INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
19–0192/2019/22–5–1059–1064
DOI: 10.17957/IJAB/15.1169
http://www.fspublishers.org

Full Length Article

High Photochemical Efficiency and Nutrient Homeostasis in Stevia (*Stevia rebaudiana*) Plant Leaf Increases Tolerance to Saline Irrigation water

Sihem Soufi*, Sarra Aabaoui, Shaima Jelleli, Salah Rezgui and Taoufik Bettaieb
Laboratory of Horticulture Sciences, National Agronomic Institute of Tunisia, Carthage University, Tunisia
*For correspondence: sihemsoufi@yahoo.fr

Abstract

The effect of irrigation with different levels of saline water on growth, protein content, photochemical efficiency, and nutrient content of Stevia (*Stevia rebaudiana* Bert.) plants was investigated in this study. The plants were irrigated with fresh water (1.22 dS m^{-1}) and saline water (3.40; 4.64; 7.72 dS m^{-1}). The results showed that root/shoot ratio, leaf number and leaf area decreased with irrigation of high saline water (7.72 dS m^{-1}). The inhibition of plant growth induced by high salinity was associated with a decrease in the photochemical efficiency of PSII. In the stem, stevia plants accumulated Na^+ and its excess accumulation induced K^+ efflux which leads consequently to an imbalance in cellular homeostasis. For all salt treatments, leaf K^+/Na^+ ratio was higher than stem and root. It appears from the present investigation that Stevia is a relatively tolerant to salt stress. Thus, saline water can be utilized in the irrigation of *S. rebaudiana* and may be exploited in crop production improvement. © 2019 Friends Science Publishers

Keywords: *Stevia rebaudiana* Bert.; Irrigation water salinity; Growth; Protein content; Ion accumulation

Introduction

Stevia rebaudiana Bert. is a perennial bushy shrub member of the Asteraceae (Compositae) family and indigenous to Paraguay specifically to the Amambay region. It also grows in Brazil and Argentina (Soejarto, 2002). Stevia leaves are the most important economic and valuable part of the plant and source of steviol glycosides (SVglys), a group of no-calorie compounds responsible for the leaves sweet taste (Kinghorn, 2004; Ramesh *et al*., 2006; Brandle and Telmer, 2007). Steviols glycosides are approximately 150 times sweetener than sugar. It could be considered as a great alternative to synthetic sweeteners. Regarding many factors, the total amount of steviols glycosides varies from 4–20% of total leaves dry weights (Brandle *et al*., 1998; Starratt *et al*., 2002). The use of these compounds as a sweeteners was already approved in several countries such as China, Australia, United States, Japan, New Zealand, Canada, South Korea, and Europe (Singh and Rao, 2005; EFSA, 2010; Wölwer-Rieck, 2012; Xu *et al*., 2018).

S. rebaudiana has wide medicinal applications, and edible values, several cultural practices were used to optimize the leaves yield and the content of rebaudioside A (Brandle *et al*., 1998; Singh and Rao, 2005). Stevia was found over an extensive variety of climatic areas around the globe and could be cultivated successfully under different growth conditions (Hajar *et al*., 2014). The plant is adapted to poor soils, did not require high nutrient requirements, but for to intensify agricultural production, crop irrigation is needed (Ramesh *et al*., 2006; Tounekti *et al*., 2018). *S. rebaudiana* has a short time span of domestication and introduction as a novel crop in the Mediterranean area especially in Tunisia. Thus, for its large scale and economic production, the use of greenhouses plantings could be considered as the most recommended framing system that provides a controlled environment suitable for optimal Stevia multiplication and plantlets production.

