DOI: 10.1007/s11099-014-0053-6 PHOTOSYNTHETICA 52 (3): 421-429, 2014

Response of two tomato cultivars to field-applied proline under irrigation with saline water: Growth, chlorophyll fluorescence and nutritional aspects

B. KAHLAOUI[*,+], M. HACHICHA[*], S. REJEB[*], M.N. REJEB[*], B. HANCHI[**], and E. MISLE[***]

National Research Institute of Rural Engeneering, Waters and Forestry (INRGREF), 17 rue Hédi Karray, BP n°10 Ariana 2080, Tunisia[*]
Faculty of Sciences, Tunis, University Campus, 1060 Tunis, Tunisia[**]
Faculty of Agricultural Sciences and Forestry, Universidad Católica del Maule, Casilla 7AD, Curicó, Chile[***]

Abstract

The response of tomato (*Solanum lycopersicum* L.) to abiotic stress has been widely investigated. Recent physiological studies focus on the use of osmoprotectants to ameliorate stress damage, but experiments at a field level are scarce. Two tomato cultivars were used for an experiment with saline water (6.57 dS m^{-1}) and subsurface drip irrigation (SDI) in a silty clay soil. Rio Grande is a salinity-tolerant cultivar, while Heinz-2274 is the salt-sensitive cultivar. Exogenous application of proline was done by foliar spray at two concentrations (10 and 20 mg L^{-1}) during the flowering stage. Control plants were treated with saline water without proline. Proline at the lower concentration (10 mg L^{-1}) increased dry mass of different plant organs (leaves, stems, and roots) and it improved various chlorophyll *a* fluorescence parameters compared with controls. Regarding mineral nutrition, K^+ and P were higher in different organs, while low accumulation of Na^+ occurred. However, Mg^{2+} was very high in all tissues of Rio Grande at the higher concentration of proline applied. Thus, the foliar spray of proline at 10 mg L^{-1} increased the tolerance of both cultivars. The growth of aboveground biomass of Heinz-2274 was enhanced by 63.5%, while Rio Grande improved only by 38.9%.

Additional key words: chlorophyll fluorescence; foliar pulverization; proline; salt tolerance; *Solanum lycopersicum*; Tunisia.

Introduction

Salinity is considered a significant factor affecting crop production and agricultural sustainability in arid and semiarid regions of the world, reducing the value and productivity of the affected land (Khadri *et al*. 2007). The identification of tolerant genotypes that may sustain a reasonable yield in salt-affected soils has been a strategy adopted by scientists to overcome salinity (Kingsbury and Epstein 1984).

Salt tolerance in plants is a complex phenomenon that involve morphological and developmental changes as well as physiological and biochemical processes. Two components have been identified as the probable cause of salt toxicity: osmotic stress and ion toxicity. The osmotic stress is associated to reduced cell wall extension and cell expansion leading to cessation of growth. The ionic effect includes interference with the transport of essential ions within the plant and lowered net photosynthetic rates in the affected plants (Greenway and Munns 1980, Khadri *et al*. 2007).

The accumulation of compatible solutes may help to maintain relatively high water contents necessary for plant growth and cellular function. Plants respond to salt stress

Received 27 November 2013, *accepted* 7 January 2014.
[+]Corresponding autor; e-mail: besma.kahlaoui@gmail.com
Abbreviations: Chl – chlorophyll; CK – control; DM – dry mass; ECw – electrical conductivity of water; F_m – maximal fluorescence in the dark-adapted leaves; F_m' – maximal fluorescence in the light-adapted leaves; F_0 – minimal fluorescence in the dark-adapted leaves; F_s – steady-state fluorescence; F_v – maximal variable fluorescence in the dark-adapted leaves; F_v' – maximal variable fluorescence in the light-adapted leaves; F_v/F_m – maximal efficiency of PSII photochemistry; F_v'/F_m' – efficiency of excitation energy capture by open PSII reaction centers; NPQ – nonphotochemical quenching; Φ_{PSII} – the quantum yield of PSII electron transport; PQ-pool – plastoquinone pool; Pro – proline; Pro10 – proline concentration of 10 mg L^{-1}; Pro20 – proline concentration of 20 mg L^{-1}; SAR – sodium adsorption ratio; ROS – reactive oxygen species; SC – salt-sensitive cultivar; SDI – subsurface drip irrigation; TC – salt-tolerant cultivar.
Acknowledgements: The study was carried out under the INRGREF/ACSAD project for saline water use in agriculture and PISEAUII project for saline water use in center of Tunisia.

by accumulating certain specific metabolites, the most conspicuous being amino acids in general, and proline (Pro) in particular. The content of free Pro has been reported to increase when plants were growing under saline stress (Lutts *et al*. 1999, Lin *et al*. 2002). It has been proposed that Pro accumulation can serve as an adaptive mechanism to salt stress in higher plants (Kumar *et al*. 2003, Nounjan *et al*. 2012). The physiological effect of Pro accumulation may be expressed in sustained photosynthesis, osmoregulation, and/or prevention of protein (including enzyme) degradation (Rajasekaran *et al*. 1997). Pro can also serve as an easily available source of nitrogen and carbon during the recovery from stress (Hellmann *et al*. 2000). The Pro accumulation in plants depends on species (Nounjan *et al*. 2012). Some authors did not observe any appreciable increase in free Pro contents (Kumar *et al*. 2003) whilst others consider enhanced content of Pro merely a stress effect, rather than a cause of stress tolerance (Kumar *et al*. 2003). In contrast, Zhu *et al.* (1998) demonstrated that the Pro overproduction enhanced root biomass and flower development in transgenic tobacco under water-stress conditions.

