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Investigation of the ameliorating effects of eggplant, datura, orange nightshade, local Iranian tobacco, and field tomato as rootstocks on alkali stress in tomato plants

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Abstract

Among the most important quality parameters of irrigation water used for greenhouse crops, alkalinity of water is considered critical due to its impact on soil or growing medium solution pH. In this study, plant growth, Fe content, photosynthetic pigment content, maximal quantum yield of PSII photochemistry (F_v/F_m), performance index (PI), leaf relative water content (LRWC), and soluble sugars concentration were investigated in nongrafted and grafted tomato (*Lycopersicon esculentum* Mill. cv. Red stone) plants onto five rootstocks of eggplant (*Solanum melongena* cv. Long purple), datura (*Datura patula*), orange nightshade (*Solanum luteum* Mill.), local Iranian tobacco (*Nicotiana tabacum*), and field tomato (*Lycopersicon esculentum* Mill. cv. Cal.jn3), exposed to 0, 5, and 10 mM $NaHCO_3$ concentrations, to determine whether grafting could improve alkalinity tolerance of tomato. Significant depression of leaf area, leaf and stem dry mass, shoot and root Fe content and LRWC under high $NaHCO_3$ level was observed in both grafted and ungrafted plants. The highest reduction in the shoot Fe content was observed at 10 mM sodium bicarbonate in control plants (greenhouse tomato). Moreover, at high HCO_3^- level, the highest percentage of LRWC reduction was also recorded in ungrafted plants. Values of F_v/F_m and PI decreased significantly at 5 and 10 mM $NaHCO_3$ irrespective of rootstock type. The present study revealed that soluble sugars content, photosynthetic pigments content, F_v/F_m and PI values in plants grafted onto datura rootstock were higher than those in nongrafted and rest of the grafted plants. Thus, the use of datura rootstock could provide a useful tool to improve alkalinity tolerance of tomato plants under $NaHCO_3$ stress.

Additional key words: chlorophyll fluorescence; grafting; *Lycopersicon esculentum*; $NaHCO_3$; performance index.

Introduction

The increase in urban population is imposing restrictions on the use of water of good quality for irrigation of cultivated plants (Carter *et al*. 2005). Water quality can determine the crops that can or cannot be grown, the methods for irrigation, and the requirement of water treatments. Among the most important quality parameters, alkalinity of water is considered critical due to its impact on soil or growing medium solution pH (Petersen 1996). Bicarbonate is the main ion that causes alkalinity and imparts buffer capacity to water, and at concentrations higher than 2 mM it can cause a significant suppression in plant growth of sensitive species due to the increase in water pH (Valdez-Aguilar and Reed 2010). The most conspicuous symptom of excessive alkalinity is the induction of interveinal chlorosis in the youngest leaves and stunted growth (Valdez-Aguilar and Reed 2007). Alkalinity-induced leaf chlorosis has been attributed to an iron (Fe) deficiency due to decreased Fe uptake (Bertoni *et al*. 1992) and/or Fe availability (Roosta 2011). Fe deficiency reflects upon the physiology and biochemistry of the whole plant, as Fe is an important cofactor of many enzymes, including those involved in the biosynthetic pathway of chlorophylls (Marschner 1995). Fe deficiency decreases the leaf photosynthetic rate (Terry 1980) by reducing the number of photosynthetic units per area (Spiller and Terry 1980) and by lowering the actual photosystem II (PSII) efficiency of the remaining units (Morales *et al*. 1998). On the other hand, PSII is well known for its sensitivity to abiotic stresses and hence it is a good choice to study response and adaptation to stress

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Abbreviations: Car – carotenoids; Chl – chlorophyll; DM – dry mass; F_v/F_m – maximal quantum yield of PSII photochemistry; FM – fresh mass; LRWC – leaf relative water content; PI – performance index; PS – photosystem.
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by plants (Strasser *et al.* 2000). Environmental stresses that affect PSII efficiency lead to a characteristic decrease in F_v/F_m (Krause and Weis 1991). The F_v/F_m is a measurement of the light energy transfer in dark-adapted samples or the photochemical quantum yield of open PSII centers (De Ell and Toivonen 2003).

Alkali stress has complex effects on root physiology (Wang *et al.* 2012). High alkalinity can cause the loss of the normal physiological functions of the roots, destruction of the root cell structure, inhibit the absorption of ions such as Cl^-, NO_3^-, and $H_2PO_4^-$, thus greatly affecting the metabolism of K^+ and Na^+ and disrupt metabolism homeostasis (Chen *et al.* 2011). Organic acid secretion from the root has a crucial role in alkali tolerance of plants (Yang *et al.* 2010). One way to avoid or reduce losses in production caused by alkalinity in high yielding genotypes would be to graft them onto rootstocks capable of reducing the detrimental effect of external pH on the shoot (Colla *et al.* 2010a).

Nowadays, grafting is used to reduce infections by soil-born pathogens and to enhance the tolerance against abiotic stresses (Schwarz *et al.* 2010). Among those are saline soils (Colla *et al.* 2010b), soil-pH (alkalinity) stress, nutrient deficiency, and toxicity of heavy metals (Savvas *et al.* 2010). In relation to alkalinity tolerance, Colla *et al.* (2010a) suggested that grafting provided an alternative way to improve watermelon alkalinity tolerance. Nevertheless, no published data is available concerning the effects of high alkalinity in the rooting medium on agronomical, physiological and biochemical responses of grafted tomato.

The purpose of this investigation is to study the effect of different rootstocks on the greenhouse-tomato tolerance to alkalinity in hydroponic system, interpreted by evaluating some vegetative parameters, photosynthetic pigments content, F_v/F_m, PI, LRWC, soluble sugars, and Fe content in grafted tomato plants.

