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GROWTH AND PHYSIOLOGICAL RESPONSES OF SOME WILD GRAPEVINE (*VITIS VINIFERA* L. SSP. *SYLVESTRIS*) GENOTYPES TO SALINITY

H. DOULATI BANEH[1*], A. HASSANI[2] and R. ABDOLLAHI[3]
[1]*Horticulture Crops Research Department, West Azerbaijan Agriculture and Natural Resources Research Center, ARREO, Urmia, Iran*
[2]*Urmia University, Faculty of Agriculture, Department of Horticulture, Urmia, Iran*
[3]*Payame Noor University, Agriculture Department, Iran*

Abstract

DOULATI BANEH, H., A. HASSANI and R. ABDOLLAHI, 2015. Growth and physiological responses of some wild grapevine (*Vitis vinifera* L. spp. *sylvestris*) genotypes to salinity. *Bulg. J. Agric. Sci.*, 21: 530–535

Salt stress is one of the most important environmental stress that influences growth and physiological characters of grapevine in saline region. Selection and use of tolerant grapevines as rootstock or own-rooted vines and finding some linked morphological and physiological indexes have especial importance. In this study effects of different NaCl concentrations (0, 50, 100 and 150 mM) on visible symptoms of salt injury and, some growth factors of 9 wild genotypes (*Vitis vinifera* ssp. *sylvestris*), were investigated. Result showed that shoot length, dry weight, fresh weight, relative water content decreased due to the increase of NaCl concentrations. Under saline conditions (from control to 150 mM NaCl treatment) the lowest decline in shoot fresh and dry weight was recorded in wild genotypes numbers 7. Soluble sugars and proline content as an osmoregulation increased markedly at the highest NaCl concentration. Wild genotypes number 4 and 7 had the lowest proline content. Based on salt symptoms on leaves, wild genotypes number 4 and 7 showed fewer symptoms and they were recommended as saline tolerant rootstocks.

Key words: Vitis vinifera, wild genotype, salinity tolerance, salt injury

Introduction

Salinity is one of the main challenges for sustainable agriculture, with decreasing effect on plant growth and specifically on horticultural crops yield. Soil salinity affects growth and yield in grapevine by osmotic and specific ion toxicities (Shani and Ben-Gal, 2005). The osmotic effect on vine growth proportional to the decrease of osmotic potential in the soil solution, operates from low values of soil salinity, and reduces leaf water potential, transpiration and photosynthesis (Urdanoz and Aragüés, 2009). During the onset and development of salt stress within a plant, all the major processes such as photosynthesis, protein synthesis and energy are influenced. Although whole plant mechanism can contribute to the avoidance of stress during the plants life cycle, tolerance can occur at the cellular level (Yokoi et al., 2002). One of the most common stress responses in high plants is over production of some various compatible organic solutes (Serraj and Sinclar, 2002). These solutes are most commonly carbohydrates, like sugars, amino acids and proteins that act as osmolytes. Proline is one of them that is known to occur widely in higher plants and normally accumulates in large quantities in response to environmental stresses (Kishor et al., 2005). In some studies a positive correlation between the accumulation of these osmolytes and stress tolerance has been reported (Yamada et al., 2003). Fozouni et al. (2012) demonstrated that proline accumulation increased significantly by increasing salinity.

One of the strategies adopted in overcoming salinity is the use of tolerant genotypes (as rootstock or own

*Correspondence author: ah_dolati@yahoo.com

root) through the characterization of local genetic resources and the selection of potential tolerant genotypes (Daldoul et al., 2010). Grapevine - *Vitis vinifera*. L. has been defined as moderately sensitive to salinity (McCarthy et al., 1992). However, it has also been reported that varieties of a species show differences in salt tolerance (Schwarz, 1995).