Biological approach to crop management for reducing salinity effect in agriculture includes the identification of plant mechanisms for salt tolerance and the selection and breeding of new cultivars and it represents one of the two essential strategies besides soil improvement (Poustini *et al*. 2004). In addition, great effort have been made to search other strategies to generate improved tolerance to salt stress in plants. Exogenous osmoprotectants have been reported for their osmoprotective role in plant response to abiotic stress and they have been suggested as an alternative approach to improve crop productivity under saline conditions (Nakayama *et al*. 2005, Nounjan *et al*. 2012). The role and mechanisms of action of exogenous osmoprotectants are complex and remain controversial. The beneficial effects of external supply of osmoprotectants vary depending on various conditions, including plant species, developmental stages, and the severity and duration of salt stress. The effectiveness of the osmoprotectants also depends on whether they are applied prior to or during stress, on methods of application, and their concentration (Nounjan *et al.* 2012). In leaf tissues of tomato, Pro does not accumulate to sufficient concentration to contribute significantly to osmotic adjustment (Aziz *et al*. 1999).

The aim of this work was to evaluate the exogenous appplication of Pro in tomato. Specifically, the objective was to test two tomato cultivars of contrasting tolerance to salinity under field conditions. Plants were irrigated with saline water (6.57 dS m^{-1}) and sprayed with Pro at 10 and 20 mg L^{-1} considering growth, chlorophyll fluorescence and nutritional aspects.

Materials and methods

Plant material: The experiment was carried out during the summer 2008 (from May to the end of August). Two tomato cultivars (*Solanum lycopersicum* L.) have been used: the salt-tolerant cultivar (TC), Rio Grande (Kahlaoui *et al.* 2011a, 2011b), and the salt-sensitive cultivar (SC), Heinz-2274 (Kahlaoui *et al.* 2012). Seeds were provided by the Laboratory of Seeds and Plant Control of the General Direction of Protection and Control of the Agricultural Production and Quality, Tunisia.

Experimental design: The experiment was located at the Cherfech Agricultural Experimental Station located 25 km north of Tunis in the Low Valley of Mejerda River. Climate of the region is Mediterranean with an annual rainfall close to 470 mm and an average yearly evapotranspiration of 1,370 mm (Penman method). The soil is a silty clay with about 40% clay, 50% silt, and 10% sand. The organic matter is about 1% and total $CaCO_3$ is about 40%. The soil pH is around 7–8 and no fertilizer was used in our experiment. The experiment was set using emitters with filters and drip lines buried at 30-cm depth as subsurface drip irrigation (SDI). Transplanting date was 2 May 2008. Plants were grown in single lines. The plants with 5–7 leaves were used. Tomato plants were spaced 1 m between rows and 0.4 m between plants. This experiment was carried out according to a randomized design with two factors (cultivar and Pro concentration). Each treatment was replicated three times and each replicate had 10 plants (30 plants per treatment for both cultivars). Treatments were two exogenous applications of Pro for each cultivar:

Pro10	10 mg(proline) L^{-1}
Pro20	20 mg(proline) L^{-1}
CK	0 mg(proline) L^{-1}

Pro spraying was performed six times from 30% anthesis at June, using 1 L per plant. In each treatment, four plants/replications were used in statistical analysis.

Irrigation water: The irrigation water came from a well with ECw = 6.57 dS m^{-1} and SAR = 11. Water chemical characteristics are described in Table 1. The total water quantity used for the whole irrigation cycle was about 700 mm (7,000 m^3 ha^{-1}).

Determination of growth: Plants were harvested and separated into roots, stems, and leaves. The dry mass (DM) was determined after drying samples at 80°C during 48 h until constant mass.

Chlorophyll (Chl) fluorescence measurements: Chl fluorescence emission from the upper surface of leaves of intact plants was measured by a modulated fluorimeter (*Mini PAM*, *Walz*, Effeltrich, Germany). The minimal (F_0)

Table 1. Characteristics of the irrigation water used. EC – electrical conductivity; SAR – sodium adsorption ratio.

pH	EC	Ionic composition [meq L^{-1}]							SAR
	[dS m^{-1}]	HCO_3^-	SO_4^{2-}	Cl^-	Ca^{2+}	Mg^{2+}	K^+	Na^+	
7.8	6.57	4.2	24.1	37.1	14.7	10.2	1.1	41.2	11

and maximal fluorescence (F_m) emissions were assessed in leaves after 30 min of dark adaptation and the maximum quantum efficiency of PSII photochemistry was calculated as $F_v/F_m = (F_m - F_0)/F_m$ (F_v – maximum variable Chl fluorescence yields in the dark-adapted state). Then, the leaves were continuously illuminated with a white actinic light (1,500 W) , which was equivalent to the actual growth light in order to measure steady-state fluorescence in the light-adapted state (F_s) and maximal fluorescence level in the light-adapted leaves (F_m'). The intrinsic efficiency of open PSII (or efficiency of excitation energy capture by open PSII reaction centres) was calculated as F_v'/F_m' (F_v' – maximum variable Chl fluorescence yields in the light-adapted state) (Genty *et al.* 1989, Harbinson *et al.* 1989).

Proline content was estimated by the method of Bates *et al*. (1973). The plant material was homogenized in 3% (w/v) aqueous sulfosalicylic acid and the homogenate was centrifuged at 720 × *g* for 10 min. The supernatant was used for the estimation of Pro content. The reaction mixture consisted of 1 ml of 140 mM acid ninhydrin and 1 ml of glacial acetic acid, which was boiled at 100°C for 1 h. After termination of the reaction in ice bath, the reaction mixture was extracted with 2 ml of toluene, and the absorbance read at 520 nm by spectrophotometer (*Spectro 2000 RS, Labomed-inc*, USA). Pro content was expressed as mg g^{-1}(DM).

