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Growth and physiological responses of grafted and non-grafted cultivars of *Ziziphus spina-christi* to salinity

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Summary

Two grafted cultivars of *Z. spina-christi* ('Danehgird' and 'Danehboland' were grafted on wild seedlings) and non-grafted seedlings were grown in soil and perlite mixture (1:1 v/v) and treated with 0 (0 dS m^{-1}), 3.2 (5 dS m^{-1}), 6.4 (10 dS m^{-1}) and 12.8 (20 dS m^{-1}) g l^{-1} NaCl. After 16 weeks, salt stress resulted in a substantial decrease in root length, stem, leaf surface, lateral root number, root, stem and leaf fresh and dry weight in both grafted cultivars and seedling rootstock. These reductions were predominance in seedling rootstock than in grafted cultivars. In all organs (leaf, stem and root) of grafted 'Danehboland' cultivar, the K^+/Na^+ ratio was significantly higher than non-grafted wild seedling in saline and non-saline conditions. The proline and soluble sugar also was significantly higher in the leaves of 'Danehboland' cultivar than non-grafted control. The results imply the predominance of the scion genotype in determining salt tolerance in comparison with rootstock seedling.

Introduction

Widespread in tropical and subtropical regions, Ziziphus *spina-christi* (L.) Desf. belonging to the Rhamnaceae family is a spiny shrub or small tree (Johnston, 1963) that are naturally distributed in southern parts of Iran and locally named 'konar'. This species is a multipurpose plant. In addition to high value of non wood products, particularly leaves and shoots saponin, tannin substrates and fruit nutrition, this species is ecologically and economically important for its tolerance to drought and salinity (Sudhersan and Hussain, 2003; Sohail, 2009). The fruit is a drupe (about 1 to 1.5 cm in diameter) and is consumed either fresh or dried by the rural population (Saied et al., 2008). In the south of Iran, a wide variability in yield, fruit size, peel colour was observed among the genotypes (Torahi, 2011). At present, the seedlings are used as rootstock for cultivars with high fruit quality (Takhti and Shekafandeh, 2012).

Salinity is a main problem in arid and semi-arid regions of the world (Gebauer et al., 2004). Global warming and low rainfall intensify this difficulty. The saline growth condition causes many unfavorable effects on plant growth and development at physiological and biochemical levels, which are due to low osmotic potential of soil solution (osmotic stress), specific ion effects, nutritional imbalance or combination of these factors (Parida and Das, 2005; Arzani, 2008). Due to water scarcity and exacerbated soil salinity in some part of Iran, cultivation of other fruit trees such as citrus is limited, so, orchardists established *spina-christi* grafting orchards in which selected genotypes with superior edible fruit are grafted on *spina-christi* seedling. Despite the fact that the most documents on salt tolerance of plants reported the important role of rootstocks, for they represent the first part to control the uptake and translocation of nutrients and salts throughout the plant (Munns, 2002; Zrig et al., 2011), there are many reports that clarify the predominant role of scion genotype in plant growth and improvement of salt effect (Moya et al., 2002; Santa-cruz et al., 2002; Chen et al., 2003). Colla et al. (2010) expressed that grafting is an integrative mutual process and both scion and rootstock can influence growth and greater root to shoot ratio and lower accumulation of Na^+ and/or Cl^- in shoots than ungrafted or self-grafted. The proline accumulation has often been proposed as a valuable marker for the selection of salt tolerance genotypes (Zid and Grignon, 1991).

In relation to *spina-christi* 'Konar', despite its highly appreciated fruits in local markets and increasing the area under cultivations, there is limited information on physiological responses and the extent of salt tolerance of these grafted plants. The objective of this research was to study the effect of different levels of NaCl salinity on growth parameters and physiological responses of three selected local genotypes non-grafted wild type, 'Danehgird' and 'Danehboland' grafted on wild *spina-christi* seedlings.

Material and methods

One year-old grafted 'Konar' plants ('Danehgird' and 'Danehboland' cultivars were grafted on wild seedlings) and non-grafted seedlings were used in this experiment. The plants were transferred into 20 liter plastic pots without drainage and filled with a mixture of soil (laom soil) and perlite (1:1, V:V).