One of the major constraints in crop production is the quality of water. The salinity of irrigation water is often considered as a main factor that limits the crop productivity (Kim *et al*., 2016). Numerous authors have reported that the salinity of irrigation water negatively affect soil–water–plant relations and it may lead to the inhibition of normal physiological activity and thereby the productivity of the crops. Salinity stress affects the photosynthetic process due to stomatal closure that limits CO_2 fixation leading to a decrease in the photosynthesis rate and causing plant growth inhibition (Arbona *et al*., 2013). As far as it is known from the studies concerning the effect of salinity levels of irrigation water on *S. rebaudiana* under greenhouse conditions are relatively scarce. Therefore, the objective of the present study was to investigate Stevia response to the different salinity levels of irrigation water applied in order to examine its extent of tolerance to salinity. The data obtained

To cite this paper: Soufi, S., S. Aabaoui, S. Jelleli, S. Rezgui and T. Bettaieb, 2019. High photochemical efficiency and nutrient homeostasis in leaf increases tolerance to saline irrigation water in stevia (*Stevia rebaudiana*) plant. *Intl. J. Agric. Biol*., 22: 1059–1064

will help to assess further understanding of the mechanisms of adaptation and tolerance to salt stress in stevia.

Materials and Methods

Plant Materiel, Experimental Design and Treatments

The pot experiment was carried out in a green house, Laboratory Horticultural Sciences, National Agronomic Institute of Tunisia (36°82' N; 10°17' E; 13 m). The experiment was set up as a completely randomized block design with four treatments and three replications. Healthy and equal-sized Stevia 8-week-old plants obtained from sowing were selected and transplanted to pots (1 L) containing sand, peat and garden soil in equal ratio (Table 1). The selected plants were left to grow in a greenhouse under natural lighting (25/15) ± 2°C (day/night) and 70% relative humidity. The stevia plants were then split randomly into three groups and exposed to different saline irrigation water with three salinity levels for 36 days. The tap water was used as control treatment. Irrigation dose was calculated and estimated to 110 mL per pot. Plants including control and treated plants were watered three times a week throughout the experimental period with the different levels of salinity.

Irrigation Water Quality

In this study irrigation water were collected from various regions from north to south of Tunisia representing different sources of irrigation. It was collected in specific containers until the laboratory. According to a standard methods, several tests were done to determine for conventional parameters including the hydrogen exponent (pH) and conductivity measured respectively according to ISO 10523 (2008) and NF EN 27-88 (1994).

Calcium, magnesium, sodium, potassium, chloride, copper, iron, manganese, nickel, zinc, chromium, arsenic, selenium and boron ions were measured by Atomic emission-ICP according to ISO 11885 (2007). Mercury measured by Atomic emission-ICP according to NF EN 1483 (1997) (Table 1).

Estimation of Plant Growth

As non-destructive way to estimate Stevia plants growth, height, number of leaves per plant and the leaf area was recorded throughout the experiment. At the end of the trial (36 days), Stevia plants were carefully uprooted and divided into stem, root and leaf. The different divided organs were oven-dried at 80°C to a constant weight and their relative dry weights (DW) were also determined.

Protein Content

After 15 days of exposure to stressful conditions, extraction of soluble proteins and antioxidant enzymes was carried out on ice unless otherwise indicated. Frozen fresh leaves samples were homogenized at 4°C with 100 m*M* potassium phosphate buffer (pH 7.8) containing 0.1% (w/v) PVPP. The homogenate was centrifuged at 14,000×rpm for 30 min at 4°C, and the supernatant was used for protein content. Total soluble protein content was analyzed according to the method of Bradford (1976). Bovine serum albumin used as the standard.

Chlorophyll Fluorescence

The efficiency of photo system II (PSII) primary photochemistry, given as *Fv/Fm*, was recorded at the end of the experiment on an attached leaf after a dark incubation period of 20 min using a chlorophyll fluorimeter (OS5p, Opti-Sciences, Hudson, NH, USA).

Na^+, K^+ and Ca^{2+} Accumulation

At 36 days of planting, the plants of each treatment were harvested and divided into roots, stems, and leaves. These organs were separately oven-dried at 70°C for 48 h. the grinded material of root, stem, and leaf were incubated for 12 h in a mixture of a concentrated nitric acid (HNO_3) and per chloric acid ($HClO_3^-$) and then completely digested at 300°C for 6 h. Na^+, K^+ and Ca^{2+}contents were determined with a flame photometer (ICPES, Flame Photometer 410, Sherwood, U.K.).

Statistical Analysis

Statistical differences were assessed following the analysis of variance PROC ANOVA using SAS 9.0 (Chicago, IL, USA) and *t*-test. PDMix800 of PROC MIXED was adopted in order to compare means at $P < 0.05$.

