Effect of soil salinity on growth, metal distribution and photosynthetic performance of two *Lycium* species

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Abstract

The effect of salinity on gas exchange and fluorescence characteristics in *Lycium barbarum* and *Lycium chinense* grown on nonsaline and saline soils was investigated. The distribution of Na⁺ between three types of substrates and different organs showed acropetal concentration gradient for Na⁺ accumulation only in *L. barbarum* grown on peat-moss-perlite and saline soil. Selective uptake of K⁺ over Na⁺ in the *L. barbarum* leaves was established. All plants grown on saline soil showed a slight inhibition of PSII activity as revealed by the quantum yield of the primary photochemical reaction. Intersystem electron transport, reduction of the end electron acceptors, and the performance index of photosynthesis declined to a greater extent in *L. barbarum* than that in *L. chinense*. The higher tolerance of *L. chinense* to salt stress was accompanied with the higher overall photosynthetic performance.

Additional key words: chlorophyll fluorescence; metal contents; net photosynthesis; salinity.

Introduction

Salinity in soil or water is one of the major negative factors of the environment, especially in arid and semiarid regions, and can severely limit crop production. In recent decades, the investigations are focused on studying biochemical and physiological traits (Munns and Richards 2007, Koyro *et al.* 2014), identification of key genes and/or discovery of distinctive molecular markers in a great number of agricultural crops for improving their salt tolerance (Kosová *et al.* 2011). Besides the naturally formed saline and sodic soils, so-called secondary salt-affected soils occur due to the application of different agricultural practices and continue to grow (Munns 2011). According to FAO Land and Plant Nutrition Management Service, the total surface of the saline regions of the world is the almost 397×10^6 ha and 2% of them are salt-affected (Dajič 2006). Deeper soil pollution and salinization require utilization of alternative fast-growing woody species with deep root systems and the ability to grow in nutrient-poor soil. Munns (2011) proposed that increased salt tolerance of perennial species (such as woody species or shrubs) used for fodder, biofuel or economic important bioactive products is a key component in reducing the spread of secondary salinity,

while increased salt tolerance of crops directly improves production in soils with primary salinity.

Lycium L. (Solanaceae) is a genus of approximately 80 species distributed mainly in Asia, America, and Africa (Bernardello 1986, Miller 2002, Levin and Miller 2005). *Lycium* species are long-lived perennial shrubs which produce red or purple, fleshy berries. *L. barbarum* and *L. chinense* are a unique nutritional and medicinal food. The plants are used in traditional Chinese medicine for the treatment of pneumonia, cough, inflammation, and diabetes mellitus. In some Asian countries, the fruits are used as a tonic and invigorating agent, stimulating the immune system. In Bulgaria, *L. barbarum* L. is cultivated as an ornamental shrub, for forming hedges, and also for stabilisation of the soil and landslides. The effects of the fruits are widely popularised in Bulgaria during the past few years and the species is strongly recommended by some private companies for a cultivation (under the name “Goji Berry”) (Petrova *et al.* 2013). As Zheng *et al.* (2010) confirmed, *L. barbarum* L. is the only halophytic species in the genus *Lycium* and its physiological characteristics of drought and salt tolerance make it an ideal plant for preventing soil desertification and alleviating the soil salinity. Overexploitation of *Lycium* natural habitats

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Abbreviations: Chl – chlorophyll; DI_o/RC – energy flux dissipation per reaction center; *E* – transpiration rate; *F*_m – maximal fluorescence yield of the dark-adapted state; *F*₀ – minimal fluorescence yield of the dark-adapted state; M-PEA – multifunctional plant efficiency analyzer; OEC – oxygen-evolving complex; PF – fast chlorophyll *a* fluorescence; PI_{ABS} – performance index; PI_{total} – total performance index; PMP – peat-moss-perlite; *P*_N – net photosynthetic rate; PQH₂ – reduced plastoquinone; Q_A – primary quinone acceptor of PSII; ROS – reactive oxygen species; WUE – water-use efficiency; Φ_{P₆₈₀} – quantum yield of the primary photochemistry reaction; Φ_{E₀} – quantum yield of the electron transport between PSII and PSI; Φ_{R₀} – quantum yield for reduction of the end electron acceptors of PSI.

caused its deterioration and decreased existing populations (Liu *et al.* 2012). That is why the development of micro-propagation technologies is necessary in future.

