Bangladesh J. Bot. **46**(2): 639-645, 2017 (June)

EFFECTS OF DIFFERENT SALICYLIC ACID DOSES ON SALT-TOLERANCE OF AMERICAN VINE ROOTSTOCKS

HATICE BILIR EKBIC*

Department of Horticulture, Faculty of Agriculture, University of Ordu, 52200, Ordu, Turkey

Keywords: Vine, Salt stress, Salicylic acid

Abstract

Three different American vine rootstocks (99 R, 420 A, 1616 C) were used for the study. Before exposing to salt stress, cuttings were immersed in to salicylic acid solutions at 4 different doses (0, 3, 6 and 9 mM). Saline water treatments lasted after 8 weeks. About 2 months later, the parameters of root and shoot chlorophyll content, leaf K (%), Ca (%), Mg (%), Na (ppm) content were investigated to assess the efficiency salicylic acid treatments. Less damage levels were observed in all three rootstocks under saline conditions with increasing salicylic acid doses and increasing root and shoot tolerance rates were observed with treatments. Treatments under saline conditions had positive effects on root and shoot development and leaf nutrient (K, Ca and Mg) contents.

Introduction

Plants were exposed to various negative conditions throughout growth and development stages. Salinity is a significant stress factor negatively effecting yield and quality in about 6% of agricultural lands worldwide (800 million ha). Sodium chloride is the most prevalent salt form in soils and plants. Salt accumulation in soils primarily resulted from irrigated agricultural practices, salinity induced by groundwaters or underground sources and salt accumulation in soil layers induced by dry conditions. Improper irrigations may also create a salinity problem and exert salt stress on plants. Saline irrigation waters may also result in significant decreases in both yield and quality (Culha and Cakırlar 2011). Salinity results in contraction of photosynthetic tissue and cell membranes and disturbs molecular structure of chlorophylls and consequently reduces photosynthesis, transpiration rate and stomal conductance (Yilmaz *et al*. 2011). Salt-induced stress caused serious physiological disorders in plants and ultimately decelerated vegetative and generative growth. Thus, salinity consequently results in pollination disorders, recess in bud formation, reduction in leaf and fruit sizes, cell deaths and resultant yellow spots in roots, buds, leaf edges and growth tips and ultimate plant died (Dölarslan and Gül 2012). Grapevine is moderately resistant to salinity. Although rootstock resistance to salinity-induced stress varies based on the rootstocks, they have quite low resistances compared to cultivars (Mullins *et al*. 1992). In recent years, practices to prevent salinity-induced damages have become prominent. Salicylic acid treatments are sometimes used to prevent salinity damage on plants. Salicylic acid generally includes a hydroxyl group or a functional derivative of it. It is plant phenolics with an aromatic chain (Harborne 1980). Various studies were preformed about the biology of salicylic acid on plants and salicylic acid was indicated as an internal plant growth regulator with various effects on several metabolic and physiological processes influencing plant growth and development. Salicylic acid was also indicated as a significant internal indicator molecule for plant resistance to stress conditions. In this respect, salicylic acid plays a great role in protein synthesis in plants exposed to biotic and abiotic stressors, promotes systemic resistance in plants and prevents plants against different abiotic stress conditions (Kök 2012).

*Author for correspondence: <haticebilirekbic@gmail.com>.

The present study was conducted to assess the efficiency of different salicylic acid doses in prevention of salinity damage on salinity-resistant 1616 C and salinity-sensitive 99 R and 420 A American vine rootstock cuttings. These American rootstock cultivars which were used in this research are expailaned below: 99 R (99 Rihcter) (*Vitis berlandieri* × *Vitis rupestris*), 420 A Mgt (*Vitis berlandieri* × *Vitis riparia*), 1616 C (1616 Couderc) (*Vitis solonis* × *V. riparia*). These rootstocks were obtained from the hybridization of the parents mentioned above about 1800 years and are so named in viticulture studies.

