyll content was determined according to Harborne[29] using spectrophotometer.

In order to determine the leaf mineral composition, representative samples of two leaves usually 4-5th mature leaves from randomly distributed shoots were collected randomly around the selected transplants nine months after beginning of the experiment in November 2003. They were washed with distilled water and oven dried at 70°C till constant weight was obtained. The samples were ground and digested with nitric acid. From these solutions, the subsequent analyses were performed. Nitrogen (N) was determined by a semi-micro Kjeldahl method. Phosphorus (P) was measured by atomic emission spectrophotometer. Potassium (K) and sodium (Na) were determined by flame photometer as described by Tandon[30]. Calcium (Ca) and magnesium (Mg) were determined by atomic absorption spectrophotometer. Chloride was determined by $AgNO_3$ titration method[30].

Statistical design and analysis: The experimental design was a Randomized Complete Block Design. Each treatment was replicated three times and each replicate was represented by two transplants. The data were subjected to analysis according to Snedecor and Cochran[31]. The Analysis of Variance (ANOVA) was used to determine significant differences. Means were compared by using the method of Least Significant Difference Test (LSD) at 5% level.

RESULTS AND DISCUSSION

Vegetative growth: The results of Table 4 elucidate that salinity markedly reduced plant height, numbers of young shoots and leaves and dry weight of Zini grapevines. It is clear that there were no significant differences detected on number of leaves per young shoot between the high salinity level (100 $mol_c\ m^{-3}$) compared with the low (20 $mol_c\ m^{-3}$) salinity stress and the control. The reduction of vegetative growth was more pronounced as the electrolyte concentration of irrigation water increased. The effect of salt stress at low levels was not obvious since low salinity levels (20 and 40 $mol_c\ m^{-3}$ treatments) gave similar height, dry weight and numbers of leaves and shoots as recorded in control pots. Concerning plant height and dry weight of the plant, there were no significant differences between the control and the 20 and 40 $mol_c\ m^{-3}$ treatments. The height was significantly reduced to 28.3 cm only when using an electrolyte solution of 60 $mol_c\ m^{-3}$ or higher. Moreover, the minimum growth rate of the plants was found at the highest electrolyte concentration. Irrigating olive transplants with 100 $mol_c\ m^{-3}$ saline water produced an approximately 50% reduction in dry weight of the plant and number of leaves per the shoot (Table 4). The reduction in the above parameters was highly and negatively correlated with promotion of salinity stress (r=-0.37). Salinity had a more pronounced effect on dry weight and plant height than other parameters, which was evident from significantly more decreased values especially at high electrolyte concentration. The transplants started to show visible signs of salt injury, manifested as marginal leaf burn, seven months after the beginning of the experiment (data not shown). It is interesting to report that the degree of injury was more severe at the highest concentrations of salts (80 and 100 $mol_c\ m^{-3}$). Vegetative parameters are highly affected by specific chloride effect of the high concentrations of the multicomponent solutions that reduce availability of water to the plants (Table 4). In other words, the inhibitory influence is thought to result from osmotic stress, chloride toxicity, or a combination of these factors. The obtained results evince the imperative need of the rational use of low salinized water with low chloride content in irrigation. Vegetative growth of plants is very well known to be sensitive to multicomponent saline solutions[4,32,33].

Table 4: Plant height, number of young shoots, number of leaves and dry weight of grapevines as influenced by electrolyte concentration of irrigation water*

Electrolyte concentration (mol$_c$ m^{-3})	Plant height (cm)	No. of young shoots/plant	No. of leaves/young shoot	Dry weight of plant (g)
Control	33.9a	3.5a	20.3a	51.7a
20	37.9a	3.8a	20.3a	51.3a
40	30.5a-c	3.8a	15.0ab	44.0a
60	28.3bc	3.0ab	14.3ab	24.5b
80	23.4c	2.3b	13.5ab	24.0b
100	23.8c	2.3b	10.3a	20.2b

*: Values followed by the same letter(s) in the same column are not significantly different ($p<0.05$)

A number of studies have demonstrated reductions in grapevine height and dry weight[9,13,34] and fruit yield[35,36] with increased salt stress. The severity of salt damage has been found to be dependent on electrolyte concentration, growth stage and the cultivars in terms of scion and rootstock. Leaf size, leaf shape and changes in internode length of grapes were potentially used as potential tools for monitoring changes in levels of salt stress in vineyards[37].

The deleterious effect of salinity on grapevine growth could be attributed to direct and indirect causes. Increasing electrolyte concentration affects plant growth directly by increasing the osmotic pressure of the saline solutions associated with reduction in water absorption[38], which retards cell division and/or elongation and indirectly by decreasing the rate of photosynthesis[20].