Field capacity of soil mixture was measured using cell pressure device (Santa Barbara Calif soil Equipment Co.) and accordingly, plants were irrigated once every three days. Commercial fertilizer NPK (20:20:20) including; iron (1 g l^{-1}), manganese (0.5 g l^{-1}), zinc (0.5 g l^{-1}), copper (0.04 g l^{-1}), boron (0.2 g l^{-1}), molybdenum (0.04 g l^{-1}) and calcium nitrate (0.5 g l^{-1}) was applied to pots with irrigation water each week.

The pots were placed into a plastic greenhouse with natural sunlight and temperature range 32± °C and 20± °C in the day and at night respectively. After 10 weeks when plants were well established, salinity treatments were applied. For preventing salinity shock, the salts were applied in three times with irrigation water. Treatments were 0 (0 dS m^{-1}), 3.2 (5 dS m^{-1}), 6.4(10 dS m^{-1}) and 12.8 (20 dS m^{-1}) g l^{-1} NaCl.

At the ends of experiment (after 16 weeks), the leaf number of all treatments were counted. Leaf area was measured by leaf area meter (AM 200, ADC Bio Scientific Ltd. Taiwan). All 8 plants in each replicate were individually harvested, the lateral roots were counted, stem and root length were measured with a ruler, then they were divided into stems, leaves and roots after recording their fresh weight, all plant organs were oven-dried at 80 °C for 48 h then weighed again.

Ion content

Dry-ashing of plant material was obtained at 500 °C for 6 hr. Sodium (Na^+) and potassium (K^+) contents of samples were measured after digestion process in 1 N nitric acid solution (HNO_3-), using the flame emission photometry. Chloride (Cl^-) content was analyzed by titration with 0.1N silver nitrate ($AgNO_3$) in the presence of potassium bichromate according to a modified colorimetric method of Mohr (Mathieu and Pieltain, 2003).

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Chlorophyll content

Fresh leaf samples were washed with deionized water prior to extraction to remove any surface contamination. One g leaf sample was squashed in 80% acetone using a pestle and mortar. The mixture was centrifuged at 4800 rpm for 20 min. The optical density of the supernant was measured at 663 and 645 nm wavelengths and chlorophyll content was calculated using the following equations.

Mg Chl g F.W. = [20.2 (OD645nm) + 8.02 (OD663nm)] × V/F.W. × 1000

Where V is final volume of solution (ml) and F.W. leaf fresh weight (mg).

Proline contents

To determine proline content of the leaves, 0.5 g plant material was homogenized in 10 ml of 3% aqueous solution of sulphosalicylic acid and the homogenate filtered through Whatman # 2 filter paper. Two ml of filterate was reacted with 2 ml acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 h at 100 °C and the reaction terminated in an ice bath. The reaction mixture was extracted with 4 ml toluene, mixed for 15~20 seconds. Chromophore containing toluene was aspirated from the aqueous phase and the absorbance was read at 520 nm in a spectrophotometer. The proline concentration (μ mole g^{-1} F.W.) was calculated based on a standard curve.

Soluble sugars content

Soluble sugars were extracted from 0.1 g fresh leaves in 5 ml of 80% ethanol placed in a water bath at 70 °C for 30 min. The insoluble remains were removed by centrifuging at 5000 g for 10 min. One ml of the resulted extract was mixed with 5ml of sulfuric acid and 1 ml of 5% phenol solution. The mixed solution was vortexed after cool down to room temperature. The absorbance was recorded at 490 nm using a spectrophotometer. The soluble sugar content of each sample was determined using a standard curve for glucose and expressed as mg glucose g^{-1} F.W. (McCready et al., 1950; DuBois et al., 1956).

Starch content

From previous section, the solid debris remaining in the centrifuge tube was washed, re-extracted and re-centrifuged four-times using 80% (V/V) ethanol. The starch content in the samples was determined colorimetrically using anthrone method (McCready et al., 1950). The absorbance was read at 630 nm in a digital spectrophotometer as described by López et al. (2002).