Materials and methods

Plants, treatments, and growth conditions: A greenhouse experiment was conducted in 2010 at the Agricollege of Vali-e-Asr University of Rafsanjan (30°23′06″N, 55°55′30″E), at 1,523 m a.s.l. To ensure similar stem diameters at the grafting time, one week before the planting of the greenhouse-tomato hybrid *Lycopersicon esculentum* Mill. cv. Red stone as a scion plant, seeds of five rootstocks of tomato were sown 5 mm deep in a circular arrangement in each bucket containing perlite medium (particles diameter of 1–2 mm). The five rootstocks used in this study were eggplant (*Solanum melongena*. cv. Long purple), datura (*Datura patula*), orange nightshade (*Solanum luteum* Mill.), local Iranian tobacco (*Nicotiana tabacum*), and field tomato (*Lycopersicon esculentum* Mill. cv. Cal.jn3) which is commonly planted in dry and saline areas of south Iran. Grafting was performed when seedlings have developed 3–4 true leaves using the touch splice and hole insertion grafting methods, while ungrafted greenhouse tomato was used as a control. Then the seedlings were transplanted into 4-L plastic containers, containing aerated nutrient solution. The basic nutrient solution used in experiment was modified Hoagland and Arnon formulation (Hoagland and Arnon 1950). This nutrient solution consisted of: 1 mM $Ca(NO_3)_2$, 1.5 mM KNO_3, 0.25 mM KH_2PO_4, 0.5 mM $MgSO_4$, 0.1 mM NaCl, 20 µM Fe-EDDHA, 7 µM $MnSO_4$, 0.7 µM $ZnCl_2$, 0.8 µM $CuSO_4$, 2 µM H_3BO_3, and 0.8 µM Na_2MoO_4. The experiment was arranged as a factorial in the framework of a completely randomized design with two factors, six grafting combination (*i.e.*, grafted or ungrafted plants) and bicarbonate (0, 5, and 10 mM $NaHCO_3$) with 3 replications consisting of 12 plants per treatment. Solutions were changed completely every week in the first 2 weeks and subsequently every 4th day. Solutions were prepared 24 h before use to allow pH stabilization. pH were recorded before renewal. Average initial pH was 7, 7.75, and 8.10 for solutions containing 0, 5, and 10 mM $NaHCO_3$, respectively. The plants were grown in a greenhouse with 13-h light phase (26 ± 3ºC) and 11-h dark phase (22 ± 3ºC). Greenhouse temperature was controlled by using cool air from central cooler. The relative humidity was 52.4–63.2%. The photosynthetically active radiation was 500 µmol m^{-2} s^{-1}.

Plant growth measurement: At the end of the experiment the shoot length, leaf area and the number of leaves produced for each treatment were recorded. Leaf area (LA) was measured with an electronic area meter (*Delta-T Devices Ltd.,* Cambridge, UK). Six weeks after transplanting, the plant organs (roots, leaves, and stem) were harvested, weighed and oven-dried (48 h at 72ºC) for determination of leaf, stem and root dry mass.

Fe analysis: Dried samples of roots and shoots were weighed separately and ground to pass a 40-mesh sieve. The ground plant samples were dry-ashed at 500ºC for 4 h; the ashes dissolved in 10 ml HCl (2N) and made the volume to 100 ml with distilled water. Content of Fe was determined by atomic absorption spectrometry (*model GBC, AVANTA,* Australia).

LRWC and soluble sugars content measurement: The fully expanded fourth leaf from the top was used for measuring LRWC as described by Weatherley (1950) and calculated according to the formula: LRWC = [(FM – DM)/(FM at full turgor – DM)] × 100, where FM is fresh mass and DM dry mass. The content of soluble sugars in leaves was measured according to the method of Irigoyen *et al.* (1992).

Chlorophyll (Chl) and carotenoids (Car) contents: Chl *a*, Chl *b*, total Chl, and Car were extracted with 80% aqueous acetone (v/v) and were quantified using of Arnon (1949) method. After filtering, absorbance of centrifuged extracts was measured at 480, 510, 645, 652, and 663 nm using a spectrophotometer (*U-2000, Hitachi Instruments,* Tokyo, Japan).

Chl fluorescence parameters measurement: F_v/F_m and PI parameters were recorded by using a portable pocket *Plant Efficiency Analyzer* (*PEA, Hansatech Instruments Ltd.*, Norfolk, UK). The pocket *PEA* optical interface was mounted directly on to the front of the pocket *PEA* control unit. It consisted of a single high intensity focused LED which was positioned vertically above the sample and provided up to 3,500 µmol m^{-2} s^{-1} intensity with a peak wavelength of 627 nm at the sample surface. Three leaves were selected from each pot and preadapted to dark period for 20 min by fixing special tags on each leaf blade before measurements were taken. During dark adaptation, all the reaction centers were fully oxidized and available for photochemistry and any fluorescence yield was quenched. After 20 min of dark adaptation, the sensor cup was fitted on the leaf for measurement. The vitality state of the tomato plants was characterized with the performance index PI (Strasser *et al.* 2000).

Statistical analysis of variance and correlation were performed by using the *SAS* software (*SAS Institute,* Cary, NC, USA), if *ANOVA* determined that the effects of the treatments were significant (P<0.05 for F-test), then the treatment means were separated by LSD test.

Results

Growth: The results obtained from this experiment showed that the stem dry mass was significantly affected by $NaHCO_3$, rootstock, with no significant $NaHCO_3$ × rootstock interaction; whereas shoot length, leaf number, leaf area, and leaf and root dry mass were highly influenced by $NaHCO_3$, rootstock and their interaction (Table 1). As shown in Fig. 1, stem dry mass decreased dramatically with increasing $NaHCO_3$ concentration in solution. The highest stem dry mass was observed in control plants (Fig. 2*A*). Whereas, even low bicarbonate concentration (5 mM) decreased leaf number, leaf area, leaf dry mass and root dry mass in control plants, but had no effect on

Table 1. Interactive effects of $NaHCO_3$ levels and different rootstocks on shoot length, leaf number, leaf area and leaf and root dry mass (DM) of tomato plants. Values are means ± SE of three replicates (n = 12). *Different letters* indicate significant differences according to LSD test (P<0.05). ** –P<0.01; * – P<0.05.