Old cultivated grapevine (*Vitis vinifera* spp. Sativa) is thought to have been domesticated from wild population of *Vitis vinifera* ssp. *sylvestris* (Lacombe et al., 2003). Populations of wild grapevine mostly distributed in different geographical environments (Grassi et al., 2003). Under these diverse climates some desirable resistance genes to biotic and abiotic stress will be existed (Liu et al., 2012). Askri et al. (2012) studied the physiological responses of some wild grapevine accessions to salt stress. They found that salinity significantly reduced vine shoot length, shoot growth rate (SGR) and relative growth rate (RGR) of the whole plant, leaves, stems and roots. Wild grapevine populations were found generally in riparian wood habitats on river margins located in Alborz and Zagros mountains in north and North-Western of Iran (Doulati et al., 2011). The problem of soil salinity is spreading fast and it is a threat for viticulture industry in various parts of the country. The availability of the wild grapevine genotypes provides an opportunity for using them in saline conditions. The main purpose of this study was to evaluate the physiological responses of some wild grapevine genotypes to salt stress and find salt tolerant genotypes.

Materials and Methods

Plant material

Nine accessions of wild grapevine (*Vitis vinifera* L. ssp. *sylvestris*) were collected from forest and wetlands regions of Zagros Mountain in Kurdistan and West Azerbaijan, Iran. In this region the annual precipitation range is from 400 to 800 mm (16 to 30 inches) and rain is mostly in the winter and spring. The winters are severe, with low temperature often below -25°C (-13°F). The region has the continental variation of the Mediterranean climate pattern (Frey and Probst, 1986). Uniform rooted cuttings were planted in pots containing a mixture of soil and sand (1:1, v/v). All vines were pruned back to a single shoot with two buds. After the good establishment, salinity treatments were (0, 50, 100 and 150 mM NaCl) started. To avoid osmotic shocks, salt concentrations were increased by 50 mM every two irrigations until a final concentration was reached to 150 Mm. Salinity treatments were imposed by adding NaCl to irrigation water. The pots were weighted daily and irrigated until their soil water content reached to 70% of field capacity. This experiment was carried out at Agricultural and Natural Resource Research Center of West Azerbaijan, Iran. The experiment designed as a Factorial Complete Randomized Block design with three replications.

Growth measurement

Shoot length of each vine was measured at the beginning of the salinity treatments and at the end of salt period. It was calculated by using the equation: Shoot length = L_f- L_i where L_i and L_f are the initial and final shoot length, respectively. Measurement of leaf area was conducted with graph paper in four selected leaves at nodes 4–5 and 7–8 in each treatment. At the end of the experiment fresh and dry weights of leaves, shoots and roots were noted.

Relative water content

Relative water content was measured as: RWC (%) = (FW−DW) / (SFW−DW)* 100 where FW is fresh weight, DW is dry weight, and SFW is saturated fresh weight of the leaves after re-hydrating samples for 24 h.

Salt injury

Visible symptoms of salt injury in leaves and shoots were scored as 1- plants with no necrotic tissues; 2- necrosis on 30% of blade and necrosis on the tip of the leaves; 3- necrosis on 50% area of the leaves and necrosis on the stem; 4- necrosis on 60-80% of the leaves and necrosis on the stem; and 5- necrosis leading to the death of the plant.

Proline and soluble sugar content

Proline content was calculated according to Bates et al. (1973). Proline concentration was determined using calibration curve and expressed as µg proline/g DW. Leaf Soluble sugar content was extracted and analyzed according to the method of Dubois et al. (1956).

Statistic analysis

Analysis of variance (ANOVA) was carried out by SAS 9.1 software and differences among means of data were compared by Duncan's Multiple Range Test At $p < 0.05$.