Analysis of Na^+, Mg^{2+}, K^+, and P in plant tissues: Leaves, petioles, stems, and roots were used for chemical analyses. Organic ions were extracted from dry matter by HNO_3 at room temperature for 48 h. K^+ and Na^+ were analyzed by flame emission using an spectrophotometer (*Jenway PFP7*, *Jenway LTD-Essex CM63LB*, England). Mg^{2+} was determined by atomic absorption spectrophotometry (*Model 2380*, *Perkin Elmer*, USA). Phosphorus was estimated by the chloro-stannous molybdophosphoric blue colour method (Gericke and Kurmies 1952) where 5 ml of extract solution is mixed with 5 ml of reagent nitrovanadomolybdate and then completed with distilled water. After waiting 1 h, samples were passed to the photoelectric colorimeter (*Model AE-11, Erma optical works LTD*, Japan).

Data analyses: Statistical processing was performed by the software *STATISTICA*, *Version 5* (*Statsoft* France, 1997). All the recorded parameters were subjected to an analysis of variance with two factors (cultivars and concentration of Pro). Mean comparisons were carried out by the LSD test at the significance level of 0.05.

Results

Growth parameters: DM of all plant organs of the SC (leaves, stems, and roots) was significantly lower compared with the TC (Table 2). The exogenous application of Pro had a significant effect on both cultivars compared to the CT. The low concentration of Pro10 increased DM in both cultivars, particularly in SC. At Pro20, this parameter decreased significantly in leaves of the TC (Table 2).

Proline content was higher in leaves of the TC than in the leaves of the SC cultivar and in the CT (Fig. 1). The exogenous application of Pro caused the Pro accumulation in leaves of both cultivars. The higher accumulation of Pro occurred in leaves of both cultivars after the application of Pro at the lower concentration.

Chl fluorescence: F_0 was higher in SC than in the TC but lower compared with the CT (Table 3). F_0 decreased after the treatment with Pro10 in both cultivars, particularly in SC. In F_m, a significant increase at the lower Pro concentration was found in both cultivars (Table 3). The increase at Pro20 was observed in both cultivars of tomato, reaching 6% increase. Both concentrations of Pro caused a significant increase of F_v/F_m, F_v/F_0, and F_v'/F_m' in both cultivars compared with the CT (Table 3).

Ion content: According to our results, Na^+ content was higher in shoots (leaves, petioles, and stems) in TC and in the roots of SC in the CT treatment. At both concentrations of Pro used, the Na^+ content was lower in all organs of the TC plants (Fig. 2*A*). Regarding SC, the lower concentration of Pro led to a decrease in the accumulation of Na^+ in all organs. The exogenous application of Pro20 exhibited a significant effect only in leaves (Fig. 2*B*). The Cl^- content was reduced significantly by Pro10 in both tomato cultivars. At Pro20, the effect of the exogenous application was significant in all organs of TC and in petioles and roots of SC (Kahlaoui *et al*. 2013).

Table 2. Effect of exogenous application of proline (Pro) on dry mass (DM) per plant of two tomato cultivars (Rio Grande and Heinz-2274) irrigated with saline water (6.57 dS m^{-1}). All values are the mean of three replications ($n = 4$). Mean ± SE with the *different letters* are significantly different at $P \leq 0.05$ according to LSD test. ns or ** – insignificant or significant differences at $P \leq 0.01$, respectively, according to the variance analysis. CK – control; Pro10 – 10 mg(Pro) L^{-1}; Pro20 – 20 mg(Pro) L^{-1}. C – cultivar.

Treatment	DM of leaves [g]	DM of stems [g]	DM of roots [g]
Rio Grande (TC)			
CK	32.56 ± 2.15^{c}	23.89 ± 1.64^{b}	16.02 ± 0.42^{b}
Pro10	42.42 ± 3.12^{e}	34.29 ± 1.69^{d}	23.92 ± 1.71^{c}
Pro20	28.78 ± 2.2^{b}	20.52 ± 0.76^{a}	15.27 ± 1.05^{b}
Heinz-2274 (SC)			
CK	23.29 ± 0.6^{a}	19.38 ± 0.8^{a}	11.85 ± 1.47^{a}
Pro10	36.51 ± 0.81^{d}	30.54 ± 3.22^{c}	22.07 ± 1.74^{c}
Pro20	24.50 ± 0.66^{a}	19.12 ± 1.08^{a}	14.75 ± 0.77^{b}
Effect of C	**	ns	ns
Effect of Pro	**	**	**
Interaction C × Pro	**	**	**

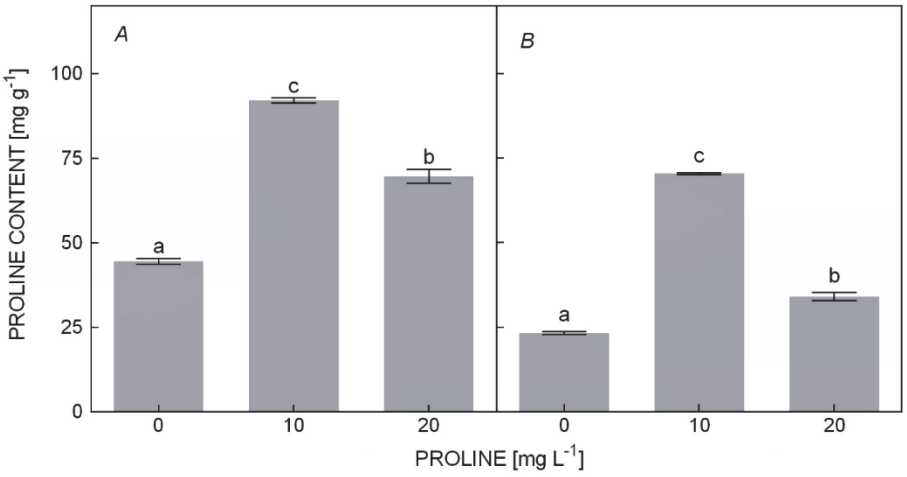

Fig. 1. Effect of the exogenous application of proline (Pro) on endogenous Pro content in leaves of two tomato cultivars (*A*: Rio Grande; *B*: Heinz-2274), irrigated by SDI and saline water (6.57 dS m^{-1}). Values represent the mean ± SE of four plants per assay and bars with *different letters* are significantly different ($P \leq 0.05$) according to the LSD test.