Rootstock	$NaHCO_3$ [mM]	Shoot length [cm]	Leaf number [$plant^{-1}$]	Leaf area [cm^2 $leaf^{-1}$]	Leaf DM [g $plant^{-1}$]	Root DM [g $plant^{-1}$]
control	0	71.13 ± 3.25^{a}	11.72 ± 0.98^{a}	92.93 ± 5.29^{a}	2.88 ± 0.20^{a}	1.14 ± 0.07^{a}
	5	68.37 ± 2.99^{a}	8.91 ± 0.46^{bcd}	71.09 ± 3.95^{b}	2.04 ± 0.30^{bc}	0.71 ± 0.09$^{c–f}$
	10	56.66 ± 0.36^{b}	7.23 ± 0.39^{efg}	48.25 ± 2.27^{c}	2.01 ± 0.09^{bc}	0.67 ± 0.03^{def}
Field tomato	0	41.41 ± 0.41^{cd}	9.16 ± 0.88^{bcd}	63.82 ± 6.73^{b}	2.12 ± 0.30^{b}	0.78 ± 0.06$^{b–e}$
	5	35.88 ± 1.44^{def}	8.25 ± 0.25^{def}	51.00 ± 1.66^{c}	1.71 ± 0.09$^{b–e}$	0.59 ± 0.11^{efg}
	10	34.76 ± 0.73^{def}	8.16 ± 0.16^{def}	46.15 ± 1.13^{cd}	1.16 ± 0.14^{fgh}	0.38 ± 0.10^{ghi}
Datura	0	23.76 ± 2.63^{gh}	9.83 ± 0.16^{b}	51.42 ± 0.94^{c}	1.83 ± 0.11^{bcd}	0.61 ± 0.13^{ef}
	5	23.37 ± 3.45^{gh}	8.80 ± 0.15^{bcd}	47.16 ± 2.05^{cd}	1.67 ± 0.15$^{b–e}$	0.57 ± 0.01^{fg}
	10	23.17 ± 2.31^{gh}	8.33 ± 0.20^{cde}	39.41 ± 2.89^{de}	1.40 ± 0.09^{def}	0.50 ± 0.02^{fgh}
Orange nightshade	0	33.16 ± 1.74^{ef}	9.66 ± 0.88^{bc}	32.41 ± 1.97^{efg}	1.65 ± 0.12^{cde}	0.98 ± 0.05^{ab}
	5	29.66 ± 0.88^{fg}	6.50 ± 0.5^{gih}	27.86 ± 0.50^{fgh}	1.16 ± 0.03^{fgh}	0.89 ± 0.07^{bc}
	10	22.66 ± 3.28^{gh}	5.36 ± 0.31^{i}	25.16 ± 0.72^{gh}	0.97 ± 0.06^{fgh}	0.54 ± 0.01^{fgh}
Tobacco	0	44.94 ± 2.23^{c}	12.77 ± 0.22^{a}	66.85 ± 2.56^{b}	2.60 ± 0.08^{a}	0.83 ± 0.08^{bcd}
	5	38.88 ± 3.46^{cde}	8.38 ± 0.45^{cde}	47.07 ± 3.35^{de}	1.33 ± 0.18^{efg}	0.58 ± 0.09^{efg}
	10	21.20 ± 3.03^{h}	7.00 ± 0.57$^{e–h}$	23.37 ± 0.69^{h}	1.00 ± 0.20^{fgh}	0.34 ± 0.02^{igh}
Eggplant	0	32.74 ± 2.08^{ef}	6.90 ± 0.1^{fgh}	33.94 ± 1.80^{ef}	1.10 ± 0.15^{fgh}	0.21 ± 0.04^{ij}
	5	23.07 ± 4.03^{gh}	6.00 ± 0.28^{gih}	27.75 ± 3.07^{fgh}	0.88 ± 0.16^{gh}	0.21 ± 0.02^{ij}
	10	20.75 ± 0/90^{h}	5.73 ± 0.37^{ih}	25.22 ± 0.36^{gh}	0.72 ± 0.03^{h}	0.15 ± 0.02^{j}
ANOVA	DF	Mean square				
Rootstock	5	2137.60**	14.94**	2281.58**	2.004**	0.484**
$NaHCO_3$	2	583.15**	44.33**	2239.25**	3.179**	0.478**
Rootstock × $NaHCO_3$	10	61.70**	3.41**	228.37**	0.200*	0.038*

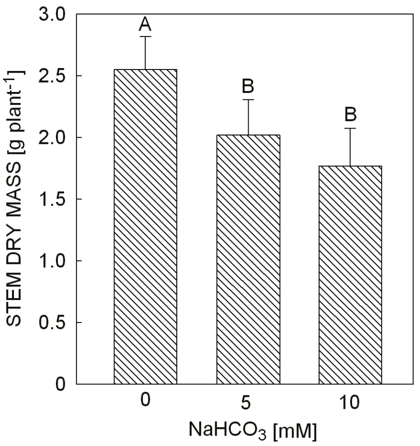

Fig. 1. Effects of $NaHCO_3$ concentrations (0, 5, and 10 mM) in the nutrient solution on stem dry mass of tomato plants. The results are the means ± SE of three replicates (n = 12). *Different letters* indicate significant differences according to LSD test (P<0.05).

shoot length (Table 1). At high concentration (10 mM) of bicarbonate in control plants shoot length also decreased (5 mM) decreased leaf number, leaf area, leaf dry mass and root dry mass in control plants, but had no effect on shoot length (Table 1). At high concentration (10 mM) of bicarbonate in control plants shoot length also decreased significantly. In the plants grafted on datura rootstock, measured vegetative traits were not significantly affected by bicarbonate application (Table 1). Considering to tolerance of rootstocks to bicarbonate, field tomato was in the second order after datura rootstocks. 10 mM $NaHCO_3$ treatment caused a significant decrease in shoot length, leaf number, leaf area, leaf and root dry mass of ungrafted and grafted onto tobacco rootstock plants (Table 1). The root-to-shoot ratio was significantly affected by rootstock, but not by $NaHCO_3$ and their interaction. The highest root-to-shoot ratio was measured in the plants grafted onto orange nightshade rootstock. However the lowest values of root-to-shoot ratio were recorded on those grafted onto eggplant and the ungrafted plants (Fig. 2*B*).