Results

Growth measurement

The effect of NaCl treatments on the measured parameters of wild grapevines genotypes are shown in Table 1. Shoot length, dry weight, fresh weight and relative water content decreased due to the increasing NaCl concentrations. Under saline condition, wild genotypes numbers 9

Table 1
Effect of salinity levels on different characteristic of wild grape genotype

Salinity, mM NaCl	Shoot length, cm	Leaf area, mm^2	Leaf DW, %	Root DW, gr	RWC, %	Soluble suger
0 mM	4.07 a	34.14 a	28.18 b	11.82 a	88.18 a	491.86 d
50 mM	0.95 b	26.70 b	25.18 b	3.83 b	80.93 b	601.19 c
100 mM	1.37 b	22.16 c	28.24 b	5.67 b	74.26 c	694.66 b
150 mM	0.96 b	19.01 c	31.59 a	4.23 b	63.41 d	777.82 a
Genotype						
G1	1.33 a	23.18 bc	29.52 a	4.91 bcd	75.17 cd	607.11 cde
G2	1.40 a	16.72 c	29.15 ab	6.62 bc	73.42 d	605.64 cde
G3	1.62 a	23.36 bc	31.80 a	7.15 b	74.92 cd	627.67 cd
G4	2.18 a	24.49 b	24.20 b	3.78 cd	78.17 bc	576.96 de
G5	2.24 a	19.45 bc	27.31 ab	3.24 d	76.17 bcd	713.6 a
G6	1.73 a	25.46 b	27.67 ab	5.43 bcd	79.35 b	725.26 a
G7	2.47 a	20.63 bc	27.13 ab	6.54 bc	83.17 a	570.82 e
G8	1.77 a	24.08 bc	28.27 ab	3.86 cd	74.17 d	653.9 bc
G9	1.77 a	52.37 a	29.64 a	15.95 a	75.75 bcd	691.48 ab

Means followed by different letters within a column are significantly different at $P \leq 0.05$

Table 2
Effect of salinity levels on shoot fresh weight (gr) of wild grape genotype

Genotype	Salinity Levels							
	0 mM		50 mM		100 mM		150 mM	
G1	5.33	bcd	1.11	ghij	0.43	ij	1.62	fghij
G2	5.94	bc	1.02	hij	1.27	fghij	2.49	fghi
G3	7.17	ab	1.68	fghij	1.28	fghij	2.39	h
G4	3.37	defg	1.8	fghij	1.43	fghij	0.48	ij
G5	3.15	efgh	0.56	ij	1.25	fghij	1.54	fghij
G6	3.45	def	0.26	j	1.71	fghij	1.83	fghij
G7	5	cde	2.01	fghij	3.14	efgh	1.44	fghij
G8	2.61	fghi	1.02	hij	1.49	fghij	1.3	fghij
G9	8	a	3.39	defg	3.42	def	2.65	fghi

Means followed by different letters within a column are significantly different at $P \leq 0.05$

Table 3
Effect of salinity levels on shoot dry weight (gr) in wild grape genotype

Genotype	Salinity Levels							
	0 mM		50 mM		100 mM		150 mM	
G1	2.9	bc	0.78	fghij	0.21	ij	1.32	efghij
G2	2.78	bcd	0.73	fghij	0.6	fghij	1.24	efghij
G3	3.73	ab	0.84	fghij	0.75	fghij	1.6	efg
G4	1.4	efghi	1.29	efghij	0.75	fghij	0.27	hij
G5	1.26	efghij	0.47	ghij	0.46	ghij	0.85	fghij
G6	1.49	efgh	0.15	j	0.92	fghij	1.11	efghij
G7	2.14	cde	1.16	efghij	1.7	def	0.71	fghij
G8	1.44	efgh	0.55	fghij	1.07	efghij	1.02	efghij
G9	4.27	a	2.19	cde	2.21	cde	1.65	efg

Means followed by different letters within a column are significantly different at $P \leq 0.05$

produced more root, shoot fresh and dry weight than other genotypes. By comparing the reduction of shoot fresh and dry weight from control to 150 mM NaCl treatment, genotype number 7 showed the lowest decline (Tables 2 and 3).

Salinity significantly affected leaf area. The lowest leaf area was recorded in wild genotype number 2 (16.72 mm^2) while significantly the highest was observed in wild genotypes number 9 (52.37 mm^2).