Table 3. Effect of exogenous application of proline (Pro) on fluorescence parameters F_0, F_m, F_v/F_m, F_v/F_0, and F_v'/F_m' of two tomato cultivars (Rio Grande and Heinz-2274) irrigated with saline water (6.57 dS m^{-1}). All values are the mean of three replications ($n = 4$). Mean ± SE with the *different letters* are significantly different at $P \leq 0.05$ according to LSD test. CK – control; Pro10 – 10 mg(Pro) L^{-1}; Pro20 – 20 mg(Pro) L^{-1}.

	F_0	F_m	F_v/F_m	F_v/F_0	F_v'/F_m'
Rio Grande					
CK	385 ± 10.84^{c}	$1{,}763 \pm 10.9^{b}$	0.781 ± 0.003^{c}	3.585 ± 0.067^{c}	0.691 ± 0.014^{b}
Pro10	325 ± 4.85^{a}	$1{,}887 \pm 8.84^{c}$	0.827 ± 0.002^{d}	4.809 ± 0.07^{e}	0.803 ± 0.002^{d}
Pro20	360.75 ± 12.5^{b}	$1{,}866 \pm 1.25^{c}$	0.806 ± 0.006^{d}	4.175 ± 0.183^{d}	0.732 ± 0.004^{c}
Heinz-2274					
CK	543 ± 18.8^{f}	$1{,}654 \pm 12.95^{a}$	0.671 ± 0.011^{a}	2.05 ± 0.109^{a}	0.658 ± 0.003^{a}
Pro10	415 ± 4.34^{d}	$1{,}877 \pm 15.25^{c}$	0.778 ± 0.01^{c}	3.53 ± 0.219^{c}	0.812 ± 0.004^{d}
Pro20	485 ± 9.64^{e}	$1{,}755 \pm 10.36^{b}$	0.723 ± 0.006^{b}	2.619 ± 0.08^{b}	0.722 ± 0.012^{c}

The K^+ content was higher in petioles and leaves of both tomato cultivars when they were irrigated with saline water (Fig. 2*C*,*D*). Pro had a significant effect on K^+ content in both cultivars. In TC compared with the CT, K^+ was greater in leaves and petioles after the treatment with both Pro concentrations. In SC, the exogenous application of Pro10 caused a significant increase of K^+ in all plant tissues with the exception of petioles. At Pro20, we noticed a significant reduction in K^+ in all organs when comparing with the CT.