Results

Effect of Different Salinity Levels of Irrigation Water on Plant Growth

The variations in plant growth of stevia showed that salt stress produced significant ($P < 0.05$) changes in stevia leaf, aerial and total dry weights (Table 2). When compared with control, leaf and total dry weights of stevia treated respectively with 3.40 and 7.72 dS m^{-1} had significantly similar dry weight. Whereas, no significant changes were even observed in root, stem dry weights and root/canopy DW ratios of all salt treated stevia remarkably inhibited by 21.6, 38.3 and 60.2% respectively comparing to control plants.

The stevia leaves response to salt stress showed increasing trend in all treated plants after 15 days of exposure to saline conditions (Table 3). The maximum increase in leaf number was reached after 30 days of treatment and achieved using treatment 4.64, 1.22 and 3.4 dS m^{-1} with a respective

Table 1: Quality of irrigation water for *Stevia rebaudiana* over the experiment period

	Treatments			
	T0 (Fresh water)	T1 (INAT)	T2 (Chott-Meriem)	T3 (Medenine)
pH	8.05 - 24.0°C	7.70 - 23.6°C	8.40 - 24.8°C	8.15 - 25.0°C
Conductivity(dS m^{-1})	1.22	3.40	4.64	7.72
Chloride (mg/L)	216	776	$1.15\ 10^3$	$2.09\ 10^3$
Calcium (mg/L)	94.1	219	263	670
Magnesium (mg/L)	27.8	91.9	203	310
Sodium (mg/L)	104	475	594	$1.03\ 10^3$
Potassium (mg/L)	4.38	7.97	2.02	28.6
Iron	0.027	<0.01	<0.01	<0.01
Zinc	0.110	<0.01	0.011	<0.01

Table 2: Effects of salinity levels on the Growth Parameters of *Stevia rebaudiana* after 36 days of Treatment

	1.22 dS/m	3.40 dS/m	4.64 dS/m	7.72 dS/m
Root/Canopy DW	0.60 ± 0.46^a	$0,47 \pm 0.12^a$	$0,37 \pm 0.06^a$	$0,40 \pm 0.15^a$
DW Total (g)	3.94 ± 1.67^b	4.08 ± 1.29^b	$5,66 \pm 0.51^a$	$3,91 \pm 0.81^b$
PA	2.65 ± 1.4^b	2.81 ± 1.0^b	4.13 ± 0.38^a	2.82 ± 0.62^b
Root DW	1.29 ± 0.67^a	1.26 ± 0.40^a	1.53 ± 0.26^a	1.08 ± 0.37^a
Stem DW	0.80 ± 0.36^a	$0,74 \pm 0.27^a$	0.97 ± 0.17^a	0.84 ± 0.33^a
Leaf DW	2.22 ± 0.36^b	2.08 ± 0.27^b	3.17 ± 0.17^a	1.99 ± 0.33^b

Letters denote a statistically significant difference ($P < 0.05$)

Table 3: Effects of salinity levels on leaf area (cm^2) and leaf number after 15 and 30 days. Values are expressed as the mean ± SE (n = 6). Bars carrying different letters are significantly different at $P < 0.05$ among treatments

		Salinity levels of irrigation water			
	Date (days after treatment)	1.22 dS/m	3.40 dS m^{-1}	4.64 dS m^{-1}	7.72 dS m^{-1}
Leaf number	15	83.4^{bc}	92.3^{abc}	95.0^{abc}	89.6^{bc}
	30	97.7^{ab}	97.8^{ab}	123.3^a	64.0^c
Leaf area (cm^2)	15	173.5^a	170.7^a	202.9^a	177.4^a
	30	194.3a	168.6^{ab}	197.4^a	131.7^a

Letters denote a statistically significant difference ($P < 0.05$)

increase of 29.82, 17.04 and 5.9%. A notable decrease of 28.57% in leaves number was recorded under higher levels of salinity. The leaf number and leaf area (Table 1) which followed almost showed the same trend as those during the first 15 days of salt treatment. At the end of the experiment, a significant and pronounced ($P < 0.05$) decrease in 25.8% in leaf area was noticed in stevia plants treated with high salinity irrigation water (Fig. 1).

