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Salinity effects on toxic ions accumulation in grape (*Vitis* L.)

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Abstract

To study the salinity tolerance of own rooted grape cuttings taken from H6 grape genotype, hydroponically grown were treated with different concentrations of NaCl during 14 days. H6 genotype was a hybrid of *V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB. Chloride (Cl^-) and sodium (Na^+) ions accumulated in the various parts of the vine with increasing external NaCl concentration. Na^+ accumulation exceeded that of Cl^- in roots of H6 genotype under salinity, but in lamina Cl^- accumulated higher than Na^+. Cl^- uptake rate was 0.9174µmol.gDW^{-1}.day^{-1} in lamina and was 0.2520µmol.gDW^{-1}.day^{-1} in root, also Na^+ uptake rate was 0.8560µmol.gDW^{-1}.day^{-1} in lamina and 0.6080µmol.gDW^{-1}.day^{-1} in root. Indeed there is no strong control of Cl^- transport from root to shoot in H6 and the roots of H6 showed a high ability to restrict Na^+ uptake compare to Cl^-. There was positive correlation ($P<0.01$, $r^2>0.9$) between Cl^- and Na^+ concentrations in root and lamina of H6 genotype.

Key words: salt, grape, Cl^- accumulation, ion uptake rate.

Introduction

Soil salinity is one of the most serious environmental threats for plant survival and crop yield. It affects 19.5% of irrigated land and 2.1% dry land agriculture across the globe [5].

Salinity limits vegetative and reproductive growth of plants by inducing severe physiological dysfunctions and causing widespread direct and indirect harmful effects, even at low salt concentrations [15]. Tissue injury is induced by both the osmotic effects of salts and specific toxic effects resulting from the accumulation of Cl^- and Na^+ [8].

Accumulation of Cl^- and Na^+ in grapevines may result in physiological disturbances leading to reductions in growth, vegetative biomass, and fruit yield [18].

When plants are growing in salinity, roots have to cope with two types of stress. The first of these is an osmotic stress resulting from salt concentration that results in lowered water potential and a consequent loss of cell turgor in roots. The second is ionic stress induced by changes in the concentrations of Na^+, Cl^- or both in the root-growing medium. Both of which contribute to its deleterious effects [14]. It has been shown that the physiological disturbances in citrus produced by salinity are associated with Cl^- build-up rather than with Na^+ accumulation [10].

Cl^- is an essential plant micronutrient, functioning as an osmotically active solute in plant vacuoles and involved in both turgor and osmoregulation [19]. But Cl^- can be toxic to plants at high concentrations, with critical concentrations estimated at 4-7 mg/g of dry weight (DW) for Cl^- sensitive species and 15-50 mg/g of DW for Cl^- tolerant species [20]. In fact, Na^+ and Cl^- are both toxic to plants at high concentrations, but some species can control Na^+ transport better than Cl^- and vice versa [11].

"Why focus on Na^+, why not consider Cl^-?" This question relates particularly to species that accumulate high concentrations of Cl^- and not Na^+ in leaves and to species that are routinely grown on Cl^- excluding rootstocks such as grapevines and citrus. For these species, Cl^- toxicity is more important than Na^+ toxicity. However, this does not imply that Cl^- is more toxic than Na^+, but rather that these species are better Na^+ than Cl^- excluders.

Indeed, Na^+ may be a more toxic solute, but because Na^+ is transported more efficiently than Cl^-, Cl^- becomes a more toxic component [11].

Vitis vinifera grapevines are classified as moderately sensitive to salinity [9]. Salinity damage is caused by chloride ions [16], as grapevines have only a modest capacity to exclude Cl^- [1].

Grapevine responses to salinity include physiological and systemic disturbances leading to reductions in both growth and yield [17]. Ion accumulation in leaves decreases growth [6]. The reduction in growth in response to salinity is usually attributed to ion toxicity and low

external osmotic potential, both of which may affect plant physiological and biochemical processes [7]. The symptoms of severe salt stress include necrotic areas on leaves: starting at the leaf margins and progressing inwards. Such visible symptoms are often referred to as 'leaf burn' [18].