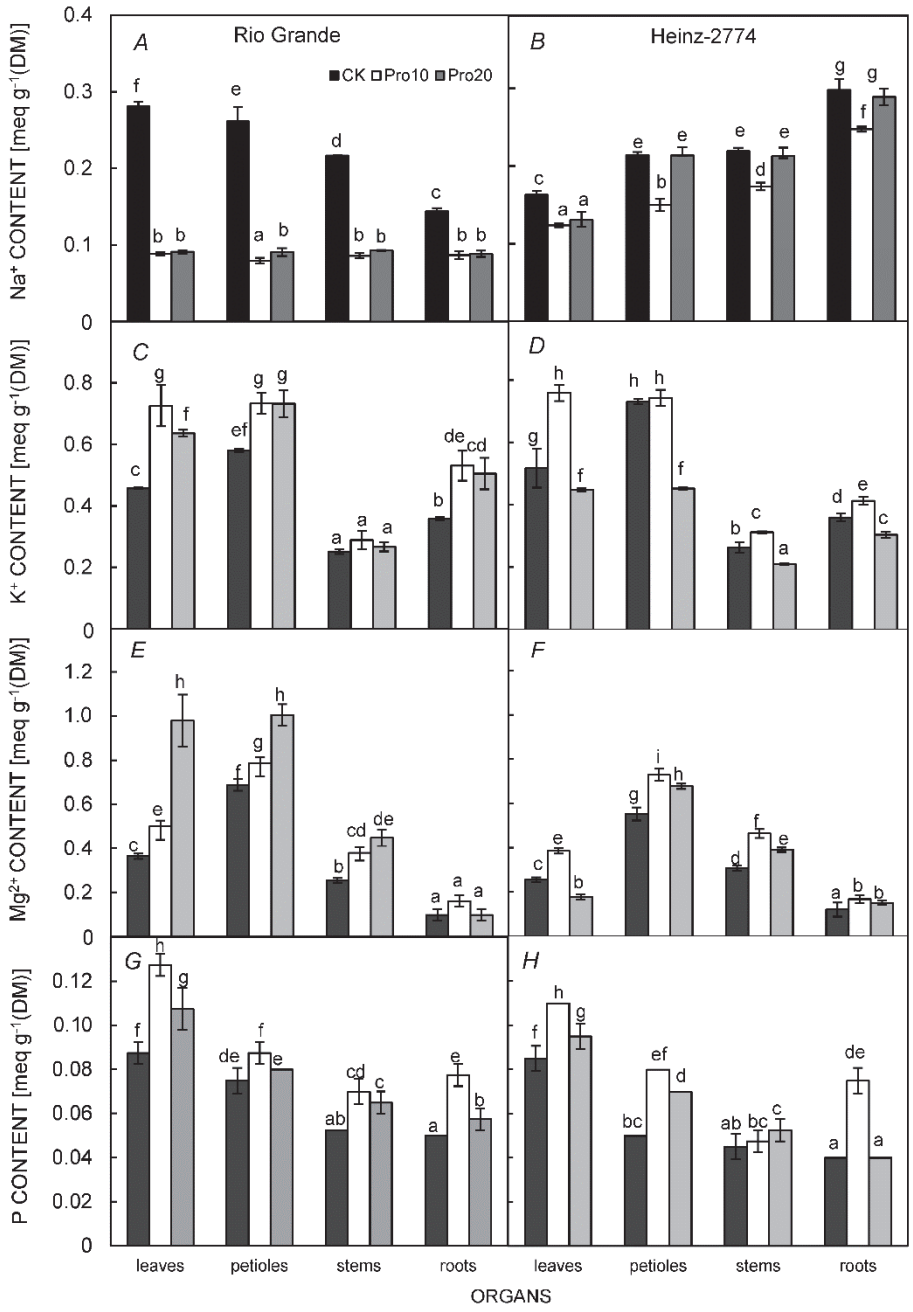

Fig. 2. Effect of exogenous application of proline (Pro) on Na^+, K^+, Mg^{2+}, and P contents in different organs of tomato cultivars (Rio Grande and Heinz-2274) irrigated with saline water (6.57 dS m^{-1}). Means comparisons were made separately for each cultivar. All values are the mean of four replications (n=4) and bars with different letters are significantly different at ($P \leq 0.05$) according to the LSD test. CK – control; Pro10 – 10 mg(Pro) L^{-1}; Pro20 – 20 mg(Pro) L^{-1}.

Table 4. Analysis of variance of the fluorescence parameters F_0, F_m, F_v/F_m, F_v/F_0, and F_v'/F_m' in two tomato cultivars (Rio Grande and Heinz-2274). * and ** – significant differences at $P \leq 0.05$ and at $P \leq 0.01$, respectively, according to the variance analysis. C – cultivar; Pro – proline.

Mean squares						
Source of variance	dl	F_0	F_m	F_v/F_m	F_v/F_0	F_v'/F_m'
C	1	92,008.6**	35,266.7**	0.039**	12.7**	0.001**
Pro	2	17,776.2**	60,386.7**	0.011**	3.6**	0.034**
Interaction C × Pro	2	2,346.2**	6,618.3*	0.0018**	0.0047*	0.009**
Error	18	126.7	1,438	$5.8.10^{-5}$	0.018	0.0017

According to our results, Mg^{2+} content increased significantly after the exogenous application of Pro. The highest increase was observed in leaves and petioles of the TC after the Pro20 treatment and in the petioles of the SC after the treatment with Pro10 (Fig. 2*E*,*F*).

Compared with the CT, P content was higher after Pro treatment. This increase was noted in all organs of TC and in leaves, petioles, and roots with the Pro10 (Fig. 2*G*,*H*).

Discussion

Exogenously applied Pro may play a significant role in enhancing crop tolerance to various abiotic stresses. According to some reports, exogenous Pro provides osmoprotection and facilitates growth of plants subjected to salt stress (Yancey 1994, Ashraf *et al*. 2008). This result was in agreement with our experiment. The 10 mg L^{-1} dose of Pro was the most effective in promoting growth in both cultivars of tomato (Rio Grande and Heinz-2274) (Table 2). The increase in growth parameters caused by Pro was in conformity with Cnoska and Hanson (1991) and Yancey (1994), where the exogenous application of Pro provided osmoprotection in cells of plant tissues. In contrast to the beneficial effect of Pro10, the inhibitory effect of Pro20 was more prominent in the leaves. These results were similar to the observations of Jain *et al.* (2001) in *Arachishypogaea*, in tomato (Heuer 2003), and in mustard (Wani *et al*. 2011). According to the work of Ashraf *et al*. (2008), the concentration of Pro used for exogenous application to *Arabidopsis* might be too high, which resulted in more harmful than beneficial effects.

Accumulation of Pro under salt stress has been correlated with stress tolerance in many plant species, and its concentration has been shown to be generally higher in salt-tolerant than in salt-sensitive plants (Petrusa and Winicov 1997, Ashraf *et al*. 2008). It was confirmed by our present study, where the high accumulation of Pro was noticed in the salt-tolerant cultivar contrary to the salt-sensitive one. However, these findings are in contrast with the results of Aziz *et al*. (1999) in tomato. They reported a negative relationship between Pro accumulation and salt tolerance. Nevertheless, Lutts *et al*. (1999) demonstrated that the salt-sensitive rice accumulated higher concentrations of Na^+ and Pro than the salt-resistant rice. They concluded that the accumulation of Pro is related to salt-stress injury. However, Vaidyanathan *et al*. (2003) and Theerakulpisut *et al.* (2005) noted that salt-sensitive rice cultivars showed higher growth inhibition and accumulated greater amounts of Pro than the tolerant ones. They concluded that high Pro contents in sensitive cultivars did not afford much protection. In our experiment, the foliar application of Pro improved the behavior of the SC irrigated by saline water, suggesting that the endogenous content of Pro might be a limiting factor for salt resistance.