Effect of Different Salinity Levels of Irrigation Water on Protein Content

The stevia plant did not produce a significant change was detected in stevia treated respectively by 1.22, 3.40 and 7.772 dS m^{-1}. In comparison to the other treatments, stevia treated with 4.64 dS m^{-1}exhibited relatively low protein content under stressful conditions.

Effects on Photochemical Efficiency of PSII (Fv/Fm)

Results highlighted an inverse relationship between salinity levels and fluorescence chlorophylienne observed after 36 days of exposure to stress conditions (Fig. 2). Whenever the level of salinity increased, the *Fv/Fm* value decrease reaching its lowest value of 0.67 at 7.72 dS m^{-1}comparing to control. It's worthnote that the statistical analysis indicated that these observations were significant ($P < 0.05$).

Effects on Ion Accumulation

Results showed that Na^+ content in root stem and leaves of *S. rebaudiana* increased with the increase of salinity (Fig. 3). However, Na^+ content in leaves and roots did not show a significant variation during salt stress. Throughout the experiment, it appears that higher levels of salinity (7.72 dS m^{-1}) significantly ($P < 0.05$) affected k^+ content especially in leaves and stem and those comapring with control and the other treatments. Higher salinty levels also increased significantly Ca^{2+} content in aerial parts. Therefore, Ca^{2+} in roots remain unchanged under salt stress. Regarding K^+/Na^+ ratios measured in the different organs, data indicates a drastic inhibition noticed throughout the experiement and those comparing to control but the ratios remain >1.

Discusion

The results from the present experiment unraveled that *S. rebaudiana* survived for more than 36 days under lower and moderate salinity levels. When comparing the obtained values for biomass production with those of control plants under the salinity levels, results indicated that stevia growth wasn't inhibited. Since no negative effects on plant growth were observed after 36 days, hence, stevia plants showed tolerance to the tested levels of salinity. However, higher

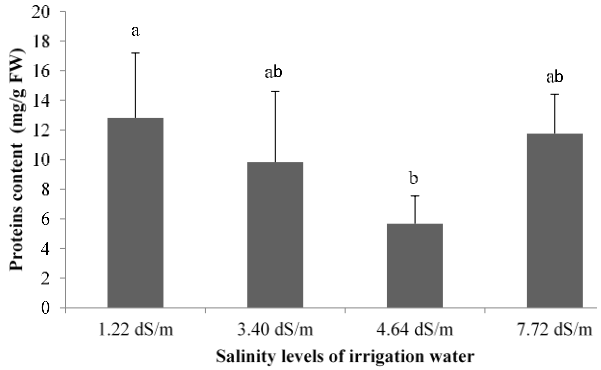

Fig. 1: Effects of salinity levels on protein content after 15 days. Values are expressed as the mean ± SE (n = 3). Bars carrying different letters are significantly different at $P < 0.05$ among treatments

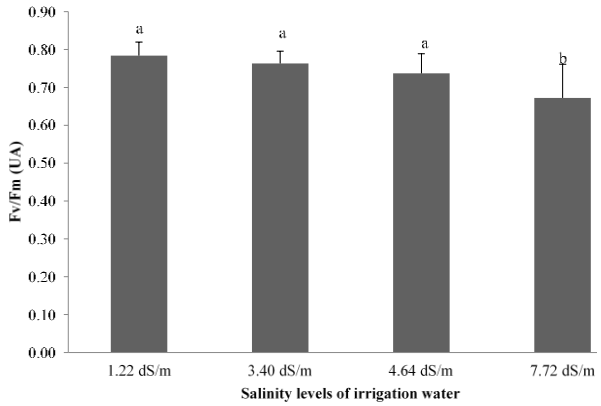

Fig. 2: Effects of different salinity level on photochemical efficiency (*Fv/Fm*) after 36 days. Values are expressed as the mean ± SE (n = 9). Bars carrying different letters are significantly different at $p < 0.05$ among treatments

salinity levels remarkebaly decreased root/ shoot ratio, leaf number and leaf area respectively by 60.02%, 28.57% and 25.8% after 30 days which indicates that stevia leaves were more succeptible to higher salinity levels. Zeng *et al*. (2013) attribuated the inhibition of stevia growth to the the damage to leaf function that occurs under salt stress conditions.