Statistical analysis

A factorial experiment 3 (two grafted and one non-grafted 'Konars') × 4 (4 levels of salinity) was arranged in complete randomized design (CRD) with 4 replicates and 2 plants in each replication. Data were subjected to analysis of variance using the SPSS software (ver. 13.0) SPSS Inc. Mean differences were determined by Tukeys tests at $p \leq 0.05$.

Results

The results showed that leaf area, stem and root length were significantly reduced by increasing salinity. However, the reduction in growth parameters varied according to cultivar and measured factor so that, in 20 dS m^{-1} NaCl, the highest stem reduction (58.1%) was observed in 'Danehboland' and lowest reduction (50.5%) in wild cultivar. In contrast, the highest root length reduction (78.6%) was observed in wild types and the lowest root reduction was observed in grafted 'Danehboland' by 44% (Tab. 1).

The effect of salinity on lateral root number, root fresh and dry weight showed a significant genotypic variation (Tab. 2). In non-saline condition, scions 'Danehboland' and 'Danehgird' promoted significantly lateral root number in comparison with control. The highest reduction in lateral root number was found in wild type rootstock (without grafting), which reached by 62.5% at 20 dS m^{-1} NaCl, whereas the lowest one was obtained in 'Danehboland' with 50% decreased when compared to control plants. Regardless of salt treatments, scion 'Danehboland' also produced more root fresh (15.4 g)

Tab. 1: Sodium chloride effect on leaf surface, stem and root length of non-grafted wild, grated 'Danehgird' and 'Danehboland' cultivars of Z. *spinachristi*.

Cultivar	NaCl dS m^{-1}	Leaf surface (mm^2)	Mean	Stem length (Cm)	Mean	Root length (Cm)	Mean
Non-grafted	0	14.0^{a-d}		80.6^{b}		19.8^{bc}	
Wild	5	13.2^{c-f}		60.1^{de}		11.4^{f}	
	10	12.6^{def}		50.0^{ef}		7.0^{g}	
	20	11.9^{f}		39.9^{fg}		4.2^{g}	
			12.9^{B}		57.6^{B}		10.6^{C}
Grafted	0	14.9^{ab}		83.0^{ab}		21.7^{ab}	
Danehgird	5	14.2^{abc}		64.3^{cd}		17.6^{cd}	
	10	13.3^{c-f}		50.1^{ef}		16.1^{de}	
	20	12.3ef		35.8^{g}		13.2^{ef}	
			13.7^{A}		58.3^{B}		17.1^{B}
Grafted	0	15.3^{a}		94.1^{a}		24.4^{a}	
Danehboland	5	14.2^{abc}		74.6^{bc}		19.8^{bc}	
	10	13.4^{b-e}		53.1^{de}		18.1^{cd}	
	20	12.4^{ef}		39.4^{fg}		13.9^{ef}	
			13.8^{A}		65.3^{A}		19.1^{A}

In each column, means followed by the same letter are not significantly different at $p \leq 0.05$, using Tukey's test. (n=8).

and dry weight (8.45 g) than scion 'Danehgird' (13.8 and 7.55 g respectively) and wild type (4.6 and 2.6 g respectively) (Tab. 2).
Leaf and stem fresh and dry weight were also significantly reduced by salinity treatments. However, this reduction is varied according to cultivar (Tab. 3). For example, in 20 dS m^{-1} NaCl wild type, 'Danehboland' and 'Danehgird' showed 77.5, 68 and 79% reduction in leaf dry weight respectively in comparison with control plants. Regardless of salinity levels, scion 'Danehboland' showed higher stem fresh (12.4 g) and dry weight (7.7 g) than scion 'Danehgird' (11 and 6.7 g respectively) and wild type (4.6 and 2.67 g).
Data showed that grafted cultivars had different behavior in Na^+ and K^+ uptake in comparison to non-grafted wild type. In all grafted and non-grafted plants, with increasing salinity in culture media the Na^+ concentrations in different organs (root, stem and leaf) increased, however, regardless of different level of salinity, the accumulation of Na^+ was significantly lower in all organs of 'Danehboland' and

Tab. 2: Sodium chloride effect on lateral root number, root fresh and dry weight of non-grafted wild, grafted 'Danehgird' and 'Danehboland' cultivars of *Z. spina-christi*.