Materials and Methods

The present study was conducted in a greenhouse constructed over implementation and experimental fields of Horticulture Department of Ordu University Agricultural Faculty during 2013-2014 vegetation period. Three different American vine rootstocks (99 R, 420 A, 1616 C) were used as the plant material of the study. American vine rootstocks were stored at +4^{o}C until planting. Cuttings were then removed from cold storage and prepared as to have two buds. Before exposed to salt stress, two-bud cuttings of 99 R, 420 A and 1616 C rootstocks were immersed into salicylic acid solutions at 4 different doses (0, 3, 6 and 9 mM) for 24 hrs to allow the cutting to take salicylic acid (SA) (SIGMA, 27301) into their structures. Cuttings were than planted into perlite-filled rooting trays. Irrigation water used to irrigate the cuttings was classified as unsaline with regard to electrical conductivity (EC) value. Saline water treatments were initiated when the buds burst and cuttings reached 2 - 3 leaf stage 9th stage specified by Eichhorn and Lorenz (1977) and treatments went on for 8 weeks. Threshold irrigation water salinity level for vineyards is defined as 1 ds/m (640 ppm). Therefore, irrigation water salinity level of 8 ds/m (5120 ppm) over this specified threshold value was applied to cuttings to exert salinity stress on plants (Kök 2012). Saline water treatments were implemented with saline water irrigations for a week, and then normal irrigation water was applied to plants. Nutrients were supplemented to plants twice with Hoagland two nutrient solution (SIGMA, H2395). Saline water treatments to cuttings were terminated after two months and they were pulled out from perlite growing medium. Then, morphological and physiological investigations were performed to assess the efficiency of salicylic acid treatments.

Within the scope of morphological investigations, shoot and root lengths (cm) were measured, shoot and root fresh weights were determined with a digital scale (±0.01 g), shoots and roots were dried separately in an oven at 65ºC for 72 hrs to get dry shoot and root weights. Number of leaves and roots were also determined. Leaf samples were taken from the mid-sections (1/3) shoots. Total leaf chlorophyll content was determined with a SPAD meter. To assess the efficiency of salicylic acid treatments, leaf K (%), Ca (%), Mg (%) contents were also determined through the readings performed on dry-ashed samples in an atomic absorption spectrophotometer. Resultant readings were then multiplied with dilution factor. To determine leaf Na contents, 0.200 g ground sample was ignited in an ash oven at 550^{0}C for 5 hrs. About 2 ml 1/3 HCl acid solution was added to burnt samples and samples were then volatilized at 45 - 50^{0}C. The volatilized samples were dissolved in 1/3 2 ml HCl again and 18 ml distilled water was added to make the final volume 20 ml. Resultant solution was filtered through blue band filter paper and readings were performed in an atomic absorption device (Chapman *et al.* 1961). Leaf damage levels were also determined in all treatments. For this attribute, the scale developed by Martinez and Alvarez (1997) for strawberry plants was modified. According to this scale, the plants without salt-induced necrotic tissues were specified as to have '0 degree' damage; the plants with slight dry outs and necrosis at leaf tips were specified as to have '1 degree' damage; the plants with necrosis over more than 50% of the leaf and stem were specified to have '2 degree' damage and the plants with mortal necrosis were specified as to have '3 degree' damage. Tolerance rates of American vine rootstock cuttings used in this study at different salicylic acid doses under saline conditions were calculated with the following equation separately for shoots and roots in dry shoot and root weight basis (Turhan *et al.* 2005):

TO = T_x / T_o , TO: Tolerance Rate , Tx: Shoot and root dry weight of the cutting exposed to certain concentration of salicylic acid (g), T_o: Shoot and root dry weight of the cutting not exposed to certain concentration of salicylic acid (g)

Experiments were conducted in randomized plots experimental design with replications and with 10 cuttings in each replication. Statistical analyses were performed with JUMP 10.0 software and means were compared by LSD test at 5% significance level.