Several studies have pointed that salt stress negatively affect DW and FW of canopy, root and leaf area and number of different plant species thereby leads to their growth inhibition (Ahmad *et al.,* 2012; Sabra *et al.,* 2012; Saleh, 2012). Different studies support also present study findings. Mathur *et al*. (2006) recorded a progressive decrease in leaf area of all moth bean genotypes with the increase of salinity levels. A notable decrease in leaves number was also recorded in brassica species namely; *Brassica oleracea capitata* and *botrytis* (Jamil *et al*., 2005).

The considerable decrease of leaf numbers and leaf area could be attributed to a certain number of factors. In fact, under salt stress sodium chloride is accumulated in

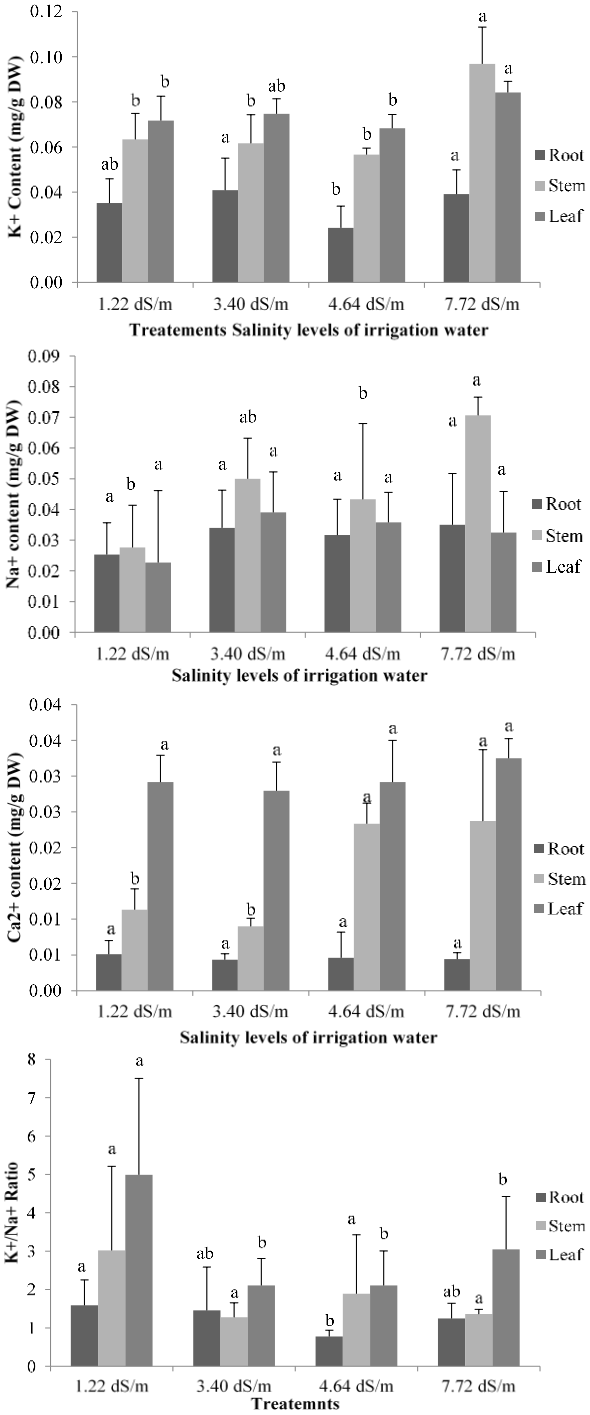

Fig. 3: Effects of different salinity level on ion contents in different organs of *Stevia rebaudiana* after 36 days of treatment. Values are expressed as the mean ± SE (n = 6). Bars carrying different letters are significantly different at $P < 0.05$ among treatments

cell walls and cytoplasm of leaves and when vacuole sap is unable to accumulate more salt, these organelles reduces sodium cells concentrations which thereby leads

to a swift leaves death (Munns, 2002). The remarkable decrease in leaf area, found throughout the experiment could be explained by the negative effect of salt stress that could be exerted on photosynthesis which ultimately leads to the inhibition of plant growth (Netondo *et al.*, 2004). These findings are in accrodance with the previous studies and pointing the fact that *S. rebaudiana* is tolerant to lower and medium salinty levels and could be thus irrigated with saline water (Zeng *et al.*, 2013; Cantabella *et al*., 2017).