Cultivar	NaCl dS m^{-1}	Lateral root number	Mean	Root F.W. (g)	Mean	Root D.W. (g)	Mean
Non-grafted	0	19.8^{d}		8.4^{d}		5.3^{de}	
Wild	5	16.2^{de}		5.4^{e}		3.1^{fg}	
	10	12.3^{e}		3.0^{f}		1.6^{gh}	
	20	7.4f		1.5^{f}		0.67^{h}	
			13.9^{C}		4.6^{C}		2.7^{B}
Grafted	0	31.7^{b}		20.7^{a}		11.8^{ab}	
Danehgird	5	25.8^{c}		15.9^{b}		8.6^{c}	
	10	19.9^{d}		11.7^{c}		6.1^{d}	
	20	13.2^{e}		6.9d^{e}		3.8^{ef}	
			22.6^{B}		13.8^{B}		7.6^{A}
Grafted	0	38.9^{a}		21.8^{a}		13.1^{a}	
Danehboland	5	32.4^{b}		17.3^{b}		10.7^{b}	
	10	26.2^{c}		12.1^{c}		6.2^{d}	
	20	18.9^{d}		7.9^{d}		3.8^{ef}	
			29.1^{A}		15.4^{A}		8.5^{A}

In each column, means followed by the same letter are not significantly different at $p \leq 0.05$, using Tukey's test. (n=8).

Tab. 3: Sodium chloride effect on leaf, stem fresh and dry weight of non-grafted wild, grafted 'Danehgird' and 'Danehboland' cultivars of *Z. spina-christi*.

Cultivar	NaCl dS m^{-1}	Leaf F.W. (g)	Mean	Leaf D.W. (g)	Mean	Stem F.W. (g)	Mean	Stem D.W. (g)	Mean
Non-grafted	0	6.2^{a}		3.4^{ab}		7.8^{ef}		4.7^{efg}	
Wild	5	3.3^{bcd}		1.9^{ed}		5.1^{gh}		2.9^{ghi}	
	10	1.8^{def}		$1.2^{c\text{-}f}$		3.5^{hi}		2.0^{hi}	
	20	1.2^{f}		0.7^{ef}		2.2i		1.1^{i}	
			3.1^{AB}		1.8^{A}		4.6^{C}		2.7^{C}
Grafted	0	4.0^{b}		2.3^{bc}		16.5^{b}		10.4^{b}	
Danehgird	5	$3.0^{b\text{-}e}$		$1.5^{c\text{-}f}$		11.2^{d}		7.4^{cd}	
	10	$2.3^{c\text{-}f}$		1.1^{def}		9.5^{de}		5.3^{ef}	
	20	1.5^{ef}		0.7^{ef}		6.8^{fg}		3.6^{fgh}	
			2.7^{B}		1.4^{B}		11.0^{B}		6.7^{B}
Grafted	0	6.8^{a}		4.1^{a}		19.1^{a}		13.3^{a}	
Danehboland	5	3.7^{bc}		1.9^{cde}		13.8^{c}		8.4^{c}	
	10	$2.3^{c\text{-}f}$		0.8^{def}		10.3^{d}		5.7^{de}	
	20	1.8^{def}		0.6^{f}		6.4^{fg}		3.5^{fgh}	
			3.6^{A}		1.8^{A}		12.4^{A}		7.7^{A}

In each column, means followed by the same letter are not significantly different at $p \leq 0.05$, using Tukey's test. (n=8).

'Danehgird' cultivars in comparison to wild type (Tab. 4, Tab. 5 and Tab. 6). For example, leaf Na^+ concentration in 'Danehboland' and 'Danehgird' was 11.4 and 12.4 mg g^{-1} D.W. respectively which was significantly lower than wild type (17.5 mg g^{-1} D.W.). In 5, 10 and 20 dS m^{-1} NaCl, leaves of all genotypes showed a significant decrease in K^+ concentration (Tab. 6). The same trend observed for accumulation of K^+ in stem, and root. Regardless of salt concentration, all organs (leaf, stem and root) of grafted cultivars maintained a higher ratio of K^+/Na^+ than wild type. (Tab. 4, Tab. 5 and Tab. 6).