In this study H6 grape genotypes were evaluated for salt tolerance. Toxic ions (Cl^- and Na^+) accumulation and their uptake rate were compared in shoots and roots.

Materials and Methods

Plant materials and growth conditions

Hardwood cuttings of H6 genotype of grape (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB) were obtained from Kahriz vineyard (Agricultural Research Center, grape genotypes collection). The cuttings were disinfected with benomyle (1% w/v) and then basal parts soaked in Indole-3-butyric acid 0.1% (w/v) for 5-10 s. All cuttings were struck in a mist house (relative humidity 80%) with a heat-bed temperature of 20-30ºC. After two weeks, the rooted cuttings were transferred into 2 L pots containing Hoagland solution. The pots were protected with Aluminum foil to avoid light effects and alga proliferation. Plants with 4-5 fully expanded leaves were treated with NaCl (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100 mM (absorption kinetics experiments) in Hoagland solution for 2 weeks. NaCl was added to the nutrient solution by incremental increases until the final desired concentrations were reached. Our experimental design was Complete Randomized Block Design (CRBD). We had three replicates per treatment and 2 pots per replicate. Plants were harvested after 2 weeks and plant parts were weighed separately and dried at 70ºC for 48 hours.

Ion Analysis

100 mg of ground tissues of all treatments were weighed into 15 ml plastic centrifuge tubes containing 10 ml deionized water. The tubes were placed in a boiling water bath for approximately 1 hour. Samples were centrifuged at 5000 rpm. The supernatant was transferred into new tubes and the volume made up to 10 ml by addition of deionized water.

0.5- ml aliquots were analyzed for chloride concentration using a Chloride Analyzer (model 926, Corning). Sodium concentrations were measured by a flame photometer (Fater 405).

Statistical analysis

All statistical analyses were done using the Statistical Package for Social Sciences (SPSS) for Windows (Version 19.0). The mean values of three replicates and the "Standard Error" of the means was calculated. One-way ANOVA was used to determine the significance of the results between different treatments and then Tukey's multiple range tests ($P<0.05$) were performed. Linear regression (slope and r^2 levels) was calculated by Graphpad Prism 5 software.

Results

Salinity effects on ionic balance

Cl^-

Cl^- content significantly ($P<0.05$) increased with increasing salt treatments in H6 genotype (Figure 1). Cl^- accumulation in shoots (petioles and laminas) was higher than roots. The difference among all treatments was significant ($P<0.05$) in shoots, except between 40 and 45 mM NaCl. The difference among some treatments in petioles, laminas and roots was not significant ($P<0.05$). Cl^- increased gradually in petioles of H6 with increasing salinity up to

50 mM NaCl and then it increased higher. With increasing salinity Cl^- increased highly in laminas and shoots, but roots showed some fluctuations.

