EFFECTS OF SALINITY ON LEAF MINERAL COMPOSITION AND SALT INJURY SYMPTOMS OF SOME IRANIAN WILD GRAPEVINE (*VITIS VINIFERA* L. SSP. SYLVESTRIS) GENOTYPES

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Abstract

Aim : To evaluate the suitability of wild grapevine genotypes to saline conditions by measuring leaf ion content and salt injury symptoms.

Methods and results : Vines of nine wild (*Vitis vinifera* L. ssp. sylvestris) genotypes were planted in pots and after the good establishment, salinity treatments (0, 50, 100 and 150 mM NaCl) started. At the end of the experimental period, the K^+, Na^+, NO_3^- and Cl^- content of leaves and visible symptoms of salt injury were recorded. Leaf Na^+ and Cl^- content increased with increasing salinity but levels and accumulation rates were different among genotypes.

Conclusion : Based on Na^+ and Cl^- content and salt symptoms, genotypes 4 and 7 showed less symptoms than the other genotypes at moderate (50-100 mM) NaCl concentration and none could tolerate high salt (150 mM) concentration.

Significance and impact of the study : Under saline conditions, ion accumulation in the leaves significantly varied among wild genotypes and some of them could be recommended as saline tolerant genotypes.

Key words : salinity, tolerance, ion content

Résumé

Objectif : Déterminer la tolérance de génotypes de vigne sauvage en conditions salines en mesurant la teneur des feuilles en ions et les dommages causés par la salinité.

Méthodes et résultats : Des plants de neuf génotypes de vigne sauvage (*Vitis vinifera* L. ssp. sylvestris) ont été plantés dans des pots et exposés au sel (0, 50, 100 et 150 mM NaCl) après une bonne implantation. À la fin de la période de traitement, la teneur des feuilles en K^+, Na^+, NO_3^- et Cl^- a été mesurée, de même que les dommages visibles causés par le sel. La teneur en Na^+ et Cl^- a augmenté en fonction de la salinité, mais à des niveaux et des taux d'accumulation différents entre les génotypes.

Conclusion : Selon les résultats de la teneur en ions Na^+ et Cl^- et des symptômes de dommages par le sel, les génotypes 4 et 7 ont montré moins de symptômes que les autres génotypes en conditions salines modérées (50-100 mM NaCl) et aucun génotype n'a toléré les conditions élevées (150 mM NaCl).

Signification et impact de l'étude : En conditions de salinité, l'accumulation des ions dans les feuilles varie selon les génotypes sauvages et certains pourraient être reconnus comme étant tolérants au sel.

Mots clés : salinité, tolérance, teneur en ions

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INTRODUCTION

Accumulation of Cl^- and Na^+ in grapevines in saline condition may result in physiological disturbances leading to reductions in growth, vegetative biomass, and fruit yield (Walker *et al.*, 2010). The specific ion toxicity occurs when the vines accumulate certain ions at levels above those known to cause detrimental effects. Grapevines are considered moderately sensitive to root-zone salinity and it has long been known that variation in salt tolerance exists between grapevine species and cultivars (Fisarakis *et al.*, 2001). Several studies revealed that salt tolerance mechanisms in grapevine involve many factors like restriction of ion absorption, translocation from roots to shoots, photosynthesis alteration and solute accumulation (Shani and Ben-Gal, 2005 ; Walker *et al.*, 2010). Some of the grapevine rootstocks have been rated as tolerant to salinity due to their ability to prevent Na^+ and/or Cl^- uptake and translocation to aerial parts of the vines (Tregeagle *et al.*, 2006). When used as a grapevine rootstock, 140 Ruggeri (*Vitis berlandieri* × *Vitis rupestris*) is known to be a good Cl^- excluder, whereas K 51-40 (*Vitis champinii* × *Vitis riparia* 'Gloire') is a poor excluder and scions grafted to it accumulate high concentrations of Cl^- when grown under saline conditions (Walker *et al.*, 2010). One of the strategies adopted in overcoming salinity is the use of tolerant genotypes through the characterization of local genetic resources and the selection of potential tolerant genotypes (Fisarakis *et al.*, 2001). In Iran, wild grapevine (*Vitis vinifera* ssp. sylvestris) populations are generally found in riparian woodland habitats in the Alborz and Zagros Mountains in the north and north-west of the country (Doulati Baneh *et al.*, 2011). The availability of these genotypes provides an excellent opportunity to determine their suitability to saline conditions. So the aim of this study was to evaluate the tolerance of nine wild grapevine genotypes to salt stress, with an emphasis on ion (Na^+, K^+, Cl^-, NO_3^-) accumulation and interaction in leaves.