With increasing salinity, the Cl^- content in all plant parts (leaf, stem and root) of grafted and non-grafted cultivars increased. But this accumulation is more pronounced in roots than stems and leaves

Tab. 4: Sodium chloride effect on root Na^+, K^+ (mg g^{-1} D.W.) and K^+/Na^+ ratio of non-grafted wild, grafted 'Danehgird' and 'Danehboland' cultivars of *Z. spina-christi*.

Cultivar	NaCl dS m^{-1}	Root Na^+ mg g^{-1} D.W.	Mean	Root K^+ mg g^{-1} D.W.	Mean	Root K^+/Na^+	Mean
Non-grafted	0	24.2^{de}		20.3^{cd}		0.8^{bcd}	
Wild	5	28.8^{c}		17.2^{ef}		0.6^{d}	
	10	34.3b		16.1^{fg}		0.5^{d}	
	20	37.9^{a}		14.7^{g}		0.4^{d}	
			31.3^{A}		17.1^{C}		0.6^{C}
Grafted	0	14.3^{g}		21.7^{bc}		1.5^{bc}	
Danehgird	5	18.1^{f}		18.7^{de}		1.0^{bcd}	
	10	22.4^{e}		17.3^{ef}		0.8^{cd}	
	20	25.4^{d}		15.3^{fg}		0.6^{d}	
			20.1^{B}		18.2^{B}		1.0^{B}
Grafted	0	9.0^{h}		30.1^{a}		3.5^{a}	
Danehboland	5	14.4^{g}		23.1^{b}		1.6^{b}	
	10	18.8^{f}		20.3^{cd}		1.1^{bcd}	
	20	22.9^{de}		16.4^{efg}		0.7^{cd}	
			16.3^{C}		22.5^{A}		1.7^{A}

In each column, means followed by the same letter are not significantly different at $p \leq 0.05$, using Tukey's test. (n=8).

Tab. 5: Sodium chloride effect on stem Na^+, K^+ (mg g^{-1} D.W.) and K^+/Na^+ ratio of non-grafted wild, grafted 'Danehgird' and 'Danehboland' cultivars of *Z. spina-christi*.

Cultivar	NaCl dS m^{-1}	Stem Na^+ mg g^{-1} D.W.	Mean	Stem K^+ mg g^{-1} D.W.	Mean	Stem K^+/Na^+	Mean
Non-grafted	0	15.2^{e}		28.8^{c}		1.9^{cd}	
Wild	5	21.7^{cd}		23.2^{d}		1.2^{def}	
	10	26.4^{b}		18.3^{e}		0.7^{ef}	
	20	31.0^{a}		15.6^{f}		0.5^{f}	
			23.6^{A}		21.5^{C}		1.1^{C}
Grafted	0	10.0^{fg}		31.6^{ab}		3.2^{b}	
Danehgird	5	15.4^{e}		27.7^{c}		1.8^{cde}	
	10	19.8^{d}		22.3^{d}		1.1^{def}	
	20	23.2^{c}		17.0^{ef}		0.7^{ef}	
			17.1^{B}		24.6^{B}		1.7^{B}
Grafted	0	7.7^{g}		34.0		4.5^{a}	
Danehboland	5	11.1^{f}		29.3^{bc}		2.7^{bc}	
	10	16.4^{e}		23.0^{d}		1.4^{def}	
	20	21.8^{d}		17.8^{ef}		0.8^{def}	
			14.2^{C}		26.0^{A}		2.4^{A}

In each column, means followed by the same letter are not significantly different at $p \leq 0.05$, using Tukey's test. (n=8).

(Tab. 7), which means the plants limited the translocation of Cl^- to aerial parts. In all cultivars, with increasing NaCl the amount of sugar increased and the amount of starch decreased and in all levels of salinity, 'Danehboland' leaves showed significantly higher sugar and starch contents in comparison with non-grafted wild type and grafted 'Danehgird' (Fig. 1). The leaf proline content increased with increasing the salinity in all cultivars and in contrast the amount of chlorophyll decreased. In all levels of salinity, scion-stock relationship had no effect on chlorophyll content in comparison with non-grafted wild type, but in 3.2 and 6.4 g l^{-1} NaCl scion 'Danehboland' cultivar showed higher proline concentration in comparison with others (Fig. 2).