Fe content: The shoot Fe content was significantly influenced by $NaHCO_3$, rootstock and $NaHCO_3$ × rootstock interaction, but its content in roots was significantly affected by rootstock and $NaHCO_3$ × rootstock interaction, without significant effects caused by $NaHCO_3$ (Table 2). Under normal growth condition, the content of shoot Fe of grafted plants onto field tomato rootstock was significantly higher than those of nongrafted and grafted onto other rootstocks. The Fe content in shoots of tomato plants decreased significantly as $NaHCO_3$ levels increased. The highest reduction in the shoot Fe content was observed in control plants (greenhouse tomato) less than 10 mM $NaHCO_3$. Also at the same stress level, plants grafted onto datura rootstocks showed the lowest

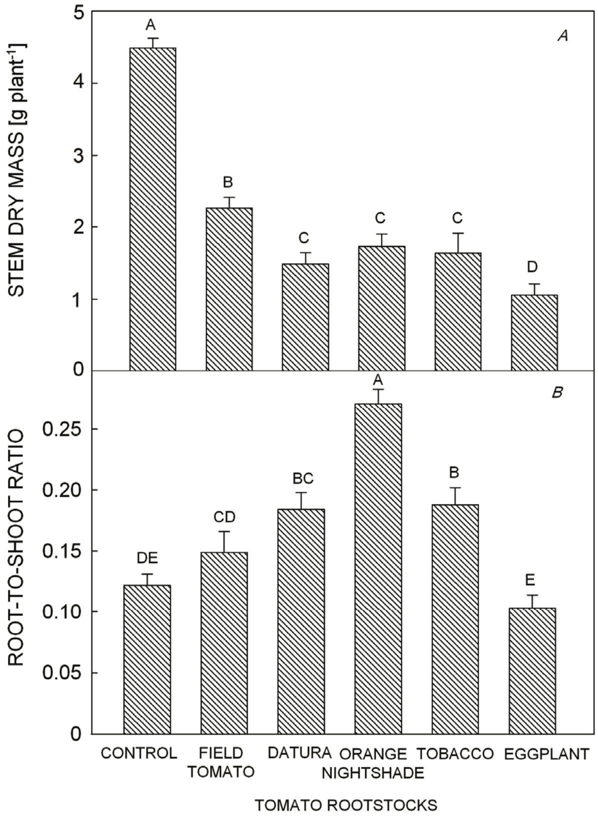

Fig. 2. The effect of different rootstocks on stem dry mass (*A*) and root- to-shoot ratio (*B*) of tomato plants, the results are the means ± SE of three replicates (n = 12). *Different letters* indicate significant differences according to LSD test (P<0.05).

reduction in the shoot Fe content compared to unstressed plants.

Data presented in Table 2 revealed that under high alkalinity level (10 mM $NaHCO_3$), the content of Fe in roots was significantly increased in plants grafted onto datura rootstock compared to unstressed plants, whereas a significant decrease in Fe content was recorded in the control plants (greenhouse tomato).

LRWC and soluble sugars content: LRWC was significantly (P<0.01) affected by $NaHCO_3$, rootstock, and $NaHCO_3$ × rootstock interaction (Table 2). With the exception of orange nightshade and field tomato in the other rootstocks an obvious decrease of LRWC was observed with 5 mM $NaHCO_3$ compared with plants grown under unstressed conditions (Table 2).

When plants were treated with 10 mM $NaHCO_3$, LRWC decreased significantly in all plants compared with plants grown under unstressed conditions, as the highest rate of decline (77.12%) can be observed in control plants (greenhouse tomato). Finally, with high $NaHCO_3$ in the nutrient solution, in comparison to control, the plants were grafted onto field tomato and datura rootstocks, showed the lower reduction of LRWC than those grafted onto other rootstocks and the ungrafted plants.

Table 2. Interactive effects of $NaHCO_3$ levels and different rootstocks on Fe content (in shoots and roots), leaf relative water content (LRWC), and chlorophyll *a* (Chl *a*) of tomato plants. DM – dry mass, FM – fresh mass. Values are means ± SE of three replicates. *Different letters* indicate significant differences according to LSD test ($P<0.05$). ** – $P<0.01$, * – $P<0.05$; ns – not significant.

Rootstock	$NaHCO_3$ [mM]	Shoot Fe [mg kg^{-1}(DM)]	Root Fe [mg kg^{-1}(DM)]	LRWC [%]	Chl *a* [mg g^{-1}(FM)]
Control	0	58.86 ± 5.88^{b}	481.0 ± 28.4^{abc}	64.28 ± 2.19^{a}	1.372 ± 0.125^{a}
	5	22.53 ± 1.41^{ik}	$375.8 \pm 40.2^{b–g}$	23.60 ± 0.68^{h}	1.096 ± 0.044^{def}
	10	16.84 ± 0.64^{k}	317.7 ± 60.5^{efg}	14.70 ± 0.73^{i}	0.997 ± 0.001^{f}
Field tomato	0	76.23 ± 3.05^{a}	$456.2 \pm 65.6^{a–e}$	60.16 ± 0.81^{ab}	$1.219 \pm 0.006^{b–e}$
	5	45.23 ± 0.92^{ef}	499.2 ± 101.7^{ab}	56.47 ± 0.82^{bc}	$1.215 \pm 0.018^{b–e}$
	10	38.11 ± 1.62^{gh}	$472.4 \pm 44.5^{a–d}$	53.93 ± 0.78^{c}	$1.214 \pm 0.023^{b–e}$
Datura	0	54.63 ± 1.53^{bc}	$327.1 \pm 31.7^{d–g}$	32.26 ± 0.88^{f}	1.287 ± 0.054^{ab}
	5	46.58 ± 1.88^{de}	296.0 ± 31.6^{fg}	27.28 ± 2.06^{gh}	1.288 ± 0.056^{ab}
	10	42.33 ± 1.45^{efg}	542.1 ± 349.9^{a}	26.94 ± 0.89^{gh}	1.241 ± 0.036^{abc}
Orange nightshade	0	39.70 ± 0.05^{fgh}	$470.6 \pm 36.9^{a–d}$	56.12 ± 0.86^{bc}	$1.208 \pm 0.035^{b–e}$
	5	26.76 ± 1.16^{j}	$397.6 \pm 31.7^{a–f}$	52.93 ± 3.35^{c}	$1.133 \pm 0.049^{c–f}$
	10	16.93 ± 1.49^{k}	$347.5 \pm 31.8^{c–g}$	45.70 ± 1.13^{d}	$1.116 \pm 0.050^{c–f}$
Tobacco	0	51.83 ± 2.71^{cd}	$414.3 \pm 56.8^{a–f}$	41.72 ± 0.34^{d}	$1.225 \pm 0.021^{a–d}$
	5	33.50 ± 1.90^{hi}	303.9 ± 37.8^{fg}	37.35 ± 2.29^{e}	1.054 ± 0.072^{f}
	10	25.48 ± 3.67^{j}	275.2 ± 28.2^{fg}	32.06 ± 1.04^{f}	0.998 ± 0.027^{f}
Eggplant	0	36.76 ± 1.89^{gh}	257.9 ± 63.9^{g}	41.95 ± 0.84^{d}	$1.220 \pm 0.001^{b–e}$
	5	27.64 ± 1.29^{ij}	$333.3 \pm 26.7^{c–g}$	31.73 ± 1.06^{f}	1.083 ± 0.067^{ef}
	10	25.40 ± 0.73^{j}	$354.0 \pm 23.4^{b–g}$	28.19 ± 2.10^{fg}	1.025 ± 0.013^{f}
ANOVA	DF	Mean square			
Rootstock	5	945.8^{**}	$29,730.5^{**}$	$1,117.9^{**}$	0.040^{**}
$NaHCO_3$	2	$3,179.3^{**}$	$5,062.8^{ns}$	$1,191.8^{**}$	0.116^{**}
Rootstock × $NaHCO_3$	10	154.9^{**}	$21,224.7^{*}$	253.8^{**}	0.015^{*}