In the present study, pot experiments were carried out to investigate differences in the growth between *L. barbarum* and *L. chinense* Mill. plants cultivated *ex vitro* on peat-moss-perlite (PMP) and two types of saline soils for a period of two vegetative seasons. PMP is preferred as a substrate for long-term cultivation of *Lycium* plants in the private companies. For this reason, differences in the distribution of Mg^{2+} , Ca^{2+} , Na^+ , and K^+ between available fractions from three substrates and different organs, as well as photosynthetic performance of both species were investigated in order to understand the salt resistance mechanisms of *Lycium* species. The hypothesis that both species adapted any sort of avoidance mechanisms towards increasing substrate salinity was tested.

Materials and methods

Plant material: Seeds and *in vivo* explants from the species of *L. barbarum* L. and *L. chinense* Mill. were used for developing of *in vitro* multiplication protocol (Dimitrova *et al.* 2016). Seedlings derived from *in vitro* micropropagation were cultivated in plastic pots filled with 1.5 kg of PMP substrate (type 0) and two soils (type 1 and 2). The experiment was set as three treatments, with seven replications and was conducted in a glasshouse (natural sunlight, temperatures of 15–35°C, relative humidity of 40–65%) for two vegetative seasons (from 1 April 2015 to 30 July 2016). The soils used in this study were taken from an area located in the vicinity of the village Belozem, Bulgaria (42°20'N, 25°3'E). A sampling strategy was carried out from the surface and at depths of 30–60 cm in different locations of the areas. As a control soil (type 0) PMP (2:1, v/v) was used. The basic component of peat moss was *Sphagnum*, supplemented with shavings, ground limestone, sand and moisture holding agent. The soil type 2 possessed exchangeable Na^+ content about 5 times higher, the sodium adsorption ratio (SAR) was about 4.5 times higher, and electrical conductivity of 14.0 mS cm^{-1} was about 2 times higher in comparison with the soil type 1 (Ivanova *et al.* 2014). On the basis of agrochemical characteristics, the soil type 1 was characterized as nonsaline, while the soil type 2 might be determined as middle alkaline (Referative basic data 2009). The pots were irrigated daily with tap water to exceeding field capacity of the soils.

Growth parameters: At the end of the experiment, the plant samples were collected, washed with tap water, and rinsed with distilled water before being separated into shoots and roots. Dry mass (DM) of shoots and roots were determined after oven drying (60°C) for 2 d until constant mass was obtained.

Plant and soil metal analysis: Total Mg^{2+} , Ca^{2+} , Na^+ , and K^+ contents in the soil samples were measured. The soil bioavailable metal fraction (free metal ions, soluble metal complexes, and metals adsorbed to inorganic soil

constituents at ion-exchange sites) was determined by extraction tests (Doumet *et al.* 2008). Total metal content in available fractions and plant organ samples was determined by atomic absorption spectrophotometric analysis (Perkin-Elmer 5000, UK), after acidic digestion with suprapur grade Fluka reagents (Doumet *et al.* 2008).

Gas-exchange measurements: Net photosynthetic rate (P_N) and transpiration rate (E) were measured simultaneously on 30 July 2016 using a portable gas analyser Li-6400 (Li-Cor Inc., Lincoln, NE, USA), with a red-blue LED light source. Measurements were made in the late morning hours between 10:00 and 12:00 under standard conditions [air temperature of 25°C, air humidity of 50% inside the gas-exchange cuvette, PAR of 1,000 $\mu mol(photon) m^{-2} s^{-1}$, and ambient concentration of 350 $\mu mol(CO_2)$]. Five to seven middle-aged, attached leaves of plants at each treatment were measured and averaged. The following index was calculated: water-use efficiency ($WUE = P_N/E$).