Tab. 6: Sodium chloride effect on leaf Na^+, K^+ (mg g^{-1} D.W.) and K^+/Na^+ ratio of non-grafted wild, grafted 'Danehgird' and 'Danehboland' cultivars of *Z. spina-christi*.

Cultivar	NaCl dS m^{-1}	Leaf Na^+ mg g^{-1} D.W.	Mean	Leaf K^+ mg g^{-1} D.W.	Mean	Leaf K^+/Na^+	Mean
Non-grafted	0	12.3^{ef}		30.7^{b}		2.5^{cde}	
Wild	5	15.7^{cd}		25.7^{c}		1.7^{ef}	
	10	18.6^{b}		22.2^{de}		1.2^{ef}	
	20	23.6^{a}		18.1^{g}		0.8^{f}	
			17.5^{A}		24.2^{C}		1.5^{B}
Grafted	0	8.3^{gh}		35.6^{a}		4.3^{ab}	
Danehgird	5	10.1^{fg}		30.3^{b}		3.0^{bcd}	
	10	13.4^{de}		24.2^{cd}		1.8^{def}	
	20	17.7^{bc}		18.4^{fg}		1.1^{f}	
			12.4^{B}		27.1^{B}		2.5^{A}
Grafted	0	7.4^{h}		36.8^{a}		5.0^{a}	
Danehboland	5	9.1^{gh}		32.4^{b}		3.6^{abc}	
	10	12.4^{ef}		26.2^{c}		2.1^{def}	
	20	16.7^{bc}		21.2^{ef}		1.3^{ef}	
			11.4^{B}		29.2^{A}		3.0^{A}

In each column, means followed by the same letter are not significantly different at $p \leq 0.05$, using Tukey's test. (n=8).

Tab. 7: Sodium chloride effect on leaf, stem and root Cl^- (%) of non-grafted wild, grafted 'Danehgird' and 'Danehboland' cultivars of *Z. spina-christi*.

Cultivar	NaCl dS m^{-1}	Root Cl^- %	Mean	Stem Cl^- %	Mean	Leaf Cl^- %	Mean
Non-grafted	0	5.1^{h}		2.9^{h}		3.0^{g}	
Wild	5	7.6^{fg}		3.9^{gh}		4.4^{fg}	
	10	8.9^{ef}		6.7^{cde}		6.6^{de}	
	20	11.6^{cd}		8.1b^{c}		6.8^{de}	
			8.3^{B}		5.4^{C}		5.2^{C}
Grafted	0	5.5^{gh}		3.6^{h}		4.5^{fg}	
Danehgird	5	7.9f		5.4^{efg}		5.5^{ef}	
	10	9.1^{ef}		7.2^{bc}		7.2^{cd}	
	20	13.1^{c}		8.8^{b}		7.3^{bcd}	
			8.9^{B}		6.2^{B}		6.1^{B}
Grafted	0	10.2^{de}		4.4^{fgh}		7.1^{cd}	
Danehboland	5	12.9^{c}		5.8^{def}		8.5^{abc}	
	10	19.5^{b}		7.8^{bc}		8.9^{ab}	
	20	22.7^{a}		9.9^{a}		9.2^{a}	
			16.3^{A}		6.9^{A}		8.4^{A}

In each column, means followed by the same letter are not significantly different at $p \leq 0.05$, using Tukey's test. (n=8).