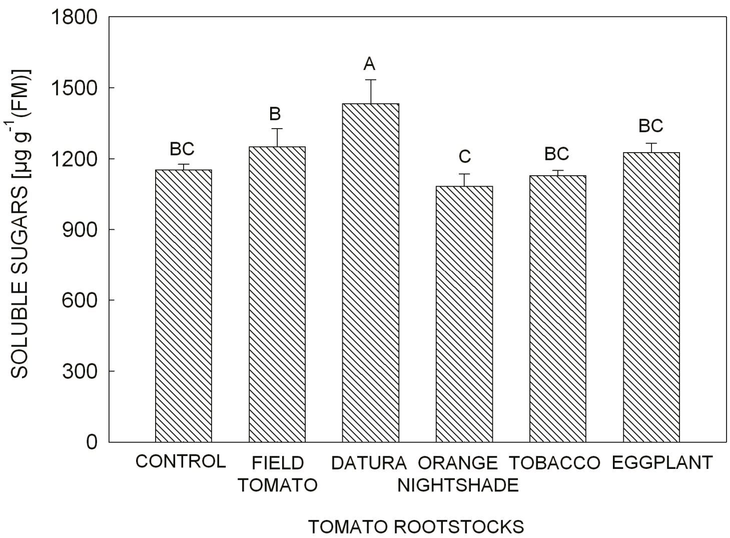

Fig. 3. The effect of different rootstocks on soluble sugars content of tomato plants, the results are the means ± SE of three replicates (n = 12). *Different letters* indicate significant differences according to LSD test ($P<0.05$).

Results showed that the soluble sugars content was significantly influenced by rootstock with values recorded for plants grafted onto datura rootstock (1,432.71 µg g^{-1}) being higher than plants grafted onto other rootstocks and ungrafted plants (1,151.46 µg g^{-1}) (Fig. 3).

Chl and Car contents: The Chl (*a*, *b*, and total) contents were significantly affected by $NaHCO_3$, rootstock had no significant impact on Chl *b* content, but had a significant effect on Chl *a*, total Chl and Car contents. Additionally, the Chl *a* content was also significantly affected by $NaHCO_3$ × rootstock interaction (Table 2). Results showed that sodium bicarbonate had no significant effect on Chl *a* content in plants grafted onto field tomato, datura and orange nightshade rootstocks. $NaHCO_3$ treatment of 5 mM decreased Chl *a* content in control and

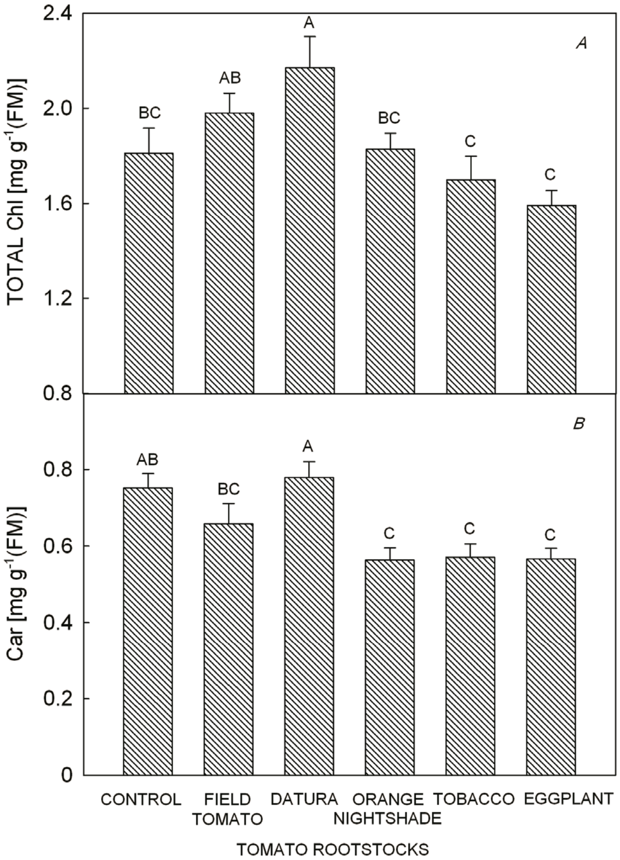

Fig. 4. The effect of different rootstocks on total chlorophyll (Chl) (*A*) and carotenoids (Car) contents (*B*) of tomato plants, the results are the means ± SE of three replicates (*n* = 12). *Different letters* indicate significant differences according to LSD test (*P*<0.05).