Chlorophyll (Chl) fluorescence measurements: The physiological state of plants at each treatment was monitored by fast Chl *a* fluorescence (PF) induction transients recorded by fluorometer M-PEA (Hansatech Instruments Ltd., King's Lynn, Norfolk, UK) after 30-min dark adaptation of seven different plants and three middle-aged leaf replicates. For the measurements, a red light beam (625 nm) was applied for 10 s at a light intensity of 4,000 $\mu mol(photon) m^{-2} s^{-1}$ (Strasser *et al.* 2004, Stirbet *et al.* 2014). The rising in PF during an illumination period of about 1 s is described by the so-called transient "JIP" curve comprised of several characteristic points (O, J, I, and P) (Strasser *et al.* 1995, 2004). Each point represented a quasi-stationary state in the fluorescence emission. At the beginning of the fluorescence measurement (point O), most of the P_{680} are opened and the fluorescence is minimal (F_0), whereas at point P the fluorescence is maximal (F_p) as most of the P_{680} are closed. Points J and I reflected the accumulation of reduced forms of Q_A and PQH_2 , respectively. PSII activity is further investigated by calculating the parameters of PF – the quantum yield of the primary photochemistry reaction (Φ_{Po}), the quantum yield of the electron transport between PSII and PSI (Φ_{Eo}), and the quantum yield for reduction of the end electron acceptors of PSI (Φ_{Ro}). These parameters represent the ratio between the energy of absorbed photons by PSII and the energy used in photochemical reactions (Strasser *et al.* 2004, Goltsev *et al.* 2014). In addition, energy flux dissipation per reaction center DI_o/RC and the photosynthetic performance indexes, PI_{ABS} and PI_{total} , were also investigated.

Statistical analysis: All data reported in this work were a mean value of at least five to seven independent experiments. The mean values $\pm$ SD and an exact number of experiments are given in the legends of the figures. The significance of differences between control and each treatment was analyzed by Fisher's LSD test ($p \leq 0.05$) after performing analysis of variance (ANOVA) multifactor analysis (Statgraphics Plus, version 5.1 for Windows).

Results

Effect of soil salinity on seedling growth: Our results showed that with increasing salinity, root and shoot DM of *L. chinense* increased, while root and shoot DM of *L. barbarum* decreased. The shoot DM of *L. barbarum* was 2.6 times higher than that of *L. chinense* after cultivation of both type of plants on PMP (Table 1).

Table 1. Changes in total dry mass (DM) of shoots and roots of *Lycium barbarum* and *Lycium chinense*, cultivated for two vegetation seasons on peat-moss-perlite (type 0), nonsaline (type 1), and saline (type 2) soils in a glasshouse. Values are means $\pm$ SD, $n = 5-7$; different letters indicate significant differences assessed by Fisher LSD test ($p \leq 0.05$) after performing one-way ANOVA analysis.

Treatment	Total shoot dry mass [g plant ⁻¹]	Total root dry mass [g plant ⁻¹]
<i>Lycium barbarum</i>		
Type (0)	0.808 $\pm$ 0.079 ^a	0.276 $\pm$ 0.027 ^a
Type (1)	2.728 $\pm$ 1.069 ^b	4.148 $\pm$ 0.512 ^b
Type (2)	0.588 $\pm$ 0.280 ^c	0.900 $\pm$ 0.079 ^c
<i>Lycium chinense</i>		
Type (0)	0.305 $\pm$ 0.022 ^a	0.291 $\pm$ 0.022 ^a
Type (1)	0.204 $\pm$ 0.131 ^a	0.288 $\pm$ 0.044 ^a
Type (2)	0.288 $\pm$ 0.169 ^b	0.358 $\pm$ 0.039 ^b

Effect of soil salinity on the distribution of metals:

The bioavailable amount of target alkaline and alkaline metals present in the fractions of the soils before planting showed that Mg²⁺, Ca²⁺, Na⁺, and K⁺ prevailed in control and saline soil (Table 2). The ratio K⁺/Na⁺ was the highest and pH was acidic in control, while pH was alkaline in nonsaline and saline soils. Higher Ca²⁺ and especially Na⁺ contents were found in the fractions of control and nonsaline and saline soils after harvesting of both type of plants. The obtained ratio of K⁺/Na⁺ in the bioavailable fractions from control and nonsaline soil was reduced about 7 times and 2–3.5 times after harvesting of *L. barbarum*

Table 2. Bioavailable alkaline and alkaline earth metal contents [mg l⁻¹] in peat-moss-perlite (0), nonsaline (1) and saline (2) soils before planting and after harvesting the plants. Values are means $\pm$ SD, $n = 3$; different letters indicate significant differences assessed by Fisher LSD test ($p \leq 0.05$) after performing one-way ANOVA analysis.