MATERIALS AND METHODS

Vines of nine wild grapevine genotypes were planted in pots containing a mixture of soil (fine loamy, super active, mixed, mesic typic calcixerepts) and sand (1:1 v/v). They were grown for 40 days and after good plant establishment (8 cm green shoot growth, 5 young leaves), salinity treatments started. The experimental design was a Factorial Complete Randomized Block design with two factors (salinity at 0, 50, 100 and 150 mM NaCl and genotype including 9 wild grapevines). This experiment was carried out at the Agricultural and Natural Resource Research Center of West Azerbaijan-Iran. At the end of the experimental period, the concentration of K^+, Na^+, NO_3^- and Cl^- in leaves was measured and visible symptoms of salt injury in vines scored as : 1- plants with no necrotic tissues ; 2- necrosis on 30 % of blade and on the tip of the leaves ; 3- necrosis on 50 % of the leaves and on the stem ; 4- necrosis on 60-80 % of the leaves and on the stem ; and 5- necrosis leading to the death of the plant. Analysis of variance (two way ANOVA) was done using SAS 9.1 software and differences among means were compared by Duncan's Multiple Range Test at $P < 0.05$.

RESULTS

There were significant differences ($P < 0.001$) between genotypes in mean leaf Na^+, K^+, Cl^- and NO_3^- content in the salinity treatments (table 1). Based on the percentage of explained variance, salinity treatment had more effect than genotype on all characters, except NO_3^- (table 2). Na^+ and Cl^- content significantly increased with increasing salinity level to varying degrees across grapevines (table 3).

Table 1. Analysis of variance on different characters affected by salinity and genotype

S.O.V	d.f	Means of Square[a]				
		Na^+	K^+	Cl^-	NO_3^-	Salt Injury
Block	2	30,93	2,23	45,13	2,22	0.07**
Salinity (S)	3	1006.00**	12.47**	1658.75**	161.97**	7.73**
Genotype (G)	8	42.47**	1.54**	67.11**	61.66**	0.32**
S×G	24	10.24**	0.82**	14.63**	12.04**	0.10**
Error	70	3,01	0,35	3,23	2,54	0,02
C.V (%)		7,07	10,34	6,51	13,16	8,62

S.O.V. : Source of variance ; d.f. : degree of freedom ; ** Significant differences at $P \leq 0.01$ [a]

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Table 2. Percentage of explained variance

S.O.V	Percentage of Explained Variance (PEV)				
	Na^+	K^+	Cl^-	NO_3^-	Salt Injury
Salinity (S)	77,8	38	80,5	16,1	78,1
Genotype (G)	8,7	12,5	8,7	42,9	8,6
S×G	6,3	20	5,68	25,1	8

S.O.V. : Source of variance

Table 3. Interaction effect of salinity and genotype on Na^+ and Cl^- content (mg g^{-1} dry weight)

	NaCl Levels							
Sample	0 mM		50 mM		100 mM		150 mM	
	Na^+	Cl^-	Na^+	Cl^-	Na^+	Cl^-	Na^+	Cl^-
G1	1.7e	4.6de	10.4c	19.4b	17.3b	27.5bcd	24.3b	33.7ab
G2	8.5cd	5.8cd	15.7ab	20.7b	21.5ab	32.6a	28.5ab	36.7a
G3	12.2ab	13.6ab	18.4a	23ab	22.6a	31ab	27.4ab	36.3ab
G4	9.5bcd	4.9de	15.4ab	18.8b	20.0ab	23.7d	26.8ab	26.6d
G5	11.4abc	12.2b	15.03ab	22.8ab	18.0ab	26.6cd	27.4ab	31.9c
G6	9.0cd	13.3ab	13.3bc	22.8ab	21.7ab	29.2abc	27.6ab	32.9abc
G7	12.9a	3.2e	18.1a	14.4c	22.2ab	24.9cd	26.3abc	29d
G8	10.1abcd	15.5a	13.8bc	26.9a	19.7ab	27.6bcd	30.2a	33.7ab
G9	7.1d	7.5c	13.5bc	18.6b	20.7ab	26.3cd	27. 7ab	33.4abc

Means followed by different letters are significantly different at $P \leq 0.05$ using Duncan's Multiple Range Test.