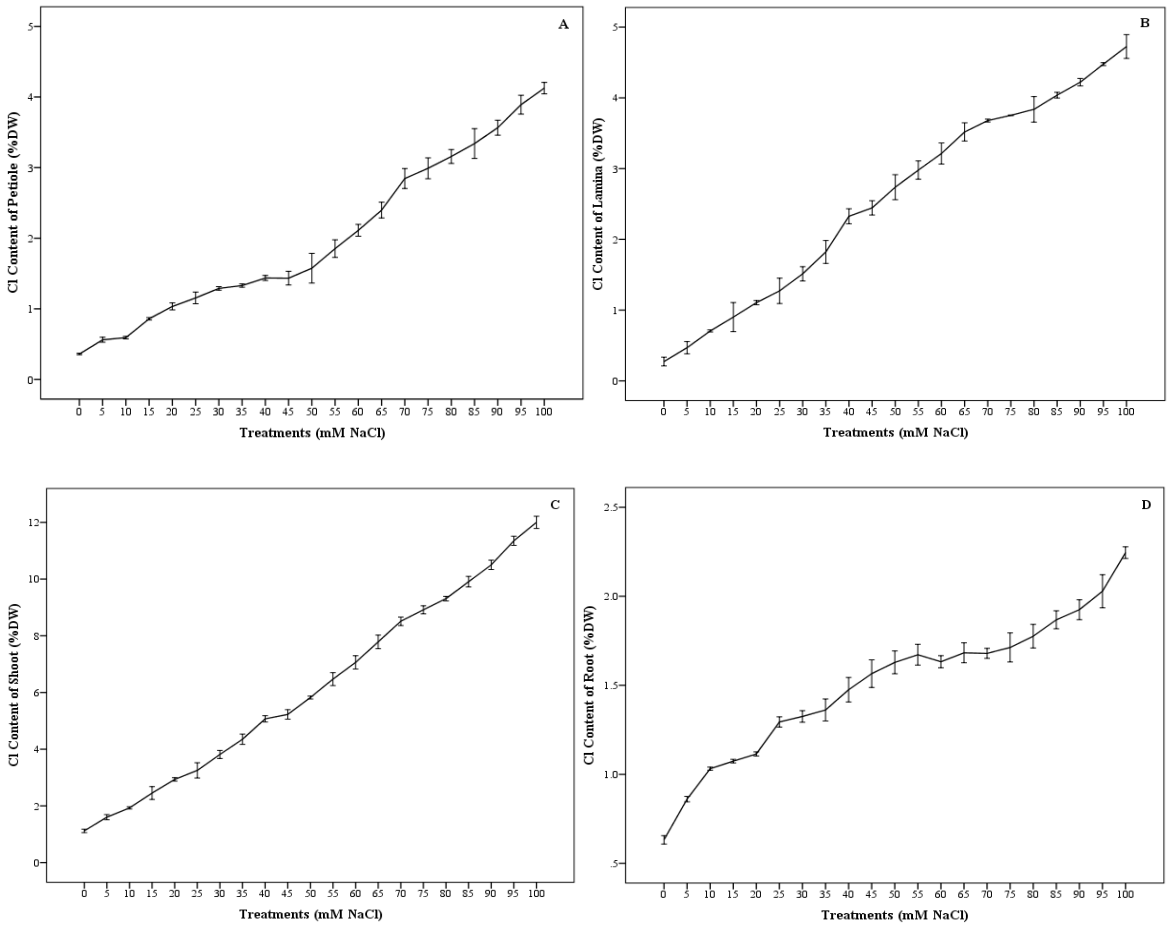

Figure 1 : Cl^- concentrations (%DW) in petioles (A), laminas (B), shoots (C) and roots (D) of H6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB) genotype at different salinity levels (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100mM NaCl) after 14 days treatment. Bars are the means ± SE (n=3, One Way ANOVA, Tukey's test).

Na^+

Na^+ content significantly ($P<0.05$) increased with increasing salinity in H6 genotype (Figure 2). Na^+ accumulation in shoots (petioles and laminas) was higher than roots. The difference among all treatments was significant ($P<0.05$) in shoots, except between 30 and 35 mM NaCl. The difference among some treatments in petioles, laminas and roots was not significant ($P<0.05$). Na^+ increased gradually in laminas and roots of H6 with increasing

salinity up to 50 mM NaCl and then it increased higher. With increasing salinity Na^+ increased highly in shoots.

