Salinity is one of the significant factors affecting the productivity of plants. Considerable attention is paid to the study of salt stress effects on the physiological symptoms in various types of plants (Munns and Gilliham 2015, Negrão et al. 2017). High salt concentrations decrease the osmotic potential of soil, which decreases the availability of water and disrupts the transport of water and nutrients to plant roots (Munns 2002, Tester and Davenport 2003). Salinity causes both water stress and osmotic stress in plants and the accumulated salt ions have a toxic effect on plants. Water deficit causes a leaf turgor decrease, further causing stomata closure and decreases of stomatal conductance (g_s); one of the factors limiting photosynthesis rates (Chaves et al. 2009). There is also an ion imbalance due to the excessive collection of Na^+ and Cl^- along with decreased absorption of other ions such as K^+, Ca^{2+} and Mn^{2+} (Flowers and Colmer 2008).

Photosynthesis is the most significant physiological process and, in all its phases, is affected by stress factors. Ashraf and Harris (2013) state that the mechanism of photosynthesis involves various components, including photosynthetic pigments and photosystems, the electron transport system, and CO_2 reduction pathways. Any damage at any level caused by a stress factor may reduce the overall photosynthetic capacity of a green plant.

Rocket, commonly also known as arugula, roquette and rucola (Eruca sativa (L.) Mill.), is an annual species belonging to the mustard family (Brassicaceae), traditionally grown in the Mediterranean region. Thanks to its excellent nutritional properties, it is increasingly becoming important for its content.
of glucosides, mineral salts and vitamin C and is therefore considered to be an excellent stomachic stimulant (Alqasoumi et al. 2009, Katsarou et al. 2016). E. sativa seed oil has antioxidant and antimicrobial properties (Khoobchandani et al. 2010) and serves to inhibit the proliferation of tumour growth (Azarenko et al. 2014).

According to Ashraf and McNeilly (2004), most of the Brassica species have been categorized as moderately salt tolerant, with a significant interspecific and intraspecific variation for salt tolerance. However, contradictory findings exist regarding the reaction of these species to salt stress at different plant developmental stages, while most others indicate that these species maintain their degree of salt tolerance consistently throughout the plant ontogeny. Bianco and Boari (1996) stated that wild rocket seeds show low germinability, but are quite tolerant to salinity, showing a significant germination decrease only for salinity greater than soil solution electrical conductivity 10 dS/m. Santos et al. (2012) stated that increasing salinity levels decreased the fresh biomass. Jesus et al. (2015) reported that, in two rocket cultivars, increasing salinity reduced the fresh and dry mass of the leaves, roots, and protein content, but proline content was increased and a change of enzyme activity took place. Ashraf (1994) stated that salt tolerant plants produced significantly greater dry biomass than the normal population E. sativa. The populations did not differ significantly in leaf osmotic potential, relative water content or leaf soluble proteins. They further stated that tolerant E. sativa populations accumulated significantly greater amounts of soluble sugars, proline and free amino acids in the leaves compared with the non-tolerant population. This proves that soluble sugars, proline and free amino acids are important components of salt tolerance in E. sativa.

The goal of our experiment was to clarify the effects of salt stress, induced by varying concentrations of NaCl, on water management and gas exchange parameters, particularly the photosynthetic assimilation of CO₂ and chlorophyll fluorescence of the PSII in rocket (Eruca sativa (L.) Mill.).

**MATERIAL AND METHODS**

**Plant material and experimental conditions.** The experiment focused on monitoring the effects of salt stress induced by NaCl on the physiological parameters of rocket (Eruca sativa (L.) Mill.) cv. Astro. The experiments took place in the greenhouse. They were conducted in semi-controlled conditions (natural light conditions, air temperature 20 ± 2/15 ± 2°C day/night, relative air humidity 65% min and 85% max). The experimental plants were grown in containers 11 × 11 cm in garden substrate (pH 5.0–6.5, nutrient content N 80–120 mg/L, P 22–44 mg/L, K 83–124 mg/L). Salt stress was induced in the BBCH 12 phase (2 fully developed leaves) in concentrations of: 0 (deionized water, control); 50, 100, 200 and 300 mmol/L NaCl. Such watering took place over the course of 50 days in the amount of 50 mL of the solution every other day. The measurement of the monitored parameters was at the end of the experiment.

**Determination of leaf relative water content.** Relative water content (RWC; %) in the leaves was established as 100 × (FM – DM)/(SM – DM), where FM represents the fresh mass of 10 leaf discs (diameter 10 mm), SM is the saturated mass of the same discs after their hydration in the dark for 4 h, and DM is the dry mass of these discs after they were oven-dried at 105°C for 48 h. RWC was established in five repetitions.

**Determination of leaf osmotic potential.** Leaf samples were placed into an insulin injection, sealed with Para film and frozen at the temperature of −18°C. Prior to actual measurement, the insulin injections were kept at the laboratory temperature until the tissue completely defrosted. The osmotic potential (ψₛ; MPa) was established based on several drops of the cells upon targets of the Whatman 1 filtration paper of 1.5 cm in diameter and measured using the WP4C Dewpoint PotentiaMeter (Decagon Devices, Inc., Pullman, USA). The measurement of these parameters took place during five repetitions of three plant samples.

**Leaf gas exchange measurements.** The net photosynthetic rate (Pₚ; μmol/m²/s) rate of transpiration (E; mmol/m²/s), stomatal conductance (gₛ; mol/m²/s) and substomatal concentration CO₂ (Cᵣ; μmol/mol) were measured on the 3rd or 4th fully expanded leaf in situ, using the portable gas exchange system LCpro+ (ADC BioScientific Ltd., Hoddesdon, UK). The gas exchange was measured from 9:00 A.M. to 11:00 A.M., Central European Time. Irradiance was 650 μmol/m²/s of photosynthetically active radiation (PAR), the temperature in the measurement chamber was 23°C and the
duration of the measurement of each sample was a 15 min interval after the establishment of steady-state conditions inside the measurement chamber. The measurements of these parameters took place on a single leaf on three plants.