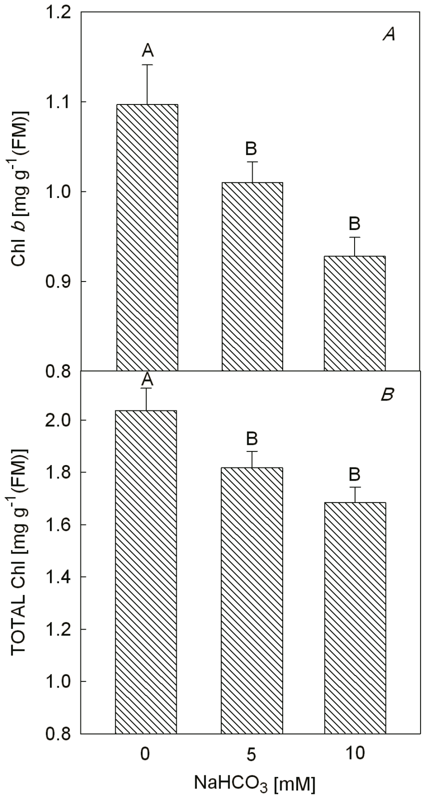

Fig. 5. Effects of $NaHCO_3$ concentrations (0, 5, and 10 mM) in the nutrient solution on chlorophyll (Chl) *b* (*A*) and total Chl (*B*) contents of tomato plants. The results are the mean ± SE of three replicates (*n* = 12). *Different letters* indicate significant differences according to LSD test (*P*<0.05).

plants grafted onto tobacco rootstocks. Meanwhile, compared to the plants grown under unstressed conditions, 10 mM $NaHCO_3$ significantly decreased Chl *a* contents in ungrafted plants and grafted onto tobacco and eggplant rootstocks (Table 2). The highest total Chl and Car contents were recorded in the plants grafted onto datura rootstock (Fig. 4*A*,*B*). Regarding to the effect of sodium bicarbonate on Chl *b* and total Chl content, increasing the concentration of $NaHCO_3$ from 0 to 10 mM in the nutrient solution decreased the Chl *b* and total Chl content in leaves significantly, however, differences between two levels of $NaHCO_3$ (5 and 10 mM) were not significant (Fig. 5*A*,*B*).

F_v/F_m and PI were significantly (*P*<0.01) affected by $NaHCO_3$ and rootstock, but not by their interaction. The highest F_v/F_m and PI values were observed in plants grafted onto datura rootstock (Fig. 6*A*,*B*). The lowest F_v/F_m values were observed in greenhouse tomato (control) plants. As shown in Fig. 6*B*, the lowest PI value was observed in plants grafted onto orange nightshade rootstock. Values of F_v/F_m and PI decreased significantly at 5 and 10 mM $NaHCO_3$ irrespective of rootstock type (Fig. 6*C*,*D*).

Correlation coefficients analysis: The correlations between shoot Fe concentration and all the photosynthetic pigments were significant with the exception of Car contents (Table 3); additionally the correlations between Fe shoot and the fluorescence indices were very significant. Total Chl content showed significant correlation with fluorescence indices and shoot Fe content. Higher correlations were found between F_v/F_m and shoot Fe content.

Discussion

Researches have indicated that plants respond to elevated $NaHCO_3$ concentrations in soil or in growing medium solution with decreased shoot and root growth (Campbell and Nishio 2000). This could be due to either HCO_3^- or Na^+ (Pearce *et al*. 1999). Tomato, petunia (Bailey and Hammer 1986), tobacco transplants (Rideout *et al*. 1995), watermelon (Colla *et al*. 2010a) and lettuce (Roosta 2011), exhibited stunted growth when growing in either soil or nutrient solution containing a high concentration of HCO_3^-. Many of the test data show high pH as a key factor in limiting plant growth and development under alkaline conditions (Yang *et al*. 2007, 2008a,b; 2009a). In the present experiment, significant depression in plant growth parameters in bicarbonate-treated tomato plants was observed, and that effect varied as a function of rootstock (Table 1). 10 mM $NaHCO_3$ treatment compared to nonstress conditions caused a significant decrease in shoot length, leaf number, leaf area, leaf and root dry mass of ungrafted and grafted tomato onto tobacco rootstock (Table 1). These phenomena may result from nutritional damage, ion imbalance, and metabolic disorders caused by alkali stress (Yang *et al*. 2009a). Moreover, a high pH may lead to the lack of protons, the destruction or inhibition of transmembrane electrochemical-potential gradients in root cells, and the loss of normal physiological root functions such as ion absorption (Yang *et al*. 2008a).

It is generally regarded that underground stresses usually lead to increasing root/shoot ratio of biomass (Szaniawski 1987), allowing the plant to have a greater root surface area for absorption of water and nutrients (Xiong *et al*. 2002). With the exception of eggplant rootstock, the tomato plants grafted onto datura, orange nightshade, field tomato, and tobacco rootstocks had

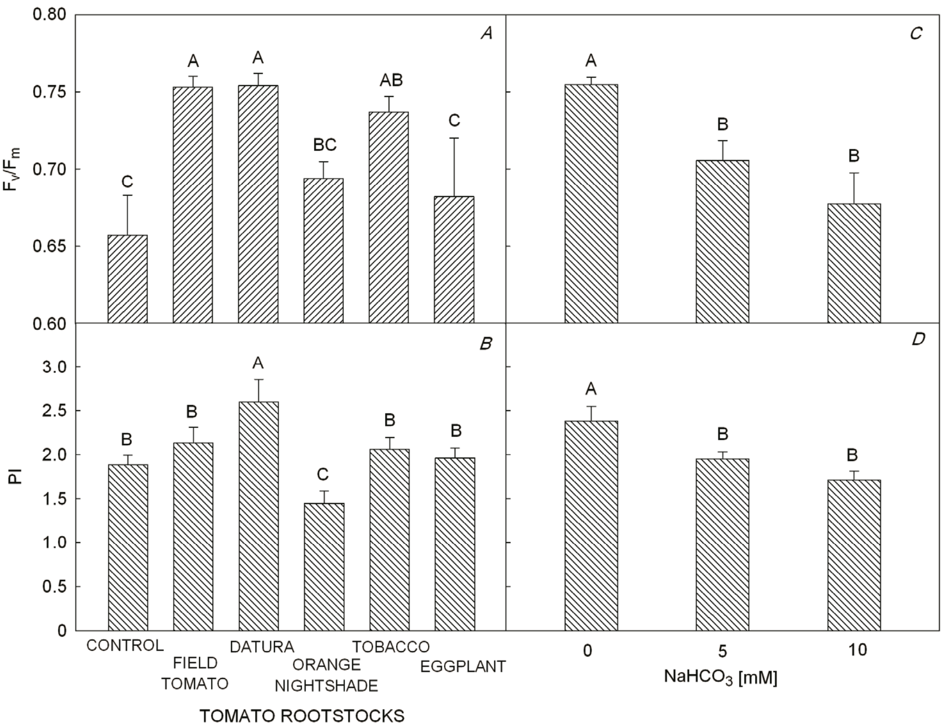

Fig. 6. Effect of the different rootstocks (*A*,*B*) and $NaHCO_3$ concentrations (*C*,*D*) on maximal quantum yield of PSII photochemistry (F_v/F_m) and performance index (PI) of tomato plants. The results are the means ± SE of three replicates (*n* = 12). *Different letters* indicate significant differences according to LSD test (*P*<0.05).