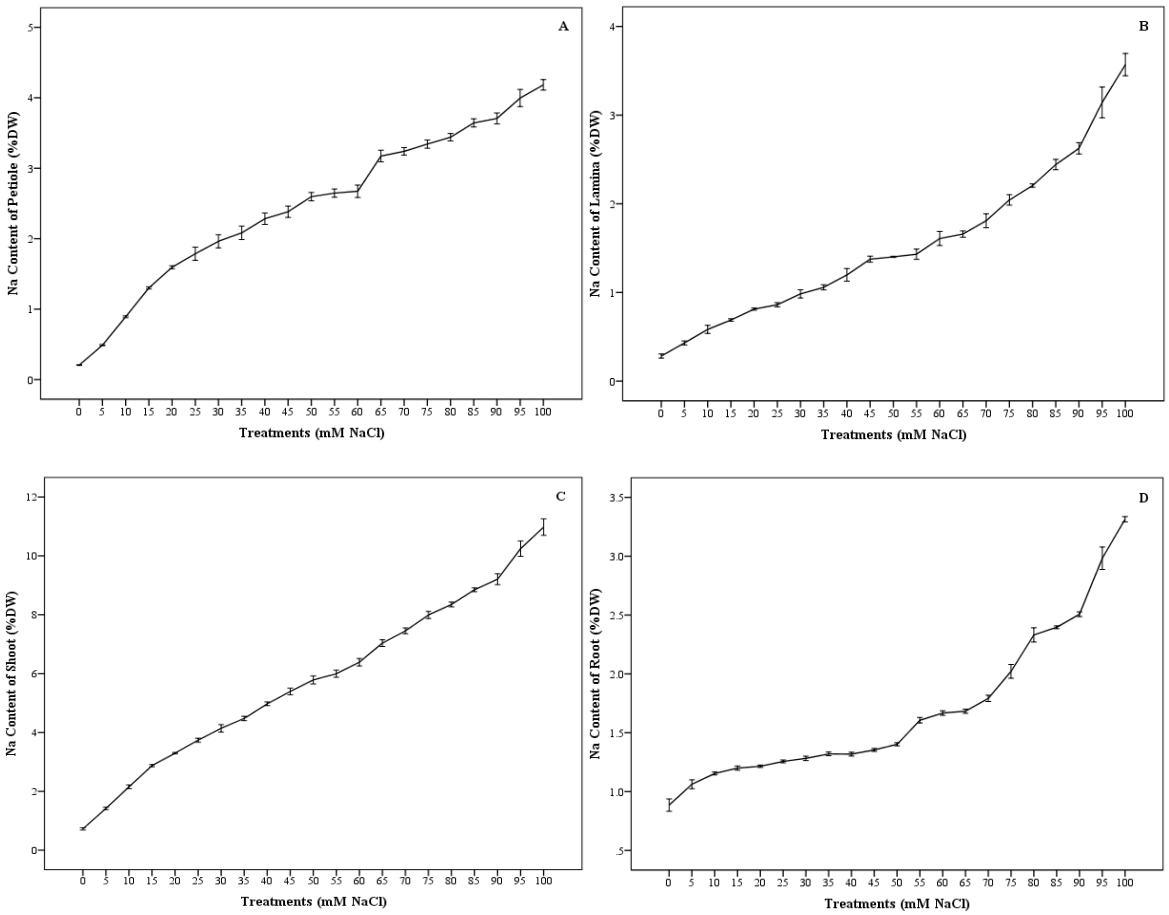

Figure 2 : Na^+ concentrations (%DW) in petioles (A), laminas (B), shoots (C) and roots (D) of H6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB) genotype at different salinity levels (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100mM NaCl) after 14 days treatment. Bars are the means ± SE (n=3, One Way ANOVA, Tukey's test).

Cl^- and Na^+ uptake rate

Cl^- and Na^+ has been significantly ($P<0.05$) increased in different parts (petiole, lamina, shoot and root) of H6 with increasing salinity in root medium (Figure 3). In all treatments Cl^- and Na^+ accumulation in lamina was higher than in root (Cl^- slope 0.92 in lamina compare to 0.25 in root and Na^+ slope 0.86 in lamina compare to 0.61 in root). The difference between

lamina and root about Cl^- was higher than Na^+, it means that roots of H6 accumulated higher Na^+ compare to Cl^-. Therefore higher Cl^- transport to lamina compare to Na^+.