Fluorescence induction patterns and derived ratios have been used as an empirical, diagnostic tool in plant physiological studies (Kocheva *et al.* 2004, Efeoğlu *et al.* 2009). Chl parameters showed that F_0 was higher in leaves of SC than of TC, indicating that PSII reaction centres are affected negatively more in the SC than in the TC. F_0 tended to decrease with the exogenous application of Pro, particularly at the Pro10 in both cultivars. However, the CT showed the higher reduction of F_m, F_v/F_m, F_v/F_0, and F_v'/F_m' in SC than in TC. PSII is a membrane-protein complex, which is believed to play an important role in the adaptation of leaf photosynthesis to environmental stresses (Loukehaich *et al*. 2011). The decrease of Φ_{PSII} might be caused by excess of light energy, which would increase the excitation pressure on PSII, raising the probability of reactive oxygen species (ROS) generation and the photoinhibition of PSII (Müller *et al.* 2001). However, the excess of light energy could be partly dissipated *via* nonphotochemical quenching. These results are in agreement with the findings of Loukehaich *et al*. (2011) on tomato crop. They showed that the parameters F_v/F_m, F_v'/F_m' decreased in tomato cultivar (T8) in 200 mM NaCl. In addition, Demetriou *et al.* (2007) and Loukehaich *et al.* (2011) showed that the organelle chloroplast is the most sensitive organelle to salt stress in the sensitive cultivar than in the tolerant cultivar of tomato. These results may confirm that salt stress might change the thylakoid structure, damage the PSII reaction centers, and the chloroplast apparatus. These results are in accordance with those of Tiwari *et al*. (1997) in rice and Loukehaich *et al*. (2011) in tomato.

We observed the trend of these parameters (F_m, F_v/F_m, F_v/F_0 and F_v'/F_m') to increase significantly with the Pro application in both cultivars, independently of the Pro concentration. The significant increase in F_0 with the corresponding decrease in F_m indicates the impairment of the LHCII (Fernandez *et al.* 1997). Similarly, F_0 increase may have several causes. The dissociation of the LHCII from the reaction centres may occur due to the presence of photoinhibited reaction centres and also due to a more reduced PQ-pool in dark-adapted leaves. Likewise, Sivakumar *et al.* (1998) showed that Pro protects PSII-mediated photochemical activities in isolated thylakoids against photodamage.

Under optimal conditions, F_v/F_m ranges from 0.80 to 0.86 in different plant species (Björkman and Demmig 1987). A decline in F_v/F_m is a good indicator of the photoinhibitory impairment when plants are subjected to a wide range of environmental stresses, including drought, salinity, and heat (Araus *et al*. 1998). In our experiment, a sustained decrease in F_v/F_m could indicate the occurrence of photoinhibitory damage. Similar results were reported by Maxwell and Johnson (2000) and Colom and Vazzana (2003) in *Eragrostis curvula*. However, the observed increase of F_v/F_m after the exogenous Pro application implied an increase in photochemical conversion efficiency of PSII in both tomato cultivars. Similarly, transgenic *Arabidopsis* (Alia *et al*. 1999), tobacco (Holmstrom *et al*. 2000), *Zea mays* (Quan *et al*. 2004), and *Brassica juncea* (Prasad and PardhaSaradhi 2004) also maintain higher F_v/F_m values than wild-type plants when subjected to photoinhibition caused by high irradiance combined with salt or low-temperature stress. F_v/F_0 is a very sensitive indicator of the potential photosynthetic activity of healthy as well as stressed plants (Ranjbarfordoei *et al*. 2006). A decrease in F_v/F_0 shows that the efficiency of the photochemical process and the electron transport chain in PSII were affected. In our experiment, the increase in F_v/F_0 reflected that the potential photosynthetic activity was strengthened by the exogenous application of Pro (Table 3).

In the present experiment, the Pro applied exogenously, particularly at the low concentration, decreased significantly the Na^+ concentration in all tissues of both cultivars. However, K^+, Mg^{2+}, and P accumulation increased, suggesting its interference in the process of osmotic adjustment. This could be attributed primarily to the ability of roots to exclude salt from the xylem sap flowing to the shoots, which would immediately imply better plant growth (Heuer 2003). Similar results were first obtained by Lone *et al.* (1987). They reported that the exogenous application of Pro to barley under saline conditions caused a significant decrease in shoot Na^+ and Cl^-, thereby causing an increase in growth. These authors suggested that the ameliorative effect of Pro in barley was due to membrane stabilization. This was further supported by Mansour (1998) in salt-stressed onion. In rice, exogenous application of 30 mM Pro offsets the adverse effects of salinity on early seedling growth and enhances the K^+/Na^+ ratio (Roy *et al*. 1993). Similar effects were observed also by Nounjan *et al*. (2012); the exogenous Pro showed the ability to alleviate the inhibitory effect of salt by reducing Na^+ uptake, which resulted in lower values of Na^+/K^+ in rice plants. Moreover, K^+ plays also other roles than osmotic, it inhibits Na^+ uptake (Fernandes-Rodrigues *et al.* 2013). Taking these arguments together, a chain of combined effects of Pro on K^+ could cause a reduction of Na^+ and therefore it decreased toxicity in plants under saline conditions.

Conclusion: The foliar spray of Pro under field conditions, particularly the lower concentration of Pro tested, increased the tolerance of salt-tolerant cultivar (Rio Grande) and lead to the amelioration of salt tolerance of the salt sensitive cultivar of tomato (Heinz-2274). The exogenous application of Pro increased the dry mass, chlorophyll fluorescence, K^+, Mg^{2+}, P and decreased the accumulation of Na^+ in different tissues of both cultivars. Commercial prospects of enhancing stress tolerance in tomato by exogenous Pro warrants further in-depth research in this area to gain a better understanding of the underlying processes for the improvement of crop production in stressful environments and in field conditions.

References

Alia, A., Kondo, Y., Sakamoto, A. *et al*.: Enhanced tolerance to light stress of transgenic Arabidopsis plants that express the codA gene for a bacterial choline oxidase. – Plant Mol. Biol. **40**: 279-288, 1999.