Results and Discussion

Salicylic acid treatments had significant positive effects on shoot length, shoot fresh weight and hoot dry weight. Optimum salicylic acid dose for shoot growth parameters varied significantly with the rootstocks. In general, the greatest shoot length (20.6 cm) was obtained from 6 mM salicylic acid treatment of 99 R rootstock cuttings. The longest shoots in 420 A and 1616 C rootstocks were obtained from 6 mM salicylic acid treatment, respectively with 15.3 and 7.5 cm. With regard to shoot fresh and dry weights, significantly higher values were observed in 6 mM salicylic acid treatments of 420 A and 1616 C rootstocks. The optimum dose for these two attributes of 99 R rootstock was identified as 3 and 6 mM. For the same rootstock, the 3 mM salicylic acid treatment yielded the best dry shoot weight (Table 1).

Joolka *et al.* (1976), Sivritepe (2000), Turhan *et al.* (2005), Urpeti and Murti (2010), Karimi and Zadeh (2013). Horvath *et al.* (2007) and Kök (2012) reported significant effects of salicylic acid treatments in improving plant tolerance levels against abiotic stress factors.

The effects of different salicylic acid doses on root length, root fresh and dry weights were found to be significant at $p < 0.05$. Compared to control treatment, entire doses had significant positive effects on root fresh weights. The greatest fresh root weight (3.802 g) was obtained from 6 mM treatment of 1616 C rootstock. The findings on root dry weights of all rootstocks were similar to findings on root fresh weights (Table 1).

Significant increases were observed in root development factors with salicylic acid treatments of the present study. Negative effects of salinity on root development were reported in several previous studies (Turhan *et al.* 2005, Urpeti and Murti 2010, Fosouni *et al.* 2012, Karimi and Zadeh 2013). Salicylic acid alone or together with auxins was reported to increase root lengths in woody species (Özeker 2005). Such outcomes support the findings of the present study.

Distinctive changes were observed in chlorophyll contents of 420 A and 1616 C rootstock with increasing treatment doses. The highest chlorophyll content was obtained from entire salicylic doses of 1616 C rootstock. The greatest chlorophyll content of 420 A rootstock was obtained from 6 mM treatment and 9 mM treatment was found to be more effective in 99 R rootstock (Table 1).

The greatest leaf Na content of cuttings grown under saline conditions was observed in control samples. Salicylic acid treatments generally reduced leaf Na contents. The greatest leaf K, Ca and Mg content of 1616 C rootstock was obtained from 6mM treatment (respectively with 0.801, 1.535 and 0.303%). While the highest K content of 420 A rootstock was observed in 3 and 6 mM salicylic acid treatments, the greatest Ca content was achieved in 3 mM treatment. The greatest K content of 99 R rootstock was observed in 9 mM treatment. However, the greatest Ca content of 99 R rootstock was obtained from 6 mM and the highest Mg content from 6 and 9 mM treatments (Table 1).

Table 1. Effects of different salicylic acid doses on morphological and physiological characters in American grapevine rootstocks under salt stress.