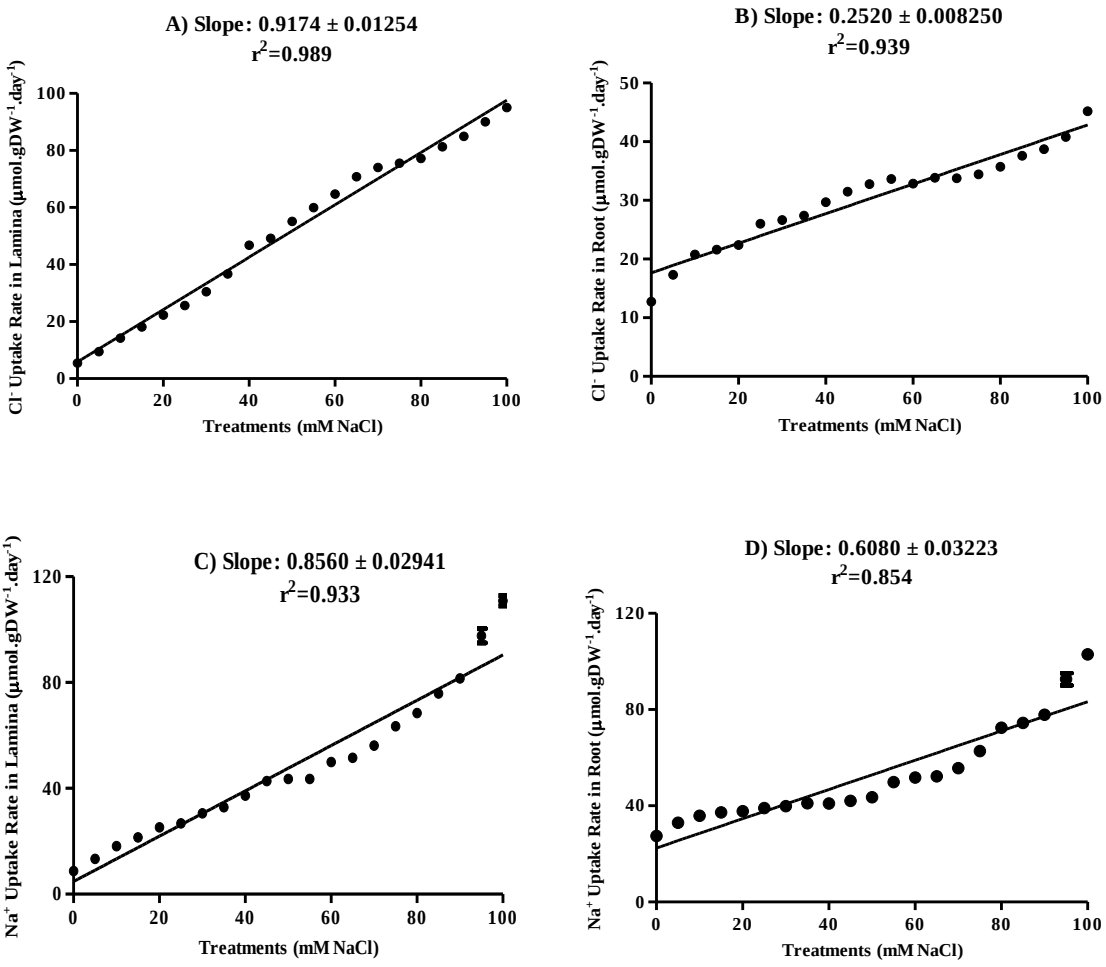

Figure 3 : Linear regression for Cl^- uptake rate (µmol.gDW^{-1}.day^{-1}) in Lamina (A) and root (B) and Na^+ uptake rate (µmol.gDW^{-1}.day^{-1}) in Lamina (C) and root (D) of H6 (*V. vinifera* cv. GharaUzum × *V. riparia* cv. Kober 5BB) genotype at different salinity levels (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 and 100mM NaCl) after 14 days treatment (n=3, Graphpad Prism 5).