Treatment	Mg ²⁺	Ca ²⁺	Na ⁺	K ⁺	K ⁺ /Na ⁺	pH
Before plant cultivation						
Type (0)	627 $\pm$ 54 ^a	5,687 $\pm$ 325 ^a	1,072 $\pm$ 106 ^a	4,636 $\pm$ 376 ^a	4.32	5.10
Type (1)	29 $\pm$ 3 ^b	10 $\pm$ 1 ^b	26 $\pm$ 3 ^b	34 $\pm$ 3 ^b	1.28	8.00
Type (2)	40 $\pm$ 4 ^c	187 $\pm$ 12 ^c	382 $\pm$ 25 ^c	50 $\pm$ 5 ^c	0.13	8.92
After harvesting of <i>L. barbarum</i>						
Type (0)	87 $\pm$ 8 ^a	587 $\pm$ 50 ^a	584 $\pm$ 46 ^a	361 $\pm$ 35 ^a	0.62	6.10
Type (1)	52 $\pm$ 4 ^b	201 $\pm$ 19 ^b	238 $\pm$ 27 ^b	88 $\pm$ 7 ^b	0.37	7.20
Type (2)	46 $\pm$ 4 ^c	151 $\pm$ 12 ^c	244 $\pm$ 21 ^c	159 $\pm$ 14 ^c	0.65	7.40
After harvesting of <i>L. chinense</i>						
Type (0)	78 $\pm$ 6 ^a	259 $\pm$ 21 ^a	487 $\pm$ 46 ^a	279 $\pm$ 26 ^a	0.57	6.02
Type (1)	51 $\pm$ 4 ^b	220 $\pm$ 18 ^b	127 $\pm$ 11 ^b	77 $\pm$ 6 ^b	0.61	7.56
Type (2)	84 $\pm$ 7 ^c	109 $\pm$ 18 ^b	238 $\pm$ 23 ^c	86 $\pm$ 8 ^c	0.36	7.30

and *L. chinense*, while in the fraction from saline soil was enhanced about 5–3 times, respectively. The pH values of the bioavailable fractions of nonsaline and saline soils decreased from alkaline to middle acidic, while pH of the bioavailable fraction of control increased slightly (Table 2). The accumulation of Na⁺ prevailed in the roots of both species grown on control and saline soil. The content of K⁺ in the roots of *L. chinense* grown on nonsaline soil was two times higher than that in the roots of *L. barbarum*. Nevertheless, the obtained ratio of K⁺/Na⁺ in the roots of *L. chinense* was 1.9 time higher (8.89) than that in *L. barbarum* (4.67) (Table 3). The results showed that both plant species grown on nonsaline soil avoided stress by excreting excess contents of alkaline metals in the rhizosphere zone (Table 2). The accumulation of Na⁺ in the leaves of *L. chinense* was higher than that of *L. barbarum* grown on control, nonsaline, and saline soils at the end of the second vegetative season. K⁺ prevailed in the leaves of *L. barbarum* grown on three types of substrates in comparison to *L. chinense*. That is why the ratio K⁺/Na⁺ was higher in the leaves of the former species than that of the latter one (Table 4). Our results showed acropetal concentration gradient of Na⁺ distribution between soil pore water and plant tissues only for *L. barbarum* grown on control and saline soil (Tables 2, 3, 4). Irrespective of salt exclusion by root membranes, leaf content of K⁺ and Na⁺ in both species grown on nonsaline soil remained similar as compared to these grown on saline soil (Table 4).

Effect of soil salinity on photosynthetic gas exchange:

The results showed gradually enhancing of P_N in the leaves of *L. barbarum* grown on different soil substrates, while in *L. chinense* the values declined (Fig. 1A). The same tendency was found for E in *L. barbarum*. This parameter was reduced in the leaves of *L. chinense* grown on saline soil in comparison to nonsaline soil (Fig. 1B). The values of WUE decreased approximately twice in *L. barbarum* grown on saline soil than that of nonsaline soil, while in *L. chinense* these differences were insignificant (Fig. 1C).