Table 3. Correlation coefficients analysis in tomato plants between leaf relative water content (LRWC), performance index (PI), leaf pigments (Car – carotenoids, Chl – chlorophyll), soluble sugars, maximal quantum yield of PSII photochemistry (F_v/F_m) and Fe content in shoot and root. *** – *P*<0.001, ** – *P*<0.01, * – *P*<0.05; ns – not significant.

	RWC	PI	Car	Total Chl	Chl *b*	Chl *a*	Soluble sugars	F_v/F_m	Root Fe
Shoot Fe	0.506^{***}	0.568^{***}	0.154^{ns}	0.559^{***}	0.365^{**}	0.583^{***}	0.074^{ns}	0.601^{***}	0.359^{**}
Root Fe	0.385^{**}	0.074^{ns}	0.184^{ns}	0.165^{ns}	0.070^{ns}	0.337^{*}	0.337^{*}	0.074^{ns}	
F_v/F_m	0.411^{**}	0.528^{***}	0.258^{ns}	0.435^{**}	0.302^{*}	0.477^{***}	0.182^{ns}		
Soluble sugars	-0.149^{ns}	0.206^{ns}	0.595^{***}	0.200^{ns}	0.209^{ns}	0.256^{ns}			
Chl *a*	0.447^{***}	0.351^{**}	0.328^{*}	0.526^{***}	0.256^{ns}				
Chl *b*	0.249^{ns}	0.306^{*}	0.358^{**}	0.460^{***}					
Total Chl	0.325^{*}	0.567^{***}	0.429^{**}						
Car	-0.108^{ns}	0.363^{**}							
PI	-0.007^{ns}								

higher values of root to shoot ratio than those ungrafted (Fig. 2). These findings concur with the results of the experiment done by Huang *et al*. (2009a) in cucumber plants. Therefore, the better growth performance of grafted- in comparison to ungrafted tomato plants exposed to alkalinity stress might be attributed, at least to some extent, to differential root growth under alkalinity stress.

The results demonstrated that the alkali tolerance of tomato plants can be improved by grafting onto datura rootstock, for this reason we have observed that the alkalinity has no significant effect on shoot length and leaf and root dry mass of these plants. Earlier findings showed that grafting of watermelon onto pumpkins rootstocks may enhance alkalinity tolerance (Colla *et al*. 2010a). Alkalinity tolerance of tomato plants grafted onto datura rootstock was due to the better uptake and translocation of Fe to the shoot. Alkalinity reduces the solubility of Fe due to the high pH associated with the consumption of H^+ by HCO_3^- (Valdez-Aguilar 2004), so that under these conditions the range of inorganic Fe availability is around 0.1–10% of the normal requirement for optimal plant growth (Römheld and Marschner 1986). Fe deficiency depresses the synthesis of chlorophyll, which results in the decrease of photosynthetic products affecting plant growth (Álvarez-Fernández *et al*. 2005). The content of Fe in shoots of ungrafted and grafted tomato plants was significantly decreased by $NaHCO_3$ treatment. The highest reduction in the shoot Fe content was observed in control plants (greenhouse tomato) under 10 mM sodium bicarbonate. On the other hand, at the same stress level, plants grafted onto datura rootstocks showed the lowest reduction in the shoot Fe content compared to unstressed plants (Table 2). The higher uptake and accumulation of Fe in tomato plants grafted onto datura rootstock was the main mechanism that reduces the detrimental effect of alkalinity (Fe-deficiency) on plant growth.

Regardless of rootstocks, roots of tomato plants accumulated larger amounts of Fe than shoots (Table 3), suggesting that the critical process leading to chlorosis in alkaline soils is Fe translocation from the root into the shoot, which can be impaired by the alkaline apoplastic pH due to high bicarbonate concentration (Colla *et al*. 2010a).

LRWC was used as a measure to estimate the stress response (Jain and Chattopadhyay 2010). During stress conditions plants need to maintain internal water potential below that of soil and maintain turgor and water uptake for growth (Ahmad and Sharma 2008). Lowering of osmotic potential by osmolyte accumulation in response to stress improves the capacity of the cell to maintain its turgor pressure at low water potential. This appears to be essential for physiological processes such as photosynthesis, enzyme activity and cell expansion (Claussen 2005). Reduction in LRWC indicates a loss of turgor that resulted in limited water availability for cell extension process (Katerji *et al*. 1997). High alkalinity (10 mM $NaHCO_3$) treatment induced significant decreases in LRWC in the stressed plants compared with those in the control plants (Table 2). Similar results were obtained by Yang *et al*. (2011). Under alkali stress, organic acids might play an important role in maintaining ion balance of cotton (Chen *et al*. 2011). In the present experiment, under 10 mM $NaHCO_3$ treatment, grafted plants onto field tomato and datura rootstocks had the lowest reduction in RWC of leaves in comparison with plants grown in normal conditions. Less reduction in LRWC at the tolerating rootstocks could be due to sufficient osmotic adjustment (*e.g.* organic acids) in plant under stress conditions. Therefore, less LRWC reduction in datura and field tomato leaves results in more tolerance of these rootstocks to alkalinity stress (Balaguer *et al*. 2002).

Parida and Das (2005) reported that lower osmotic potential allows leaves to withstand a greater evaporative demand without loss of turgor. This requires an increase in osmotically active solutes either through uptake of inorganic ions or synthesis of metabolically compatible solutes (Munns and Tester 2008). Soluble sugars are considered to be a compatible solute and their major functions are osmoprotection, osmotic adjustment, carbon storage and radical scavenging (Qun *et al*. 2010, Huang *et al*. 2009b). In the present study, plants grafted onto datura rootstock had higher soluble sugars content in the leaves compared with other plants (Fig. 3). In accordance with the present result, other researchers also reported that tomato plants grafted onto *S. lycopersicum* have higher soluble sugar content than self-rooted plants under NaCl stress (Chen *et al*. 2005). Benefits of accumulation of soluble sugars mentioned above might be part of the reason for the increased alkalinity tolerance of tomato plants grafted onto datura rootstock.