	420 A				99 R				1616 C			
	Control	3 mM	6 mM	9 mM	Control	3 mM	6 mM	9 mM	Control	3 mM	6 mM	9 mM
Root length (cm)	6.3 efg	7.3 de	6.1 fg	5.5 g	7.9 cd	10.5 ab	11.1 ab	11.6 a	5.8 g	8.9 c	10.2 b	7.1 def
Root fresh weight (g)	0.562 f	0.833 ef	0.805 ef	1.081 e	2.139 d	2.396 cd	2.625 c	3.228 b	0.612 f	2.311 cd	3.802 a	2.388 cd
Root dry weight (g)	0.158 fg	0.135 g	0.126 g	0.083 g	0.366 cd	0.446 bc	0.373 cd	0.698 a	0.179 fg	0.333 de	0.543 b	0.247 ef
Shoot length (cm)	11.2 d	14.8 c	15.3 bc	14.5 c	16.9 b	19.2 a	20.6 a	16.6 b	4.9 fg	6.1 ef	7.5 e	4.1 g
Shoot fresh weight (g)	1.458 e	3.137 bc	3.530 b	2.747 c	3.764 ab	4.451 a	4.309 a	3.590 b	1.928 de	2.526 cd	3.183 bc	1.962 de
Shoot dry weight (g)	0.385 fg	0.559 cde	0.633 bcd	0.449 efg	0.725 ab	0.855 a	0.757 ab	0.660 bc	0.159 i	0.311 gh	0.499 def	0.236 hi
Chlorophyll content	3.55 g	3.85 fg	4.03 defg	3.97 efg	4.73 cd	4.70 cde	4.44 cdef	4.78 c	6.14 b	8.86 a	9.14 a	9.50 a
K (%)	0.728 cd	0.798 a	0.793 a	0.759 b	0.653 e	0.635 e	0.660 e	0.727 d	0.758 bc	0.755 bcd	0.801 a	0.662 e
Ca (%)	1.104 f	1.246 cd	1.075 f	1.123 ef	1.413 b	1.364 bc	1.476 ab	1.395 b	1.106 f	1.241 de	1.535 a	1.137 def
Mg (%)	0.439 ab	0.416 b	0.360 c	0.461 a	0.344 cd	0.325 de	0.357 c	0.361 c	0.257 g	0.272 g	0.303 ef	0.278 fg
Na (ppm)	2204 ab	2312 ab	1594 c	2083 b	2421 a	2156 ab	1571 c	1708 c	1412 cd	1117 de	724 f	959 ef

LSD 5% (Root length): 1.2
LSD 5% (Shoot length): 0.1
LSD 5% (Chlorophyll content): 0.73
LSD 5% (Mg): 0.031
LSD 5% (Root fresh weight): 0.406
LSD 5% (Shoot fresh weight): 0.693
LSD 5% (K): 0.030
LSD 5% (Na): 312
LSD 5% (Root dry weight): 0.108
LSD 5% (Shoot dry weight): 0.138
LSD 5% (Ca): 0.119

Salicylic acid treatments had significant effects on leaf nutrient content of different vine rootstocks grown under saline conditions. Leaf Na contents were higher in control group of all rootstocks than the other treatments. Increasing leaf K, Ca and Mg contents were observed with salicylic acid treatments. In general, a decrease was observed in leaf K contents with increasing NaCl concentrations. Such an antagonistic effect was also reported by Downton (1985), Günes *et al*. (1996) and Fisarakis (2004). Decreasing leaf Ca and Mg contents under saline conditions were also reported by several researchers (Lahaye and Epstein 1971, Awada *et al*. 1995, Fisarakis *et al*. 2004). Again with salicylic acid treatments, decreases in leaf Ca and Mg contents under saline conditions were also prevented in this study.

Compared to control treatment, the cuttings exposed to salicylic acid treatments were found to be significantly more tolerant to salinity. Control cuttings of 420 A rootstock were found to be the most sensitive to salinity with a degree of damage of 2.9. In this rootstock, degree of damage decreased to 0.8 with 9 mM salicylic acid treatment (Fig. 1).

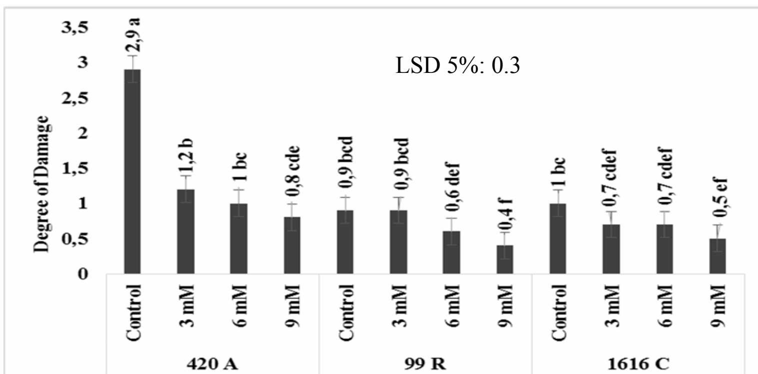

Fig. 1. Effects of salicylic acid treatments on degree of damage of cuttings under salt stress.