Table 3. Accumulation of alkaline and alkaline earth metal contents [mg g^{-1} (DM)] in the roots of *Lycium barbarum* and *Lycium chinense*, grown on peat-moss-perlite (0), nonsaline soil (1), and saline soil (2). Values are means $\pm$ SD, $n = 3$; different letters indicate significant differences assessed by Fisher LSD test ($p \leq 0.05$) after performing one-way ANOVA analysis.

Treatment	Mg ²⁺	Ca ²⁺	Na ⁺	K ⁺	K ⁺ /Na ⁺
After harvesting of <i>L. barbarum</i>					
Type (0)	0.88 $\pm$ 0.09 ^a	7.48 $\pm$ 0.67 ^a	1.84 $\pm$ 0.06 ^a	18.78 $\pm$ 1.75 ^a	10.21
Type (1)	2.00 $\pm$ 0.20 ^b	5.40 $\pm$ 0.46 ^b	1.84 $\pm$ 0.11 ^b	6.96 $\pm$ 0.59 ^b	4.67
Type (2)	2.71 $\pm$ 0.32 ^c	7.39 $\pm$ 0.59 ^a	2.95 $\pm$ 0.22 ^c	11.15 $\pm$ 1.09 ^c	3.78
After harvesting of <i>L. chinense</i>					
Type (0)	1.27 $\pm$ 0.11 ^a	9.13 $\pm$ 0.87 ^a	2.88 $\pm$ 0.26 ^a	9.02 $\pm$ 0.85 ^a	3.13
Type (1)	1.95 $\pm$ 0.20 ^b	11.04 $\pm$ 1.09 ^b	1.51 $\pm$ 0.13 ^b	13.42 $\pm$ 1.11 ^b	8.89
Type (2)	2.06 $\pm$ 0.22 ^b	8.58 $\pm$ 0.79 ^a	1.77 $\pm$ 0.14 ^c	11.50 $\pm$ 1.12 ^c	6.50

Table 4. Accumulation of alkaline and alkaline earth metal contents [mg g^{-1} (DM)] in the shoots of *Lycium barbarum* and *Lycium chinense*, grown on peat-moss-perlite (0), nonsaline soil (1), and saline soil (2). Values are means $\pm$ SD, $n = 3$; different letters indicate significant differences assessed by Fisher LSD test ($p \leq 0.05$) after performing one-way ANOVA analysis.

Treatment	Mg ²⁺	Ca ²⁺	Na ⁺	K ⁺	K ⁺ /Na ⁺
After harvesting of <i>L. barbarum</i>					
Type (0)	5.50 $\pm$ 0.49 ^a	9.57 $\pm$ 0.86 ^a	1.69 $\pm$ 0.16 ^a	43.95 $\pm$ 3.45 ^a	26.01
Type (1)	5.72 $\pm$ 0.43 ^b	11.95 $\pm$ 1.16 ^b	1.49 $\pm$ 0.17 ^a	52.04 $\pm$ 4.52 ^b	28.28
Type (2)	5.17 $\pm$ 0.39 ^c	7.76 $\pm$ 0.68 ^c	2.39 $\pm$ 0.21 ^b	57.51 $\pm$ 5.09 ^c	24.06
After harvesting of <i>L. chinense</i>					
Type (0)	5.83 $\pm$ 0.51 ^a	17.01 $\pm$ 1.15 ^a	31.45 $\pm$ 2.96 ^a	14.41 $\pm$ 1.35 ^a	0.46
Type (1)	5.83 $\pm$ 0.43 ^a	23.98 $\pm$ 2.06 ^b	20.95 $\pm$ 2.01 ^b	32.45 $\pm$ 2.99 ^b	1.55
Type (2)	6.05 $\pm$ 0.62 ^a	18.37 $\pm$ 0.79 ^c	22.29 $\pm$ 2.16 ^b	36.35 $\pm$ 3.24 ^c	1.63

Effect of soil salinity on Chl fluorescence parameters:

Fast Chl fluorescence (PF) is based on the emission from reaction centers of PSII after illumination with actinic red light (about 625 nm). The “JIP” transient curves for plants grown on control soil are typical for non-stressed plants and all JIP characteristic points are clearly visible (Fig. 2). In *L. barbarum* and *L. chinense*, salinity stress resulted in the earlier appearance of O–J phase, accompanied by a slight increase in F_0 and the J peak (Fig. 2). The O–J phase was further characterised by an early rise in the transient fluorescence curve, which was detected as K-band at about 300 μs . After salinity stress, a significant increase in F_p (F_m – maximal fluorescence) and in the I–P phase was observed for *L. chinense*. In *L. barbarum*, F_m decreased and a shift in the point P from 300 ms towards later stages of the transient curve was observed.

Fig. 3 represents different parameters of PF, including Φ_{p_0} , Φ_{E_0} , Φ_{R_0} , DI_0/RC , PI_{ABS} , and PI_{total} . Our results showed that salt stress lowered the parameters of Φ_{p_0} , Φ_{E_0} , and Φ_{R_0} (Fig. 3A–C) in both plant species in a different way. Compared to Φ_{p_0} (where inhibition values reached 3.73% for *L. barbarum* and 0.54% for *L. chinense* of the control, respectively), the effect of salt stress on Φ_{E_0} was much stronger (23.6% and 5.4%, respectively), whereas that on Φ_{R_0} was smaller (14.5% and 2.9%, respectively). The DI_0/RC was stimulated during the salt stress – 37.6% and 13.0%, respectively (Fig. 3D). The parameters PI_{ABS} and PI_{total} are considered as the most sensitive among all parameters concerning the photosynthetic activity of

PSII and the efficiency of the electron transport reactions between PSII and PSI. In *L. barbarum* leaves, the values for these parameters decreased by about 57.0 and 48.9%, while for *L. chinense* the reduction is 22.6 and 19.3%, respectively (Fig. 3E,F).

Discussion

Seedling growth is normally limited by increasing concentration of NaCl (Sreenivasulu *et al.* 2000). Our results showed that with increasing salinity root and shoot DM of *L. chinense* increased, while root and shoot DM of *L. barbarum* decreased (Table 1). Time-dependent changes of growth and development of plants exposed to salt stress have been reviewed (Munns 2002). In the first few minutes, cells lose water and shrink, whereas over hours they regain their volume, but the expansion rates are limited. Over days and weeks, reduced cell elongation and cell division result in slower leaf appearance and inhibition of shoot growth. The shoot and root DM increased in *L. chinense* grown on saline soil in comparison to nonsaline soil. The shoot and root DM of *L. barbarum* grown on nonsaline soil were higher in comparison with control, but the results obtained for *L. chinense* were opposite (Table 1).

In the present study, we found that the accumulation of Na⁺ in the leaves of *L. chinense* was higher than that of *L. barbarum* grown on control, nonsaline, and saline soils (Table 4). Besides salt exclusion in the roots, namely occurred at nonsaline soil (Table 2), low Na⁺ contents in

Fig. 1. Changes in: rate of net photosynthesis (P_N) – A, transpiration rate (E) – B, and water-use efficiency (WUE) – C, measured in intact leaves from *Lycium barbarum* and *Lycium chinense*, cultivated for two vegetation seasons on peat-moss-perlite (type 0), nonsaline (type 1), and saline (type 2) soils in a glasshouse. Mean values $\pm$ SD ($n = 25$). Values with the same letter are not significantly different when means are separated by Fisher's LSD test ($p < 0.05$).

leaves can also be achieved by salt retention in the lower plant parts and also through abscission of old leaves once they accumulate large quantities of salts. Salt excretion is a very efficient avoidance mechanism, which occurs at the cell level, but also at whole organism level, and can be connected with the regulation of K^+/Na^+ selectivity (Jeschke and Hartung 2000). Another mechanism, which prevents ion accumulation in the photosynthetic tissues, is an intraplant allocation that can keep an excess of salts within their roots and lower parts of the shoots (Dajič 2006). We observed yellowing and later abscission of older leaves in both plant species during the vegetation period. The leaf abscission was observed mainly in *L. barbarum* grown on the saline soil as compared to nonsaline soil; it can be considered as the avoidance mechanism by which younger leaves are preserved from the action of high salt concentrations.