Chl and Car are the main photosynthetic pigments of higher plants. In green plants Fe and Chl concentrations are often well correlated (Miller *et al*. 1982). Similarly, in the present study the correlation between Fe concentration in shoots and Chl content quite evident (Table 3). The solubility of Fe is known to decrease with increase in pH and bicarbonate content, which are inter-related through pH-buffering by equilibrium between H_2CO_3, HCO_3^-, and CO_3^{2-} (Bloom 2000). Thus, under Fe deficiency conditions, the reduction in leaf Fe concentration is often accompanied by a marked reduction of Chl levels (Dasgan *et al*. 2003), by a significant, although less intense, decrease in the Chl fluorescence (Nedunchezhian *et al*. 1997), and by a reduction in photosynthesis (Marschner 1995). At alkalinity stress, the contents of Chl and Car in the barley plants decreased sharply with increased stress in comparison to salinity stress. These results indicate that high pH might decrease contents of photosynthetic pigments (Yang *et al*. 2009b). Alkalinity-induced leaf chlorosis has been attributed to a Fe deficiency due to decreased Fe uptake (Bertoni *et al*. 1992) and/or Fe availability (Roosta 2011). Therefore, the bicarbonate ions interfere with the uptake and transport of Fe by tomato plants (Table 2), Chl content of plants decreased as shown in Table 2 and Fig. 4*A*,*B* was not unexpected (Gogorcena *et al*. 2004).

In accordance with our result in the datura rootstock (Table 2, Fig. 5*A*), Pestana *et al*. (2005) reported that the 'Troyer' citrange rootstock was more effective in overcoming the effects of the presence of bicarbonate since these plants accumulated a greater amount of Fe and Chl in the shoots.

According to the present result with the effect of different rootstocks on Car content of tomato plants (Fig. 5*B*), an increase in Car due to grafting was observed in two tomato cultivars grafted onto a tomato hybrid rootstock, under both nonsaline and saline conditions (Fernandez-Garcia *et al*. 2004). Although the effects of rootstock on Chl and Car levels have not been amply discussed in relevant resources, the increase in the level of Chl and Car in tomato due to datura rootstock by means of their effects on photosynthesis and consequently on other characteristics of the scion is most probably one important result obtained in this study regarding grafted tomato plants.

In the present study values of F_v/F_m decreased significantly at 5 and 10 mM $NaHCO_3$ irrespective of rootstock type (Fig. 6*C*), suggesting the occurrence of photoinhibition, and this could be a consequence of damage to PSII (Demmig-Adams and Adams 1992). The highest F_v/F_m values were observed in plants grafted onto datura and field tomato rootstocks, which was attributed to higher Chl *a* content in these rootstocks (Redondo-Gómez *et al*. 2007). Values of F_v/F_m below 0.80 were recorded for the plants of all treatments. This suggests that photosynthetic apparatus was not fully developed or slightly injured, which could occur in plants cultivated under greenhouse conditions (Klamkowski *et al*. 2009). In this experiment, significant correlation was observed between F_v/F_m values and Fe content in shoots of tomato plants (Table 3). Moreover, plants grafted onto both rootstocks (datura and field tomato) had more Fe content than those grafted onto other rootstocks and ungrafted plants (Table 2). PSI and PSII complexes are both Fe-containing proteins, Fe in PSII is important for water splitting (Hulsebosch *et al*. 1996). Bertamini *et al*. (2001) proved that the significant decrease in photosynthetic electron transport is mainly due to the loss of PSII activity in Fe-deficient grapevine leaves, and the loss of PSII activity is due to the loss of D1 protein and 33 kDa protein of the water-splitting complex. Our results strongly proved this conclusion. But this might not be the only factor limiting the electron transport in PSII.

PI is a sensitive indicator of photosynthesis (Strasser *et al.* 2000). On the other hand, PI is a more complex parameter reflecting overall efficiency of light absorption as well as both light- and dark redox reactions (Strauss *et al*. 2006). Therefore, PI is a potential indicator of current physiological status of a plant, reflecting both disturbance and acclimation of photosynthetic apparatus by changing environmental conditions (Clark *et al*. 2000). Moreover, PI is found to be a very sensitive parameter in different crops and in most of environmental stresses (Strasser *et al*. 2000, Jiang *et al*. 2006), which is in accordance with our results achieved on grafted and ungrafted tomato plants under alkalinity stress. In this experiment, PI value decreased significantly in response to increase of $NaHCO_3$ concentration in tomato plants (Fig. 6*D*). Deng *et al*. (2010) concluded that the performance index (PI) gradually decreased with increasing of salinity-alkalinity, so that under severe salinity-alkalinity stress in comparison to control PI significantly decreased. They also stated that nonstomatal limitation, *i.e*. decreased photosynthetic activity in PSII plays an important role in decreased photosynthetic rate at high salinity-alkalinity. The nonstomatal factors mainly depend on the cumulative effects of leaf water and osmotic potential, biochemical constituents (Sultana *et al*. 1999), contents of photosynthetic pigments (Yang *et al*. 2008a), ion toxicities in the cytosol (James *et al*. 2006), *etc*. We can conclude that reduction of photosynthetic pigments under $NaHCO_3$ treatments might be the part of the reason for PI reduction, which was confirmed by the results of correlation analysis. Significant correlations were observed between PI and total Chl content (Table 3). Our results showed that the tomato plants with datura as rootstock exhibited a higher value for total Chl content and greater PI than other plants (Figs. 4*A*, 6*B*). The derived PI illustrated the enhanced vitality of tomato plants with grafting onto datura rootstock.

In conclusion, this study showed that plants grafted onto datura rootstock exposed to excessive external $NaHCO_3$ level were capable of maintaining better vegetative growth, strong capacity to accumulate Fe in the aerial part, and the lower reduction of LRWC in comparison to those grafted onto other rootstocks and the ungrafted plants. Moreover, the present study revealed that soluble sugars content, photosynthetic pigments content, F_v/F_m, and PI values in plants grafted onto datura rootstock were higher than those in nongrafted and other rootstocks grafted plants. Overall, the use of datura rootstock could provide a useful tool to improve alkalinity tolerance of tomato plants under $NaHCO_3$ stress.

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