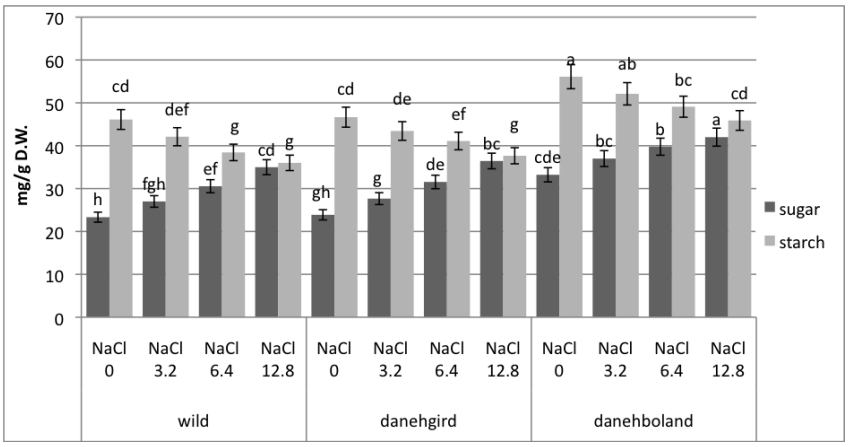

Fig. 1: Sodium chloride effect on leaf sugar, starch content mg g^{-1} D.W. of non-grafted wild, grafted 'Danehgird' and 'Danehboland' cultivars of *Z. spina-christi*. In each measured factor (sugar, starch), columns followed by the same letter are not significantly different at $p \leq 0.05$, using Tukey's test. The data are the means±SE (n=8).

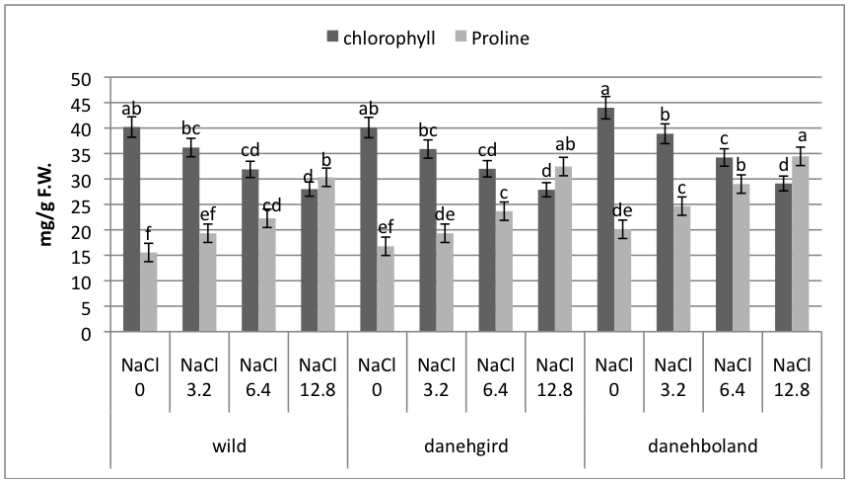

Fig. 2: Sodium chloride effect on leaf total chlorophyll (mg g^{-1} F.W.) and leaf proline content (μmole g^{-1} F.W.) of non-grafted wild, grafted 'Danehgird' and 'Danehboland' cultivars of *Z. spina-christi*. In each measured factor (chlorophyll, proline), means followed by the same letter are not significantly different at $p \leq 0.05$, using Tukey's test. The data are the means±SE (n=8).

Discussion

Salt stress resulted in a considerable decrease in leaf surface, shoot and root length, lateral root number, root, stem an leaf fresh and dry weight in both grafted cultivars and seedling rootstock of *spina-christi* 'Konar'. These reductions were predominant in seedling rootstock than in grafted cultivars (Tab. 1, Tab. 2 and Tab. 3), which means that different cultivars grafted on the same seedling rootstock improved the growth behavior of the root system. In respect to some traits such as lateral root number, shoot and root length, grafted 'Danehboland' cultivar even achieved better than grafted 'Danehgird'. Hence, the scion genotype played a major role in establishing the growth rate of grafted 'konars', which is in accordance with the results obtained in salt stress citrus (Banuls and Primo-Millo, 1995; Chen et al., 2003) and tomato (Santa-Cru, 2002). In grape vines, Sivritepe et al. (2010) explained the preference of the scion genotype by determining variation in leaf-levels physiological characters of grafted vines.