When salinity rose from 0 to 50 mM and then from 50 to 150 mM, fresh root weight decreased but at the moderate level increased except for genotypes number 4, 7, 8 and 9 (Table 4).

Relative water content was significantly affected by salinity. By increasing salinity from 0 to 150 mM NaCl relative water content was decreased in all genotypes but there were some variation between them. As a result, the lowest value of RWC was recorded in wild genotypes number 2 and 8, respectively and the highest was recorded in wild genotypes number 7 (Table 1).

Salt injury

The rate of injury symptoms was increased in all genotypes in salt treatments. At 150 mM, all genotypes, except number 7, were sensitive and showed high injury symptoms (necrosis on 80% of the leaves and necrosis on the stem). At 100 Mm, genotypes 4 and 7 had the lowest damage and more plant viability. Genotypes 1, 5, 6 and 8 showed the highest injury and even couldn't tolerate 50 mM NaCl concentration (Table 5). In this study all wild grape genotypes showed high salt injury symptoms in leaves and shoots (leaf burn, defoliation and shoot necrosis) at 150 mM NaCl concentration, meanwhile these genotypes don't tolerate this concentration. At 100 Mm NaCl concentrations, the lowest salt injury was recorded in wild genotypes number 4 and 7.

Table 4
Effect of salinity levels on root fresh weight (gr) in wild grape genotype

Genotype	Salinity Levels							
	0 mM		50 mM		100 mM		150 mM	
G1	37.4	bcd	2.75	j	4.49	hij	7.15	hij
G2	39	bcd	2.88	j	10.17	ghij	12.87	fghij
G3	55.05	a	4.73	hij	9.47	ghij	14.2	fghij
G4	17.65	efghij	5.83	hij	9.29	ghij	3.59	ij
G5	20.27	efghi	2.38	j	7.27	hij	7.75	hij
G6	21.12	efgh	9.63	ghij	9.8	ghij	10.27	ghij
G7	27.68	def	10.17	ghij	20.12	efghi	5.48	hij
G8	20.66	efghi	3.52	ij	8.13	hij	6.27	hij
G9	48.79	ab	32.31	cde	43.15	abc	25.51	defg

Means followed by different letters within a column are significantly different at $P \leq 0.05$

Table 5
Effect of salinity levels on salt injury in the leaves of wild grape genotype

Genotype	Salinity 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 within a column are significantly different at $P \leq 0.05$

Proline and soluble sugar content

Proline content in the leaves of all tested genotypes increased under salt stress and the highest content was recorded in 150 mM NaCl treatment (Table 5). The increasing proline in wild genotype number 8 at 150 mM NaCl was the highest compared to other genotypes (Table 6). Soluble sugar content was significantly increased by NaCl treatments (Table 1). The highest content was recorded in 150 mM NaCl treatment. The lowest and highest values were recorded in wild genotypes number 8 and 6, respectively.

Discussion

Reduction of dry matter weight under salt stress is previously reported (Shani and Ben-Gal, 2005). According to Munns (2003), the decrease in plant biomass due to salinity may be related to low external water potential, ion toxicity, indirect effect on nutrients uptake and ion imbalance.

In this study, plant growth parameters in all genotypes reduced under salinity treatments. However, the extent of the reduction was different among them. Shannon and Grieve (1999), Kingsbury and Epstein (2006) reported that the range of salt toleration varied greatly from species to species, and even between cultivars within species. It has been suggested that independent effects of osmotic and ionic reactions and hormonal effects of roots control the stem physiology and growth (Voetberg and Sharp, 1991). Our results were in agreement with Askri et al. (2012). They observed that salinity significantly reduced vine shoot length, whole plant size, leaves, stems and roots in wild grapevine accessions. It's known that plant growth reduction due to salinity can be attributed to the osmotic effects of salt (Kingsbury and Epstein, 2006). Excess salt also increased expenditure of energy on maintenance respiration or ion transport, and reduced energy for the translocation of carbohydrates and diversion of photosynthates from growth to osmoregulation (Allen et al., 1994).