The adaptation of stevia to the tested salinity levels was also reflected in proteins contents of present study. The maintenance of proteins content under the applied stress could be considered as another tolerance mechanism involved. The present study demonstrated that exposure of *S. rebaudiana* plants to a higher salinity stress levels was accompanied by an alterations to a certain extent in photochemical efficiency of PSII as it was observed by the decrease in *Fv/Fm*. Generally, under normal growth conditions higher plants exhibited a *Fv/Fm* is close to 0.83 (Björkman and Demmig, 1987). It can be noticed from the obtained results that *Fv/Fm* mesurement were lower than 0.8. This indicates that under higher salinity levels stevia reaction centers are photochemically altered.The results obtained, regarding the lower *Fv/Fm* value agree with those reported by Hua-Xin *et al*. (2004), where salt stress had significant on effects *Rumex patientia* PSII photochemical activity. In terms of chlorophyll florescence, stevia plants may be ranked among the relatively lower and moderate salt-tolerant species.

Plants exhibited a nutritional deficiencies that could be considered as negative consequences to exposure to salt stress conditions. In the present study Na^+,was accumulated in stem under higher salinity levels (7.72 dS m^{-1}). Associating these findings to the previous ones, it is obvious that Na^+ accumulation n the aerial part could indicate that stevia was unable to avoid the toxic effect of higher salinity levels. Although, Muhling and Lauchli (2002) reported that the inhibition of photosynthesis under increasing salinity is mostly due to higher Na^+ content in leaves. The present study findings disagree with previvously that under the 2 and 5 g/L NaCl treatments stevia avoid toxic effects by reducing the translocation of Na^+ to the leaves and stems and thereby showed tolerance mechanism (Munns and Tester, 2008; Acosta-Motos *et al.*, 2015). Meanwhile, Ca^{2+} and K^+ accumulation in stem indicates that stevia plants try to adjust osmotic and ion homeostasis under these stressful conditions.

Concluion

The present study clearly demonstrated that *S. rebaudiana* could be ranked among the moderately tolerant plant to the applied salt stress. Thus, saline water can be utilized in the irrigation of *S. rebaudiana* and may be exploited in crop production improvement.

References

Acosta-Motos, J.R., P. Díaz-Vivancos, S. Alvarez, N. Fernandez-García, M.J. Sanchez-Blanco and J.A. Hernandez, 2015. NaCl-induced physiological and biochemical adaptative mechanisms in the ornamental *Myrtus communis* L. plants. *J. Plant Physiol.*, 183: 41–51

Ahmad, P., K.R. Hakeem, A. Kumar, M. Ashraf and N.A. Akram, 2012. Salt-induced changes in photosynthetic activity and oxidative defense system of three cultivars of mustard (*Brassica juncea* L.). *Afr. J. Biotechnol.*, 11: 2694–2703

Arbona, V., M. Manzi, C.D. Ollas and A. Gomez-Cadenas, 2013. Metabolomics as a tool to investigate abiotic stress tolerance in plants. *Intl. J. Mol. Sci*., 14: 4885–4911

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

Bradford, M.M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilising the principal of protein-dye binding. *Anal. Biochem*., 72: 248–254

Brandle, J.E. and P.G. Telmer, 2007. Steviol glycoside biosynthesis. *Phytochemistry*, 68: 1855–1863

Brandle, J., A. Starratt and M. Gijzen, 1998. *Stevia rebaudiana*: its agricultural, biological, and chemical properties. *Can. J. Plant Sci*., 78: 527–536

Cantabella, C., A. Piqueras, J.R. Acosta-Motos, A. Bernal-Vicente, J.A. Hernandez and P. Diaz- Vivancos, 2017. Salt-tolerance mechanisms induced in *Stevia rebaudiana* Bertoni: Effects on mineral nutrition, antioxidative metabolism and steviol glycoside content. *Plant Physiol. Biotechnol.*, 115: 484–496

E.F.S.A. (European Food Safely Authority – Panel on food additives and nutrient sources added to food), 2010. Scientific opinion on the safety of steviol glycosides for the proposed uses as a food additive. *EFSA J*., 8: 1537–1584