The most important parts of the plant exposed with soil-related stress factors such as salinity are root systems. The enhanced salt tolerance of grafted 'konar' cultivars may be associated with the root system. In fact, in non-saline condition, grafting significantly increased lateral root and root dry weight in comparison with non-grafted seedling rootstock. In salt stress condition, two of the above factors decreased, but the reduction in grafted plants was significantly smaller than in non-grafted seedlings. In tomatoes, He et al. (2009) also observed that root dry mass declined at high NaCl in comparison with non-saline conditions, but the decrease was smaller in rootstock-grafted plants.

In non-saline condition, the Na^+ ion content in root of the grafted 'konar' cultivars were significantly lower than Na^+ ion content in root of seedling rootstock, that means, scion genotypes limited the Na^+ uptake from root zone. In the same trend, in salt stress condition (5, 10, 20 dS m^{-1} NaCl) the accumulation on Na^+ in root of grafted plants were significantly less than non-grafted controls. In addition, Na^+ content in stems and leaves of rootstock seedlings and in grafted 'konar' cultivars were less than their roots in all levels of salt treatments conditions. The preservation of Na^+ in the roots of two grafted 'Konars' aid to decline its concentration in the leaves that may help to avoid affecting plant metabolism processes. In this respect, Tabatabaie (2010) previously reported similar results with olive tree cultivars subjected to high salt stress, where salt tolerance

in olive trees was hypothesized to be associated with the ability to reduce the uptake and/or transport of saline ions.

In both grafted cultivars and seedling rootstock, with increasing salinity, the leaf K^+/Na^+ ratio decreased, which can be attributed to a failure in the K^+/Na^+ selectivity mechanisms. In the leaves of two grafted cultivars the K^+/Na^+ ratio was recorded above 2 in all salt concentrations, except in 20 dS m^{-1} NaCl for grafted 'Danehboland' and in 10 dS m^{-1} and 20 dS m^{-1} in grafted 'Danehgird' (Tab. 6). A high K^+/Na^+ ratio in the leaves is often considered as a salt-tolerance marker (CHARTZOULAKIS et al., 2002; DASGAN et al., 2002; MEENA et al., 2003). So, scion genotypes had an ameliorating effect on K^+/Na^+ ratio which helps the grafted plants do better their metabolic functions.

The finding from chloride analysis showed that both grafted 'konars' and seedling rootstock exhibited a significant variation at all salinity levels and in all plant parts (leaf, stem and root). Grafted and ungrafted 'konar' plants limited the translocation of Cl^- to the aerial parts; which is an important mechanism to avoid the deleterious effects of salinity in plants (CHARTZOULAKIS, 2005), however, grafted 'Danehboland' cultivar accumulated significantly more Cl^- in their different parts than non-grafted seedlings. It is interesting to note that this grafted cultivar had higher root, stem and leaf fresh and dry weight and also accumulated more k^+ than non-grafted rootstock. It seems that this cultivar uses Cl^- to balance electrical charge and pH of their cells (ROUSSOS et al., 2006). K^+ is the major cation within plants which counter balance negative charge of anions and stabilizes the pH, osmotic potential and turgor pressure within cells. It also plays a crucial role in the activation of the enzyme involved in the metabolism (CHELLI-CHAABOUNI et al., 2010).

In the present study, a positive correlation was recorded between proline and NaCl concentration in the leaves of non-grafted wild seedlings and two grafted cultivars. The same trend was observed between soluble sugar and salt concentration. It has been shown that these compatible solutes play a major role in osmoregulation (HARE et al., 1998; PARIDA and DAS, 2005; REJSKOVÀ et al., 2007). In this regard, grafted 'Danehboland' achieved better than grafted 'Danehgird' and non-grafted wild type. The results are in agreement with those previously reported by HOKMABADI et al. (2005) on high accumulation of leaf proline in the most tolerant *P. vera* variety. In contrast, a negative correlation was recorded between NaCl concentration, starch and total chlorophyll. Since chlorophyll and proline are synthesized from glutamate pathway, the increase in the synthesis of proline in stress conditions resulted in declining of chlorophyll synthesis. In relation to leaf starch content, scion 'Danehboland' promoted higher starch accumulation, which confirms the importance role of scion in accumulation of starch in leaves of grafted cherry reported by GONCALVES et al., 2005.

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