Proline content: Proline accumulation is one of the major common metabolic responses of higher plants to water deficits and salinity stress[19]. Figure 1 shows that proline content in the control plants was quite low comparatively to treated plants. It ranged from 0.42 to 1.36 mg g^{-1} fresh weight. After salt treatment, proline contents increased with increasing electrolyte concentration of irrigation water. Proline starts to accumulating when the plants were subjected to 60 mol$_c$ m^{-3} solution. The highest proline content was found in the plants irrigated with 80 and 100 mol$_c$ m^{-3} (Fig. 1); that also exhibited the lowest plant height, numbers of young shoots and leaves and dry weight. The increase in proline content was highly correlated with inhibition of the vegetative growth. These data indicate that proline accumulation is not a salinity tolerance mechanism of grapevines, but it merely an indication of salt injury and could be involved in osmotic adjustment. In other words, the current investigation suggests that accumulation of proline in grapes is a consequence of stress and does not lead to salt tolerance. Similar results have been found in grapes[3] and other plant species[4].

Chlorophyll content: Salinity has a very drastic reducing effect on the total content of chlorophyll (Fig. 2). Data of Fig. 2 shows significant differences in total chlorophyll content among the different treatments. It decreased with increasing electrolyte concentration of irrigation water.

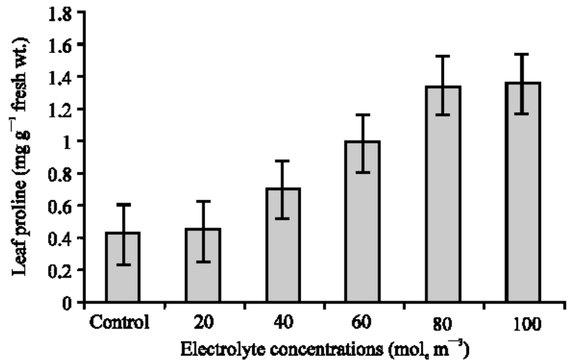

Fig. 1: Leaf proline content of grapevine plants as influenced by electrolyte concentration of irrigation water

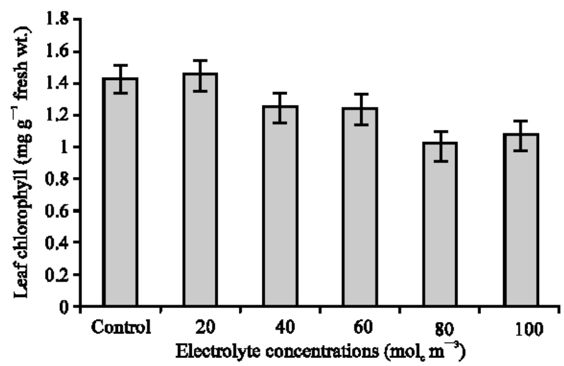

Fig. 2: Leaf chlorophyll content of grapevine plants as influenced by electrolyte concentration of irrigation water

Electrolyte concentration of 40 mol$_c$ m^{-3} was able to significantly reduce the chlorophyll content. The minimum chlorophyll content was reached by using 80 and 100 mol$_c$ m^{-3} concentrations (Fig. 2). The decrease in chlorophyll content was negatively correlated with electrolyte concentration of irrigation water (r= -0.28). It is worth noting that the results of Abou Sayed-Ahmed[23] and Singh *et al.*[3] on several cultivars of grapevines confirm these results. In this regard, the reduction in chlorophyll content of salinized plants was attributed to the inhibitory influence of osmotic stress on absorption of iron that involved in cloroplast formation[39].

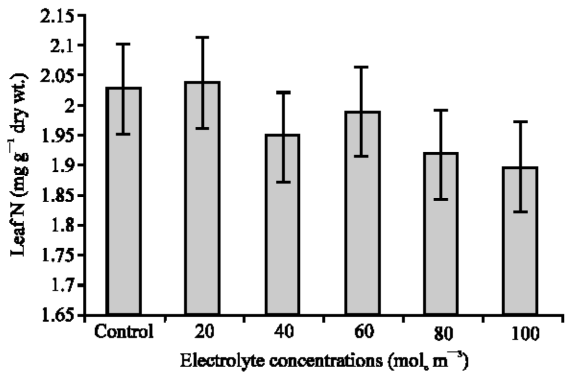

Fig. 3: Leaf nitrogen content of grapevine plants as influenced by electrolyte concentration of irrigation water