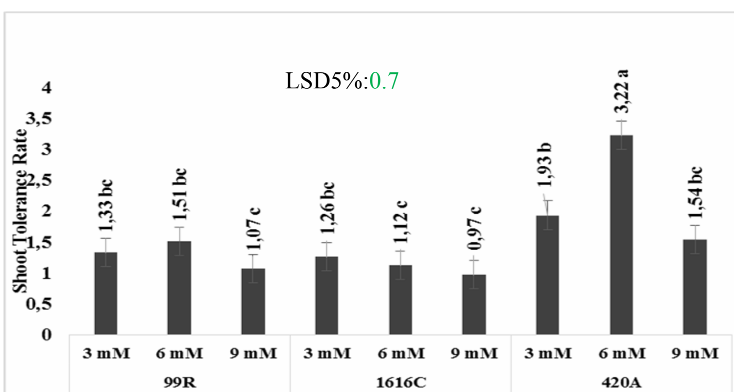

Fig. 2. Effects of salicylic acid treatments on shoot tolerance rate of cuttings under salt stress.

It was observed in this study that external salicylic acid treatments might significantly increase shoot tolerance rates of 3 rootstocks with different tolerance levels against salinity. With regard to shoot tolerance levels, the most effective salicylic acid doses were identified as 3 and 6 mM for 99 R, 3 mM for 1616 C and 6 mM for 420A rootstock (Fig. 2).

Such sensitivity of 420 A rootstock against salinity was also reported by Turhan *et al*. (2005). Homrouni *et al*. (2008) carried out a study to determine the salt stress tolerance of vine genotypes and reported decreasing growth and development, vigor and rooting with increasing salinity levels. Salem *et al*. (2011) and Turhan *et al*. (2005) reported decreasing vigor with increasing salinity levels. Current findings revealed that salicylic acid treatments were highly effective in preventing damaging effects of salinity. Salicylic acid is synthesized by several plants and various studies were carried out about the effects of salicylic acid treatments in preventing damages under biotic and abiotic stress conditions. With the present study, both the growth and development of salt-resistant rootstocks were improved and negative effects of salinity were prevented in salt-sensitive rootstocks through salicylic acid treatments.

References

Awada S, Campbell F, Dudley M, Jurinak JJ and Kham MA 1995. Interactive effects of sodium chloride, sodium sulfate, calcium sulfate and calcium chloride on snapbean growth, photosynthesis and ion uptake. J. Plant Nutr. **18**: 889-900.

Chapman HD, Pratt PF and Parker F 1961. Methods of analiysis for solis, plant and waters. Univ. of California, Division of Agricultural Sciences 309. Riverside/ USA.

Culha S and Çakırlar H 2011. Tuzluluğun bitkiler üzerine etkileri ve tuz tolerans mekanizmaları. Akü. Febid **11**: 11-34. Ankara.

Downton WJS 1985. Growth and mineral composition of the sultana grapevine as influenced by salinity and rootstock. Austr. J. Agric. Res. **36**: 425-434.

Dölarslan M and Gül E 2012. Toprak bitkisi ilişkileri açısından tuzluluk. Türk Bilimsel Derlemeler Dergisi **5**(2): 56-59.

Eichhorn KW and Lorenz DH 1977. Phanologische entwi cklungsstadilen der rebenachrichtenbl. Dtsch. Pflanzenschutz (Braunschweigh) **29**: 119-120.