Araus, J.L., Amaro, T., Voltas, J. *et al*.: Chlorophyll fluorescence as a selection criterion for grain yield in durum wheat under Mediterranean conditions. – Field Crop. Res. **55**: 209-223, 1998.

Ashraf, M., Athar, H.R., Harris, P.J.C., Kwon, T.R.: Some prospective strategies for improving crop salt tolerance. – Adv. Agron. **97**: 45-110, 2008.

Aziz, A., Martin-Tanguy, J., Larher, F.: Salt stress-induced proline accumulation and changes in tyramine and polyamine levels are linked to ionic adjustment in tomato leaf discs. – Plant Sci. **145**: 83-91, 1999.

Bates, L.S., Waldren, R.P., Teare, I.D.: Rapid determination of free proline for water-stress studies. – Plant Soil **39**: 205-207, 1973.

Björkman, O., Demmig, B.: Photon yield of O_2 evolution and chlorophyll fluorescence characteristics at 77K among vascular plants of diverse origins. – Planta **170**: 489-504, 1987.

Conka, L.N., Hanson, A.D.: Prokaryotic osmoregulation: genetics and physiology. – Annu. Rev. Microbiol. **45**: 569-606, 1991.

Colom, M.R., Vazzana, C.: Photosynthesis and PSII functionality of drought-resistant and drought-sensitive weeping lovegrass plants. – Environ. Exp. Bot. **49**: 135-144, 2003.

Demetriou, G., Neonaki, C.. Navakoudis., E., Kotzabasis, K.: Salt stress impact on the molecular structure and function of the photosynthetic apparatus -the protective role of polyamines. – Biochim. Biophys. Acta **1767**: 272-280, 2007.

Efeoğlu, B., Ekmekçi,Y., Çiçek, N.: Physiological responses of three maize cultivars to drought stress and recovery. – S. Afr. J. Bot. **75**: 34-42, 2009.

Fernandez, R.T., Perry, R.L., Flore, J.A.: Drought response of young three apple trees on three rootstocks. II. Gas exchange, chlorophyll fluorescence, water relations, and leaf abscisic acid. – J. Am. Soc. Hortic. Sci. **122**: 841-848, 1997.

Fernandes-Rodrigues, C.R., Nascimento Silva, E., Ferreira-Silva, S.L. *et al*.: High K^+ supply avoids Na+ toxicity and improves photosynthesis by allowing favorable K^+:Na^+ ratios through the inhibition of Na^+ uptake and transport to the shoots of *Jatrophacurcas* plants. – J. Plant Nutr. Soil Sci. **176**: 157-164, 2013.

Genty, B., Briantais, J.M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta **990**: 87-92, 1989.

Gericke, S., Kurmies, B: [Colorimetric phosphoric acid-determination with ammonuim-vanadate-molybdate for their use in plant analysis.]. – Zeitschr. Pflanz. Dung. Bod. **59**: 235-247, 1952. [In German]

Greenway, H., Munns, R.: Mechanisms of salt-tolerance in nonhalophytes. – Annu. Rev. Plant Phys. **31**: 149-190, 1980.

Hamilton, E.W., Heckathorn, S.A.: Mitochondrial adaptations to NaCl. Complex I is protected by antioxidants and small heat shock proteins, whereas complex II is protected by proline and betaine. – Plant Physiol. **126**: 1266-1274, 2001.

Harbinson, J., Genty, B., Baker, N.R.: Relationship between the quantum efficiencies of photosystems I and II in pea leaves. – Plant Physiol. **90**: 1029-1034, 1989.

Hellmann, H., Funck, D., Rentsch, D., Frommer, W.B.: Hypersensitivity of an arabidopsis sugar signaling mutant toward exogenous proline application. – Plant Physiol. **122**: 357-367, 2000.

Heuer, B.: Influence of exogenous application of proline and glycinebetaine on growth of salt-stressed tomato plants. – Plant Sci. **165**: 693-699, 2003.

Holmstrom, K.O., Somersalo, S., Mandal, A. *et al*.: Improved tolerance to salinity and low temperature in transgenic tobacco producing glycinebetaine. – J. Exp. Bot. **51**: 177-185, 2000.

Jain, M., Mathur, G., Koul, S., Sarin, N.B.: Ameliorative effects of proline on salt stress- induced lipid peroxidation in cell lines of groundnut (*Arachis hypogea* L). – Plant Cell Rep. **20**: 463-468, 2001.

Kahlaoui, B., Hachicha, M., Rejeb, R. *et al*.: Effect of saline water on tomato under subsurface drip irrigation: nutritional and foliar aspects. – J. Soil Sci. Plant Nut. **11**: 69-86, 2011a.

Kahlaoui, B., Hachicha, M., Rejeb, S. *et al*.: Effect of saline water on tomato under subsurface drip irrigation: yield and fruit quality. – Aust. J. Basic Applied Sci. **5**: 517-529, 2011b.

Kahlaoui, B., Hachicha, M., Rejeb, S., Rejeb, M.N.: Effect of drip irrigation and subsurface drip irrigation on tomato crop. – In: Ashraf, M., Öztürk, M., Ahmad, M.S.A., Aksoy, A. (ed.): Crop Production for Agricultural Improvement. Pp. 705-720. Springer, Dordrecht, New York 2012.

Kahlaoui, B., Hachicha, M., Teixeira, J.*et al*.: Response of two tomato cultivars to field-applied proline and salt stress. – J. Stress Phys. Bioch. **9**: 257-265, 2013.

Khadri, M., Tejera, N.A., Lluch, C.: Sodium chloride-ABA interaction in two common bean (*Phaseolus vulgaris*) cultivars differing in salinity tolerance. – Environ. Exp. Bot. **60**: 211-218, 2007.