Na^+ accumulation in leaves of salt treated vines varied based on the genotype. In the control treatment (0 mM NaCl), genotype 1 showed the lowest and genotypes 3 and 7 the highest Na^+ content. By increment of NaCl concentration to 50 mM, these two genotypes (3 and 7) still had the highest Na^+ concentration. In severe salt stress (100 and 150 mM NaCl), genotype 1 had the lowest Na^+ content. By comparing Na^+ increment rate in leaves of all genotypes from control to 150 mM NaCl, it was revealed that genotype 7 accumulated the lowest Na^+ content and genotype 1 the highest (table 3). Although genotype 1 had the lowest Na^+ content in all treatments, it showed the most salt injury symptoms.

The accumulation of Cl^- in leaf tissue of all wild genotypes was increased dramatically as salinity rose from 0 to 50 mM and then from this point to 150 mM increased moderately. The lowest Cl^- content in the control treatment was recorded in wild genotypes 7, 1 and 4, respectively. Cl^- content in leaves of genotypes 7 and 4 was the lowest in all salinity treatments, whereas in genotype 1 Cl^- content increased, especially at 100 and 150 mM NaCl. From control to 150 mM NaCl, genotypes 2 and 3 accumulated much higher concentrations of Cl^- than did the other genotypes (table 3).

The leaf K^+ accumulation pattern from 0 to 150 mM NaCl varied among wild genotypes. From 0 to 50 mM and 100 mM NaCl treatments, the K^+ content of leaves increased. In this NaCl range, the highest K^+ accumulation was determined in wild genotypes 6, 9, 3 and 4, respectively, and the lowest in genotypes 7 and 8 (table 4). At 150 mM NaCl, the K^+ content declined in all genotypes. Salinity significantly decreased the leaf NO_3^- content of all wild genotypes, but the extent of this reduction varied depending on the genotype (table 4).

By increasing the salt concentrations, injury symptoms were extended to leaves and shoots of all genotypes. Wild genotypes 1, 5, 6 and 8 could not tolerate 50 mM NaCl and all of them were dried, while genotypes 4 and 7 showed fewer injury symptoms and continued to be tolerant up to 100 mM NaCl. At 150 mM NaCl, genotypes 2 and 7

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Table 4. Interaction effect of salinity and genotype on NO_3^- and K^+ content (mg g^{-1} dry weight)

Sample	NaCl Levels							
	0 mM		50 mM		100 mM		150 mM	
	NO_3^-	K^+	NO_3^-	K^+	NO_3^-	K^+	NO3-	K^+
G1	6.6cd	0.8abc	6.6ab	1.2b	5.5a	1.2b	4.3a	0.7ab
G2	10.7ab	0.8abc	8.0 a	1.2b	3.6bc	1.1bcd	2.4bc	0.8ab
G3	5.6de	0.7bcd	4.1c	1.3ab	3.9b	1.1bcd	2.3bc	0.6b
G4	7.6c	0.6cd	7.4a	1.2b	4.4ab	1.1bcd	3.6ab	0.8ab
G5	2.4f	1.0ab	1.4d	1.3ab	2.1cd	1cd	2.0bc	0.9ab
G6	9.8b	0.37d	4.4bc	1.3ab	3.9b	1.1bcd	4.3a	1.0a
G7	12.2a	0.9abc	6.5ab	1.1b	3.5bc	0.9d	3ab	0.7ab
G8	4.0ef	1.2a	4.7bc	1.3ab	1.7d	1.2bc	0.8c	0.9ab
G9	4.6e	1.1bab	6.1abc	1.6a	4.7ab	1.5a	2.9ab	0.92a

Means followed by different letters are significantly different at $P \leq 0.05$ using Duncan's Multiple Range Test.