In this experiment, increasing salinity level had a decreasing effect on leaves RWC. It may be attributed to status of stomata and the increasing of leaves transpiration rate. Osmotic regulation is an indication response to osmotic stress. When water content is limited by salinity stress, osmotic potential is declined and this in turn causes the reduction of RWC. Osmotic regulation depends upon the genotype as well as on decreased rate of water potential and this is safe to say that maintaining high RWC of the leaf is one of the salt tolerance mechanisms in grapevine.

Adding salt to the plants excessively causes salt injury and premature senescence in older leaves. Salt injury is more likely related to chloride, since Cl^- absorbs more quickly than sodium (Downton and Millhouse, 1983).

Under high salt stress, plant cells decrease their osmotic potential by accumulating some solutes such as proline and soluble sugars (Youssef and Al-Fredan, 2008). Proline plays an adaptive role in mediating osmotic adjustment and protecting the sub-cellular structures in stressed plants (Voetberg and Sharp, 1991). Cramer et al. (2007) reported that in salinity condition organic acids, amino acids and sugars (glucose and malate) are produced in grape seedlings. Soluble sugars bring stability to proteins and membranes. Total sugar and proline content gradually increased under NaCl stress that contributed to leaf osmoregulation. Fozouni et al. (2012) reported that proline and soluble sugars increased by increasing NaCl concentration in four vinifera grapevine cultivars and this increase in salt-tolerance cultivars (Red Rishbabab and Sahebi) was higher than salt-sensitive cultivars (Red Sultana and Dastarchin). In many plants there is a positive correlation between the accumulation of proline and stress tolerance but it is so difficult to prove this correlation in grapevine.

Table 6
Effect of salinity levels on Proline ($\mu g g^{-1} dw$) content in the leaves of wild grape genotype

Genotype	Salinity Levels							
	0 mM		50 mM		100 mM		150 mM	
G1	1.55	lmno	2.63	ghijkl	3.3	efghi	4.53	cd
G2	1.65	lmno	2.4	k	3.07	fghij	4.6	cd
G3	1.47	lmnop	2.67	ghijkl	2.93	ghijk	4.3	cde
G4	1.11	mnop	2.06	jklmn	3	fghijk	4.47	cde
G5	0.95	nop	2.01	jklmn	4.17	cdef	6.97	b
G6	0.84	nop	1.7	lmno	3.61	defg	6.1	b
G7	0.32	p	1.15	mnop	1.79	klmn	3.1	fghij
G8	0.53	op	2.3	hijklm	5	c	9.43	a
G9	0.52	op	2.2	jklmn	3.45	defgh	7.1	b

Means followed by different letters within a column are significantly different at $P \leq 0.05$

Higher concentrations of carbohydrates in response to salinity are probably due to reduced growth (Munns, 2003). It can be also attributed to less movement of sugars from leaves, less consumption in leaves and roots because of less growth and hydrolysis of starch (Sanchez et al., 2005). Downton (1997) has recommended that amounts of starch decreased by increasing salt concentration. Since the soluble carbohydrates play a significant role in the balance of oxidants and antioxidants pool in plants. It has been suggested that sugar signaling and sugar-modulated gene expression are related to the control of oxidative stress in plants (Couée et al., 2006).

Conclusion

Salinity treatments significantly decreased growth parameters such as shoot, root fresh and dry weight, leaf area. There are a lot of parameters for recognizing tolerant and sensitive genotypes. Wild genotypes number 4 and 7 showed less symptoms and will be recommend as saline tolerant genotypes. Although proline and soluble sugars were significantly increased with salinity stress, this accumulation was less in salt tolerant genotypes. Increasing of these solutes was a normal response to stress in all tested genotypes so it will not be a precise marker for screening salt tolerant genotypes in grapevine.

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