Hajar, E.W.I., A.Z.B. Sulaiman and A.M.M. Sakinah, 2014. Assessment of heavy metals tolerance in leaves, stems and flowers of *Stevia Rebaudiana*. *Plant Proc. Environ. Sci*., 20: 386–393

Hua-Xin, C., L. Wei-Jun, A. Sha-Zhou and G. HuiYuan, 2004. Characterization of photosystem II photochemistry and thermostability in salt treated *Rumex* leaves. *J. Plant Physiol*., 161: 257–264

Jamil, M., C.C. Lee, S.U. Rehman, D.B. Lee, M. Ashraf and E.S. Rha, 2005. Salinity (NaCl) tolerance of brassica species at germination and early seedling growth. *Electr. J. Environ. Agric. Food Chem.*, 4: 970–976

Kim, H., H. Jeong, J. Jeon and S. Bae, 2016. Effects of irrigation with saline water on crop growth and yield in greenhouse cultivation. *Water*, 8: 1–9

Kinghorn, A.D., 2004. *Stevia: The Genus Stevia*. CRC Press, London and New York.

Mathur, N., J. Singh, S. Bohra, A. Bohra and A. Vyas, 2006. Biomass production, productivity and physiological changes in moth bean genotypes at different salinity levels. *Amer. J. Plant Physiol.*, 1: 210–213

Muhling, K.H. and A. Lauchli, 2002. Effect of salt stress on growth and cation compartmentation in leaves of two plant species differing in salt tolerance. *J. Plant Physiol.*, 159: 137–146

Munns, R., 2002. Comparative physiology of salt and water stress. *Plant Cell Environ.*, 25: 239–250

Munns, R. and M. Tester, 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, 59: 651–681

Netondo, G.W., J.C. Onyango and E. Beck, 2004. Crop physiology and metabolism Sorghum and salinity II – gas exchange and chlorophyll fluorescence of sorghum under salt stress. *Crop Sci.*, 44: 806–811

Ramesh, K., V. Singh and W.M. Nima, 2006. Cultivation of Stevia (*Stevia rebaudiana* (Bert.) Bertoni): a comprehensive review. *Adv. Agron.*, 89: 137–177

Sabra, A., F. Daayf and S. Renault, 2012. Differential physiological and biochemical responses of three Echinacea species to salinity stress. *Sci. Hortic.*, 135: 23–31

Saleh, B., 2012. Effect of salt stress on growth and chlorophyll content of some cultivated cotton varieties grown in Syria. *Commun. Soil Sci. Plant Anal.*, 43: 1976–1983

Singh, S.D. and G.P. Rao, 2005. Stevia: The herbal sugar of 21st Century. *Sugar Tech*, 71: 17–24

Soejarto, D., 2002. Botany of Stevia and *Stevia rebaudiana*. *In: Stevia: The genus Stevia*, pp: 18–39. Kinghorn, A. (Ed.). London, New York

Starratt, A.N., C.W. Kirby, R. Pocs and J.E. Brandle, 2002. Rebaudioside F, a diterpene glycoside from *Stevia rebaudiana*. *Phytochemistry*, 59: 367–370

Tounekti, T., M. Mahdhi, T.A. Al-Turki and H. Khemira, 2018. Physiological responses of the halophyte *Salvadora persica* to the combined effect of salinity and flooding. *Intl. J. Agric. Biol*., 20: 2211–2220

Wölwer-Rieck, U., 2012. The leaves of *Stevia rebaudiana* (Bertoni), their constituents and the analyses thereof: a review. *J. Agric. Food Chem.*, 60: 886–895

Xu, L., J. Liu, Z. Zhang, F. Yu, J. Guo and H. Yue, 2018. Effect of salt stress on growth and physiology in *Melia azedarach* seedlings of six provenances. *Intl. J. Agric. Biol*., 20: 471–480

Zeng, J., A. Cheng, D. Lim, B. Yi and W. Wu, 2013. Effects of salt stress on the growth, physiological responses, and glycoside contents of *Stevia rebaudiana* Bertoni. *J. Agric. Food Chem.*, 61: 5720–5726

[Received 29 Jan 2019; Accepted 22 Apr 2019; Published (online) 10 Nov 2019]