Fisarakis I, Nikolaou N, Tsikalas P, Therios I and Stavrakas D 2004. Effect of salinity and rootstock on concentration of potassium, calcium, magnesium, phosphorus and nitrate-nitragen in thompson seedless grapevine. J. Plant Nutrition **27**(12): 2117-2134.

Fozouni M, Abbaspour N and Baneh HD 2012. Short term response of grapevine grown hydroponically to salinity: mineral composition and growth paremeters. Journal Vitis **51**(3): 95-101.

Günes A, Inal A and Alpaslan M 1996. Effect of salinity on stomatal resistance, proline and mineral composition of pepper. J. Plant Nutr. **19**: 389-396.

Hamrouni L, Abdallah FB, Abdelly C and Ghorbel A 2008. *In vitro* culture a simple and efficient way for salt-tolerant grapevine genotype selection. Comptes Rendus Biolgies **331**(2): 152-163.

Harborne JB 1980. Plant phenolics. *In:* Secondary Plant Products. E. A. Bell, B. V. Charlwood (Eds), Springer Verlag, Berlin, Germany. pp. 329-402.

Horvath E, Szalai G and Janda T 2007. Induction of abiotic stress tolerance by salicylic acid signaling. J. Plant Growth Regul. **26**: 290-300.

Joolka NK, Singh J and Khera AP 1976. Growth of grapevines (*vitis vinifera* l.) as affected by sodium chloride and sodium sulphate salts. Haryana J. Hort. Sci. **5**(3/4): 181-188.

Karimi H and Yusef-Zadeh H 2013. The effect of salinity level on the morphological and physiological traits of two grape (*vitis vinifera* l.) cultivars. International Journal of Agronomy and Plant Production **4**(5): 1108-1117.

Kök D 2012. Farklı salisilik asit dozlarının asma anaçlarının tuzluluğa dayanımı üzerine etkileri. Tekirdağ Ziraat Fak. Dergisi **2**(9): 32-40.

Lahaye PA and Epstein E 1971. Calcium and salt tolerance by bean plants. Physiol. Plant **25**: 213-218.

Martinez-Barraso MC and Alvarez CE 1997. Toxicity symptoms and tolerance of strawberry to salinity in the irrigation water. Scientia Horticulturae **71**: 177-188.

Mullins MG, Bouquet A and Williams LE 1992. Biology of the grapevine. Cambridge University Press. U.K. pp. 239.

Ozeker E 2005. Salisilik asit ve bitkiler üzerine etkileri. Ege Üniv. Ziraat Fak. Derg. **42**(1): 213-223.

Salem AT, Abldel-Aal YA, Abdel-Mohsen MA and Yasin WH 2011. Tolerance of flame seedless grapes on own root and grafted to irrigation with saline solutions. Journal of Horticultural Science and Ornamental Plants **3**(3): 207-219.

Sivritepe N 2000. Asmalarda tuzdan kaynaklanan ozmotik stresin teşvik ettiği fizyolojik değişimler ve tuza dayanımdaki rolleri. Turkish Journal of Biology **24**: 97-104.

Turhan E, Dardeniz A and Müftüoğlu NM 2005. Bazı amerikan asma anaçlarının tuz stresine toleranslarının belirlenmesi. Journal of Atatürk Central Horticultural Research Institute **34**(2):11-19

Urpeti KK and Murti GSR 2010. Response of grape rootstocks to salinity: Changes in root growth, polyamines and absisic acid. Biologia Planttarum **54**(4): 730-734.

Yılmaz E, Tuna M and Bürün B 2011. Bitkilerin tuz stresi etkilerine karşı geliştirdikleri tolerans stratejileri, gösterdikleri tolerans stratejileri. C.B.Ü. Fen Bilimleri Dergisi **7**(1): 47-67.

(Manuscript received on 23 March, 2016; revised on 18 January, 2017)