Discussion

Salt tolerance in glycophytes is associated with the ability to limit uptake or transport of ions (mainly Na^+ and Cl^-) from the root zone to aerial parts [7]. Clearly, higher chloride or sodium accumulation in leaves leads to greater leaf physiological disturbance.

The physiological and biochemical basis of the growth reduction are not fully understood. The visible symptoms (marginal chlorosis and necrosis on leaves) are, in most cases, due to the accumulation of toxic concentrations of chloride in leaves. In H6 genotype we observed chlorosis at high NaCl concentrations (>50mM NaCl) because of high accumulation of toxic ions specially Cl^- in lamina.

Ehlig [4] reported salt burn symptoms in grapevine leaves when lamina Cl^-concentrations were in the range of 1.24-1.9% DW. Nevertheless, uninjured leaves may sometimes have higher Cl^- accumulation than injured leaves of the same species. Cl^- concentrations lower than 1% of DW is regarded as normal for vines under salts tress, while concentrations higher than 1.5% of DW are considered as excessive [13]. Lamina Cl^- concentrations of the genotype studied here were higher than 1.5% at high NaCl concentrations (>30mM NaCl). Therefore Cl^- concentrations in leaves was toxic at high salinity.

Petiole analysis provides a good evaluation of whether vines are suffering from salt stress [1]. But high petiole Cl^- concentrations does not necessarily mean that the vines are «stressed». Still, petiole Cl^- analysis is a good screening method for assessing genotypes for chloride exclusion ability, i.e., the ability to restrict the uptake and root-to-shoot transport of Cl^-. The higher Cl^- concentrations in petioles and laminas reflect the poor capacity of vines for Cl^- exclusion [3]. Cl^- accumulation in lamina is an important criterion to survey root control capability. In our study, Cl^- concentrations in petioles was lower than laminas, it means that petioles can't restrict Cl^- transport to laminas. Cl^- concentrations in laminas was two-fold more than roots, therefore the roots can't accumulated higher Cl^- and Cl^- transport to lamina.

Na^+ was significantly ($P<0.05$) increased in all parts of H6 genotype with increasing salinity. The distribution of sodium differed markedly from that of chloride [3, 2].

Previous studies [6, 18] showed that grape laminas accumulated higher levels of Cl^- than Na^+ under salinity. Our results was verified them, the laminas of H6 accumulated higher Cl^- compare to Na^+.

Leaf burn is more likely due to Cl^-, even if it occurs at lower concentration than Na^+ [6]. H6 leaves accumulated higher Cl^- compare to Na^+, and therefore leaf burn is related to Cl^-.

The preferential accumulation of Na^+ in the root system previously has been reported by Downton [3] in grapevine. The differences in behavior of plant species towards Na^+ accumulation within their various organs are related to their resistance to salinity. Our results were consistent with them, because H6 genotype showed higher Na^+ accumulation in roots compare to Cl^-. Therefore the roots of H6 preferred Cl^- between toxic ions.

The correlation between Cl^- and Na^+ contents in all plant parts (petioles, laminas, shoots and roots was positive ($P<0.01$, $r^2>0.9$) in H6 genotype.

In conclusion, salinity resulted in increased Cl^- and Na^+ concentrations in all organs. In our studied genotype, Cl^- accumulation in shoots was higher than in roots. This suggests that there is no strong control of Cl^- transport from root to shoot in H6. Accumulation of higher Cl^- concentration in laminas indicated that the roots of H6 showed a higher ability to restrict Na^+ uptakes compare to Cl^-.

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