Table 5. Effect of salinity levels on salt injury in leaves of wild grapevine genotypes

Genotype	NaCl Levels			
	0 mM	50 mM	100 mM	150 mM
G1	1e	5a	5a	5a
G2	1e	4b	5a	4b
G3	1e	4b	3c	5a
G4	1e	2d	2d	5a
G5	1e	5a	5a	5a
G6	1e	5a	5a	5a
G7	1e	3c	2d	4b
G8	1e	5a	5a	5a
G9	1e	4b	4b	5a

Means followed by different letters are significantly different at $P \leq 0.05$ using Duncan's Multiple Range Test.

were not completely dried and all the other genotypes were dead (table 5).

As shown in table 6, mean Cl^- content was higher than Na^+ content in all genotypes except genotype 7. And genotypes with high Cl^- content (3, 8, 6 and 2, respectively) showed more salt injury.

DISCUSSION

High NaCl concentration in the nutrient solution leads to increased Na^+ and Cl^- content in leaves. Wild genotypes 4 and 7 showed the lowest Cl^- content in their leaves. They may have the ability to limit the accumulation of chloride. Genetic differences in capability of plant species and varieties to restrict Na^+ and Cl^- uptake from the soil or to reduce ion transport to the xylem could be an influential factor in diminishing the accumulation of those ions in leaves. Fisarakis *et al.* (2001) found consistently higher accumulations of Cl^- and Na^+ in roots as compared to the leaves of 'Sultana' vines and suggested that capability to store Na^+ in roots is a tolerance characteristic of rootstocks.

The data presented here suggest that leaf K^+ content was reduced only at 150 mM NaCl and increase in K^+ concentration at lower NaCl levels was in contrast with the results of Garcia and Charbaji (1993), who reported Na-K antagonism, even at low NaCl doses. Singh *et al.* (2000) reported that leaf K^+ concentration was not decreased by salinity and plants were able to maintain a high level of K^+ under salinity stress.

The reduction of NO_3^- concentration in all genotypes under salinity is due probably to $NO_3^-Cl^-$ antagonism. There is a possibility that growth reduction is not entirely due to Cl^- toxicity, but may be partially due to Cl^- induced NO_3^- deficiency. Results presented in

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Table 6. Mean accumulation of anions and cations in leaves of wild grapevine genotypes

Genotype	Na^+	K^+	Cl^-	NO_3^-	Salt Injury
G1	13.44d	0.99bc	21.34c	5.74a	4a
G2	18.55abc	0.99bc	23.98ab	6.18a	4a
G3	20.17a	0.93c	25.99a	3.96b	3b
G4	17.94bc	0.92c	18.54d	5.77a	2c
G5	17.98bc	1.04bc	23.37bc	2.01c	4a
G6	17.92bc	0.94c	24.58ab	5.60a	4a
G7	19.90ab	0.89c	17.88d	6.29a	2c
G8	18.48abc	1.15ab	25.95a	2.82c	4a
G9	17.28c	1.28a	21.43c	4.59b	3b

Means followed by different letters are significantly different at $P \leq 0.05$ using Duncan's Multiple Range Test.

our study showed that high external NaCl concentration probably reduces NO_3^- accumulation in wild grapevine genotypes, which is consistent with previous studies (Fisarakis *et al.*, 2005).

Sodium and chloride effects are involved in direct toxicity or disturbance in the nutritional balance of vines. In this study, all wild grapevine genotypes showed severe salt injury symptoms (leaf burn, defoliation and shoot necrosis) at high salt concentration. At 100 mM NaCl, genotypes 4 and 7 had the lowest Cl^- content, but their Na^+ content was as high as that of the other genotypes.

CONCLUSION

There are a lot of parameters that determine whether a genotype is tolerant or sensitive to salinity. The ability of genotypes to control Na^+ and Cl^- absorption and translocation is one of the most important factors. The higher the ability of plant to prevent the uptake of these ions, the higher the tolerance. Salt injury is related to the accumulation of Na^+ and Cl^- ions in leaves but seems more likely associated with Cl^- content. As shown in our study, the Cl^- content was higher than the Na^+ content in all genotypes under salinity treatments. Also leaf Cl^- content of the more tolerant genotypes 4, 7 was lower than that of the other genotypes in all NaCl treatments, but their leaf Na^+ content was as high as the others. It is also revealed that all tested wild grapevine genotypes cannot tolerate 150 mM NaCl.

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