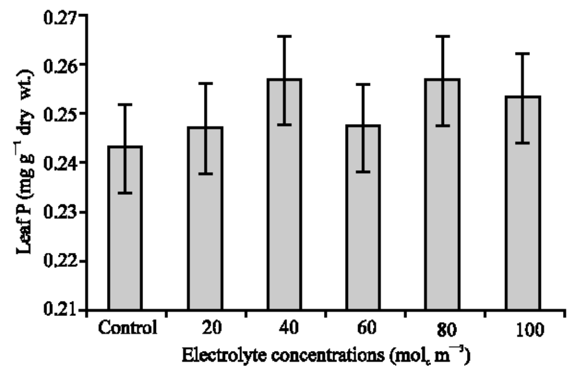

Fig. 4: Leaf phosphorus content of grapevine plants as influenced by electrolyte concentration of irrigation water

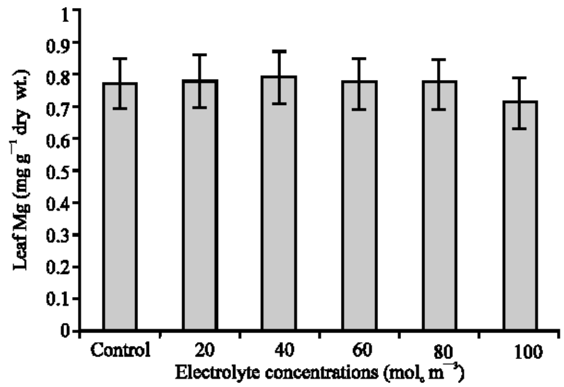

Fig. 5: Leaf magnesium content of grapevine plants as influenced by electrolyte concentration of irrigation water

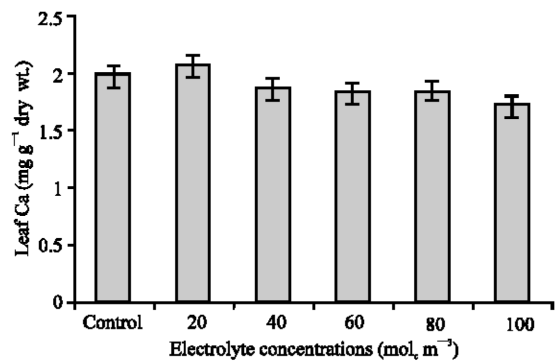

Fig. 6: Leaf calcium content of grapevine plants as influenced by electrolyte concentration of irrigation water

Mineral composition: Chemical analysis of grapevine leaves and roots (Fig. 3-9) showed that increased levels of irrigation water salinity had clear effect on mineral composition. Leaf N, P and Mg contents of Zini grapevines were not significantly affected by the different salinity treatments (Fig. 3-5). On the other hand, many authors stated that leaf N and Mg contents of other grape cultivars were significantly reduced under salinity conditions[23]. The Ca and K contents of the leaves decreased with the increase in electrolyte concentration of irrigation water (Fig. 6 and 7). At low concentrations, the K contents were lower than those of the control plants, but the differences observed were not significant. Salinity at concentrations of 60 and 40 mol$_c$ m^{-3} and above was able to significantly reduce leaf K and Ca, respectively. The reduction in leaf K content with increasing salinity level was in agreement with results reported by Garcia and Charbaji[15], Abou Sayed-Ahmed[23] using other grapevine cultivars.

In contrast to its inhibitory effect on tissue contents of Ca and K, salinity at concentrations of 40 mol$_c$ m^{-3} and above increased Na and Cl concentrations in both leaves and roots of grape transplants (Fig. 8 and 9). These increases of Na and Cl were associated with the relative tolerance of the transplants. The highest Na and Cl contents were found in leaves and roots of the plants irrigated with 80 and 100 mol$_c$ m^{-3} solutions (Fig. 8 and 9). Root Na concentrations, ranging from 0.38 at control treatment to 1.01 mg g^{-1} dry weight at 100 mol$_c$ m^{-3} concentration, were slightly more than those in leaves, indicating a partial ability of Zini plants to retard translocation of Cl from roots to shoots and leaves. High Cl concentrations in grapevine leaves in high- salt root media have been previously reported[40]. Leaf K content was negatively correlated with leaf Na content (r= -0.61) suggesting antagonism between these elements[15]. These results indicate that Zini grapevines had the ability to store sodium and chloride in its roots, thus preventing of

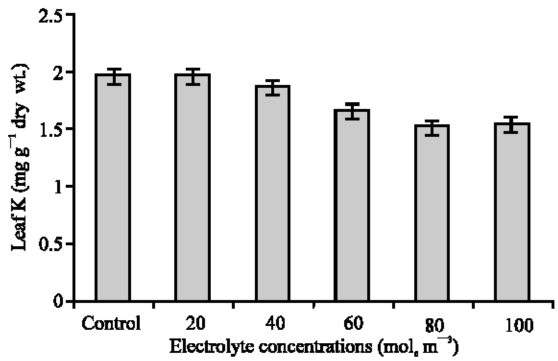

Fig. 7: Leaf K content of grapevine plants as influenced by electrolyte concentration of irrigation water