Kingsbury, R.W., Epstein, E.: Selection for salt resistant spring wheat. – Crop Sci. **24**: 310-315, 1984.

Kocheva, K., Lambrev,P., Georgiev, G., Goltsev, V., Karabaliev, M.: Evaluation of chlorophyll fluorescence and membrane injury in the leaves of barley cultivars under osmotic stress. – Bioelectrochemistry **63**: 121-124, 2004.

Kumar, S.G., Reddy, A.M., Sudhakar, C.: NaCl effects on proline metabolism in two high yielding genotypes of mulberry (*Morus alba* L.) with contrasting salt tolerance. – Plant Sci. **165**: 1245-1251, 2003.

Lin, C.C., Hsu, Y.T., Kao, C.H.: The effect of NaCl on proline accumulation in rice leaves. – Plant Growth Regul. **36**: 275-285, 2002.

Lone, M.I., Kueh, J.S.H., Wyn Jones, R.G.W., Bright, S.W.J.: Influence of proline and glycinebetaine on salt tolerance of cultured barley embryos. – J. Exp. Bot. **38**: 479-490, 1987.

Loukehaich, R., Elyachioui, M., Belhabib, N., Douira, A.: Identifying multiple physiological responses associated with salinity-tolerance for evaluating three tomato cultivars selected from Moroccan territory. – J. Anim. Plant Sci. **10**: 1219-1231, 2011.

Lutts, S., Majerus, V., Kinet, J.M.: NaCl effects on proline metabolism in rice (*Oryza sativa* L.) seedlings. – Physiol. Plantarum **105**: 450-458, 1999.

Ma, Q.Q., Wang, W., Li, Y.H. *et al.:* Alleviation of photoinhibition in drought-stressed wheat (*Triticum aestivum*) by foliar-applied glycinebetaine. – J. Plant Physiol.**163**: 165-175, 2006.

Mansour, M.M.F.: Protection of plasma membrane of onion epidermal cells by glycinebetaine and proline against NaCl stress. – Plant Physiol. Bioch. **36**: 767-772, 1998.

Maxwell, K., Johnson, G.N.: Chlorophyll fluorescence - a practical guide. – J. Exp. Bot. **50**: 659-668, 2000.

Müller, P., Li, X.P., Niyogi, K.K.: Nonphotochemical quenching. A response to excess light energy. – Plant Physiol. **125**: 1558-1566, 2001.

Nakayama, H., Horie, T., Yonamine, I. *et al*.: Improving salt tolerance in plant cells. – Plant Biotechnol. **22**: 477-487, 2005.

Nounjan, N., Nghia, P.T., Theerakulpisut, P.: Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. – J. Plant Physiol. **169**: 596-604, 2012.

Petrusa, L.M.,Winicov, I.: Proline status in salt tolerant and salt sensitive alfalfa cell lines and plants in response to NaCl. – Plant Physiol. Bioch. **35**: 303-310, 1997.

Poustini, K., Siosemardeh, A., Ranjbar, M.:Proline accumulation as a response to salt stress in wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance. – Genet. Resour. Crop Ev. **54**: 925-934, 2004.

Prasad, K.V.S.K., Saradhi, P.P.: Enhanced tolerance to photo-inhibition in transgenic plants through targeting of glycine-betaine biosynthesis into the chloroplasts. – Plant Sci. **166**: 1197-1212, 2004.

Rajasekaran, L.R., Kriedemann, P.E., Aspinall, D., Paleg, L.G.: Physiological significance of proline and glycinebetaine: maintaining photosynthesis during NaCl stress in wheat. – Photosynthetica **34**: 357-366, 1997.

Ranjbarfordoei, A., Samson, R., Van Damme P.: Chlorophyll fluorescence performance of sweet almond [*Prunus dulcis* (Miller) D. Webb] in response to salinity stress induced by NaCl. – Photosynthetica **44**: 513-522, 2006.

Roy, D., Basu, N., Bhunia, A., Banerjee, S.K.: Counteraction of exogenous l-proline with NaCl in saltsensitive cultivar of rice. – Biol. Plantarum **35**: 69-72, 1993.

Sivakumar, P., Sharmila, P., Saradhi, P.P.: Proline suppresses rubisco activity in higher plants. – Biochem. Biophys. Res. Co. **252**: 428-432, 1998.

Theerakulpisut, P., Bunnag, S., Kong-Ngern, K.: Genetic diversity, salinity tolerance and physiological responses to NaCl of six rice (*Oryza sativa* L.) cultivars. – Asian J. Plant Sci. **4**: 562-573, 2005.

Tiwari, B.S., Bose, A., Ghosh, B.: Photosynthesis in rice under a salt stress. – Photosynthetica **34**: 303-306, 1997.

Vaidyanathan, H., Sivakumar, P., Chakrabarty, R., Thomas, G.: Scavenging of reactive oxygen species in NaCl-stressed rice (*Oryza sativa* L.) differential response in salt-tolerant and -sensitive varieties. – Plant Sci. **165**: 1411-1418, 2003.

Wani, A.S., Irfan, M., Hayat, S., Ahmad, A.: Response of two mustard (*Brassica juncea* L.) cultivars differing in photosynthetic capacity subjected to proline. – Protoplasma **249**: 75-87, 2011.

Yancey, P.H.: Compatible and counteracting solutes. In: Strange, K. (ed.): Cellular and Molecular Physiology of Cell Volume Regulation. Pp. 81-109. CRC Press, Boca Raton 1994.

Zhu, B.C, Su, J., Chan, M.C. *et al*.: Overexpression of a D1-pyrroline-5-carboxylate synthetase gene and analysis of tolerance to water- and salt-stress in transgenic rice. – Plant Sci. **139**: 41-48, 1998.