The accumulation of K^+ and the ratio K^+/Na^+ is higher in the leaves of *L. barbarum* grown on three types of substrates than that of *L. chinense* (Table 3). This ratio is an important selection criterion for salt tolerance (Ashraf and Orooj 2006). The K^+/Na^+ ratio decreased by increasing salt concentration in safflower cultivars grown in perlite medium moistened by Hoagland's solution, but the highest values determined plant species more tolerant to salt stress (Erdal and Çakırlar 2014). The obtained ratio of K^+/Na^+

Fig. 2. Induction JIP curves of the fast chlorophyll fluorescence in intact leaves from *Lycium barbarum* (A) and *Lycium chinense* (B), cultivated for two vegetation seasons on peat-moss-perlite (type 0), nonsaline (type 1), and saline (type 2) soils in a glasshouse. Mean values $\pm$ SD ($n = 5-7$). Standard deviations are not shown for clearer view.

in *L. barbarum* was higher in comparison to *L. chinense* grown on control, nonsaline, and saline soils. Nevertheless, we found the enhanced root and shoot DM accumulation in *L. chinense* with increasing salinity (Table 1).

Salinity reduces P_N and g_s in most plant species (Ashraf and Shahbaz 2003, Koyro 2006). The P_N and E were reduced under high salinity in the leaves of *L. chinense*, but in the leaves of *L. barbarum*, these parameters were enhanced slightly (Fig. 1A,B). The WUE changed negligibly in the leaves of *L. chinense* grown on nonsaline and saline soil, while in the leaves of *L. barbarum* it was reduced approximately twice under the same conditions (Fig. 1C). The investigations showed a strict correlation between plant growth and photosynthetic activity under saline conditions (Ashraf and O'Leary 1996, Koyro and Huchzermeyer 2004, Geissler *et al.* 2009). Biomass production depended mainly on the ability to keep a high net photosynthesis by low water loss rates; several plants revealed a combination of low P_N , minimum E , high stomatal resistance, and minimum internal CO_2 concentration at their threshold salinity tolerance. The results showed that balance between water loss and CO_2 uptake helps to find a weak spot in the mechanism of adjustment of photosynthesis to high salinity (Badawi *et al.* 2004). Our results showed that in spite of lower P_N and E , measured in the leaves of *L. chinense* at the high saline level, these plants showed better water regime than that of *L. barbarum* under the same conditions (Fig. 1C).

Fig. 3. Parameters of the fast chlorophyll fluorescence (quantum yield, energy dissipation, and performance index), measured in intact leaves of *Lycium barbarum* and *Lycium chinense*, cultivated for two vegetation seasons on peat-moss-perlite (type 0), nonsaline (type 1), and saline (type 2) soils in a glasshouse. Mean values $\pm$ SD ($n = 5-7$). Values with the same letter are not significantly different when means are separated by Fisher's LSD test ($p < 0.05$).

We established a significant increase in F_m (F_p) for *L. chinense*, but the decline in this parameter for *L. barbarum* after salinity stress (Fig. 2). It is well known that F_m decreases in response to different stress conditions (Strauss *et al.* 2007, Strasser *et al.* 2010, Venkatesh *et al.* 2012) and this decrease is mainly due to denaturation of Chl-associated proteins including proteins of LHCII as well as D1 core protein of PSII (Yamane *et al.* 1997). In *L. barbarum* and *L. chinense*, salt stress resulted in the earlier appearance in O–J phase, accompanied by a slight increase in F_0 and J peak (Fig. 2). Such an initial increase of F_0 is reported after drought stress in *Haberlea rhodopensis* (Strasser *et al.* 2010), cold stress in *Arabidopsis thaliana* (Mishra *et al.* 2011, Nellaepalli *et al.* 2012), and chilling stress in *Arabis alpina* (Kolaksazov *et al.* 2014). It is mostly due to the accumulation of reduced plastoquinone (PQH_2) (Tóth *et al.* 2005). Furthermore, the large amount of reduced PQH_2 after chilling stress in *A. thaliana* is accompanied by partial blocking of the electron flow from PSII to PSI, thus leading to phosphorylation of LHCII (Nellaepalli *et al.* 2012). Phosphorylation of LHCII causes dissociation of this complex from PSII and presumably protects plants from excessive light, through a migration of LHCII to PSI (Nellaepalli *et al.* 2012). Reduced PQH_2 was accumulated too after moderate heat stress in *A. thaliana* and facilitated LHCII phosphorylation (Nellaepalli *et al.* 2011).