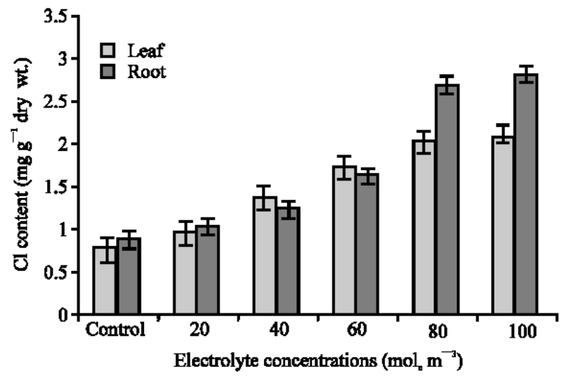

Fig. 9: Leaf and root Cl contents of grapevine plants as influenced by electrolyte concentration of irrigation water

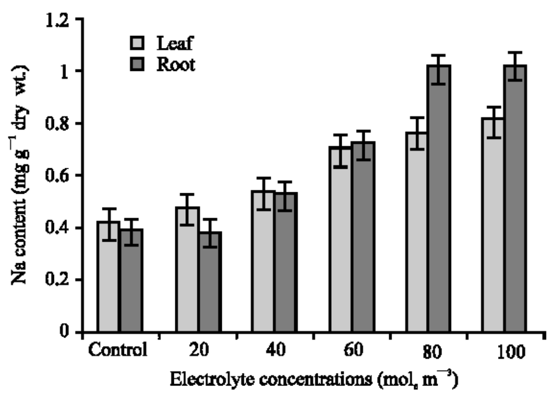

Fig. 8: Leaf and root Na contents of grapevine plants as influenced by electrolyte concentration of irrigation water

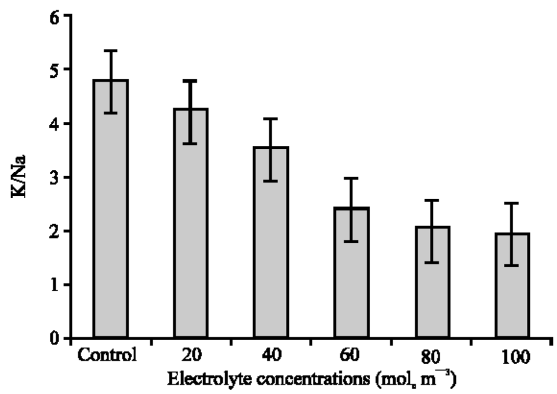

Fig. 10: Leaf K/Na ratio of grapevine plants as influenced by electrolyte concentration of irrigation water

higher quantities of them from translocation to the leaves. This partial exclusion of Na and Cl seems to be an adaptive mechanism to prohibit accumulation under saline conditions. The decrease of K concentration in leaves with the increase of electrolyte concentration of irrigation water suggests that K exclusion is an extra mechanism for osmoregulation during adaptation to salinity. It could be concluded from these results that sensitivity of Zini to salinity could be attributed to the combined effect of Cl toxicity and osmotic stress. In addition, the results may point that the local cultivar may be more tolerant to saline conditions if the irrigation water was not dominated by high proportions of Cl. The inability of grapevine to limit Cl uptake from the irrigation water may be attributed to the determinal effect of Cl on plant photosynthesis[41] and growth.

On the basis of root Cl content of different grape cultivars; Miklos *et al.*[8] concluded that there are two different defending mechanisms, i.e. Cl exclusion and Cl accumulation. These results also suggest that Zini grapevine tested here is less tolerant than other cultivars indicated in the literature[13,23]. So, new cultivars tolerant against salts must be developed and used where salinity problem dominated.

With respect to other grape cultivars, leaf Na and Cl concentrations increased linearly with increasing salinity level[11,13,23,42,43], regardless of the chemical composition of the irrigation water.

Leaf K/Na ratio declined in proportion to increasing electrolyte concentration of irrigation water from 4.8 in control to 1.9 in salt treated plants (100 mol$_c$ m^{-3} concentration) (Fig. 10), mainly because of the accumulation of Na in leaves. The ability to maintain a high K/Na ratio and the reduction of Na translocation from the root to the shoot contribute to the overall salt tolerance of the plants[44]. The results indicated in this study are consistent with previous findings[4]. Furthermore, the Na/K content of cortical cell vacuoles of grapevines has been reported to higher in the outer cortex than the inner cortex[16].

In conclusion, the present results have shown the depressing effect of irrigation water salinity on vegetative growth, mineral composition, ionic imbalance, proline and chlorophyll contents of grapevines.

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