The O–J phase can be further characterised by an early rise in the transient fluorescence curve, which is detected at about 300 μs (Strasser *et al.* 2004). This rise is called

K-band and is widely accepted as a sign of dissociation of the oxygen-evolving complex (OEC) (Guissé *et al.* 1995). Our results showed that after salt stress, a slight decrease in F_m and in the I–P phase occurred in *L. barbarum* (Fig. 2). Normally, the last part of the JIP curve represents a reduction of the end electron acceptors from the PSI side (Schreiber *et al.* 1989, Schansker *et al.* 2003). The decrease of I–P phase, which is accompanied with a decrease of F_p , could be due to a strong inhibition of the PSI acceptor side and was observed in pea (Schansker *et al.* 2005). In our experiments, the decline was observed with increasing salinity level only in *L. barbarum*. During salt stress, the JIP-curves for *L. chinense* did not change as compared with control. Additionally, the I–P phase and the P peak increased more for the nonsaline soil than that for saline soil in *L. chinense* (Fig. 2).

PSII activity can be further investigated by calculating other parameters of PF (Fig. 3). Briefly, Φ_{Po} can be considered as one of the most stable photosynthetic parameters and it is widely used in monitoring the effect of different environmental stresses on photosynthetic behaviour in plants (Strasser *et al.* 2004). Φ_{Po} is related to the deactivation or full degradation of PSII reaction centers and for that reason. Consequently, the insignificantly changing of this parameter (between nonsaline and saline soil) indicated that PSII was not affected by the salt (Fig. 3B,C). Thus, our results confirmed the accumulated body of evidence that parameters of Φ_{Eo} and Φ_{Po} , which reflect process in the photosynthetic electron chain closer to PSI, were more

sensitive to salt compared to Φ_{Po} (Venkatesh *et al.* 2012). Concerning the energy dissipation flux per reaction center of PSII (DI_o/RC), representing the amount of energy, which the plant is not able to utilize in photochemical reactions, our results showed that this parameter was mostly stimulated in *L. barbarum* compared to *L. chinense* (Fig. 3D). In *L. barbarum* samples, both parameters – PI_{ABS} and PI_{total} decreased strongly, while in *L. chinense*, the reduction was 2.5 times lower (Fig. 3E,F).

Several conclusions could be drawn from these results. (1) The performance index (PI_{ABS}), which represents the overall activity of PSII and the intersystem electron flow, was strongly inhibited in *L. barbarum* during salt stress, but it was not correlated with its effect on CO_2 net photosynthesis. (2) The quantum yield of the primary photochemical reaction (Φ_{Po}) of PSII displayed the lowest decline among all PF parameters, thus indicating higher tolerance of PSII to salt stress in both *Lycium* species. (3) The electron transport between PSII and PSI as revealed by the quantum yield Φ_{Eo} was more inhibited in *L. barbarum* than that in *L. chinense*. (4) The electron transport to the end electron acceptors of PSI as revealed by the quantum yield Φ_{Ro} was inhibited to a great extent in *L. barbarum* than that in *L. chinense*. (5) We can assume that during salt stress in *L. barbarum*, enhanced amount of reduced PQH₂ is accumulated, which leads to lower rate of the electron transport to PSI. All these changes in the intermediate electron transport and especially in the function of PSI can be considered as a protection mechanism against accumulation of ROS in the photosynthetic apparatus during salt stress. *L. chinense* is more tolerant than *L. barbarum* in spite of higher accumulation of Na⁺ in the leaves of *L. chinense* compared to *L. barbarum* grown on control, nonsaline, and saline soils. With increasing salinity level, root and shoot dry mass of *L. chinense* increased, while root and shoot dry mass of *L. barbarum* decreased.

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