**Chlorophyll fluorescence measurements.** The minimum chlorophyll fluorescence ($F_{0}$) and the maximum chlorophyll fluorescence ($F_{m}$) were also measured in situ with the portable fluorometer OSI 1 FL (ADC BioScientific Ltd., Hoddesdon, UK) with 1 s excitation pulse (660 nm) and saturation intensity 3000 μmol/m²/s after 20 min dark adaptation of the 3rd or 4th fully expanded leaf. The maximum quantum efficiency of photosystem II (PS II) $F^\prime \pi /F^\prime m$ was calculated as $F^\prime \pi /F^\prime m (F^\prime \pi = F^\prime m - F^\prime o)$. The measurement of these parameters took place on five repetitions on three plants.

**Statistical analysis.** A statistical evaluation of the experiment was made using the analysis of variance (ANOVA), with the LSD test, correlation and regression analysis. Statistical analyses were performed using Statistica 9.0 CZ for MS Windows software (Tulsa, USA).

**RESULTS AND DISCUSSION**

**Water status.** In comparison to the control group, the RWC was significantly reduced all the way to the level of 200 and 300 mmol/L NaCl (Table 1), while there was no further significant difference ($P \leq 0.001$) between these two concentrations. In comparison with the control, the RWC decrease at these concentrations was by 27.1% and 22.7%, respectively. There were no significant differences in the RWC between the 0, 50 and 100 mmol/L NaCl, while Al Gehani and Ismail (2016) stated that RWC decrease occurred in rocket due to salt stress already at the concentration of 40 mmol/L NaCl.

The osmotic potential values significantly decreased ($P \leq 0.001$) in comparison with the control, with the increasing concentration of NaCl. The osmotic potential values at the concentration 50 mmol/L, compared to the control, were not significantly different (Table 1). Figure 1 depicts a linear regressive model of the increasing NaCl concentration (independently variable) and the decrease of osmotic potential of the leaf ($r^2 = 0.9963$) and RWC ($r^2 = 0.9145$), as dependent variables. Also a high correlation between RWC and $\psi_n$ was established (Table 2). The decrease of osmotic potential is considered to be an osmotic adaptation and is one of the defense strategies against salt stress (Hajlaoui et al. 2010). Osmotic adjustment involves the net accumulation of solutes in a cell in response to salinity. Pérez-Pérez et al. (2009) stated that, consequently, the osmotic potential decreases, which in turn attracts water into the cell and enables turgor to be maintained.

**Gas exchange.** Generally, $CO_2$ exchange was regarded as an important indicator of the growth of plants, because of its direct link to net productivity (Ashraf 2004). It was proven that stomatal conductance, substomatal concentration $CO_2$, transpiration and rate of photosynthesis are all parameters affected by salt stress (Sudhir and Murthy 2004). As apparent from the measured values, a significant limitation of these factors takes place with the increasing concentration of NaCl and there is a correlation between salinity and gas exchange parameters (Figure 2). Stomatal conductance decreased from 0.16 mol/m²/s in the control to 0.03 mol/m²/s in the 300 mmol/L NaCl treatment, representing a reduction by 81.3%. The statistically significant decrease ($P \leq 0.001$) of stomatal conductance, as compared to the control, was measured from the concentration of 100 mmol/L NaCl and higher. The statistically insignificant

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**Table 1.** Relative water content (RWC); leaf osmotic potential ($\psi_v$); minimum chlorophyll fluorescence ($F_0$); maximum chlorophyll fluorescence ($F_m$); variable chlorophyll fluorescence ($F_v$) and maximum quantum efficiency of PS II ($F^\prime \pi /F^\prime m$) of plants in relation to NaCl concentration (means ± standard error)

<table>
<thead>
<tr>
<th>NaCl (mmol/L)</th>
<th>RWC (%)</th>
<th>$\psi_v$ (MPa)</th>
<th>$F_0$</th>
<th>$F_m$</th>
<th>$F_v$</th>
<th>$F^\prime \pi /F^\prime m$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>75.5 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.2 ± 0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>208.2 ± 7.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1076.0 ± 18.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>867.8 ± 17.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.81 ± 0.006&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>50</td>
<td>74.9 ± 2.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.6 ± 0.11&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>215.7 ± 16.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>952.3 ± 71.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>736.7 ± 37.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.77 ± 0.022&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>100</td>
<td>72.9 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.8 ± 0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>225.2 ± 20.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>701.4 ± 58.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>476.2 ± 37.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.68 ± 0.004&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>200</td>
<td>52.8 ± 2.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5 ± 0.08&lt;sup&gt;b&lt;/sup&gt;</td>
<td>220.7 ± 19.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>633.0 ± 35.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>412.3 ± 17.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.65 ± 0.013&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>300</td>
<td>48.4 ± 3.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.2 ± 0.09&lt;sup&gt;d&lt;/sup&gt;</td>
<td>238.2 ± 24.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>630.1 ± 65.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>391.8 ± 40.8&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.62 ± 0.002&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values within a column marked with the same letter are not significantly different ($P \leq 0.001$)
Photosynthetic rates significantly decreased ($P \leq 0.001$) with the increasing concentration of NaCl, beginning with the concentration of 100 mmol/L NaCl, but no significant differences in photosynthetic rates were measured between the concentrations of 100, 200 and 300 mmol/L NaCl. The average decrease of photosynthetic rates in these treatments in comparison with the control was by 43%. The effect of salinity stress on the rate of photosynthesis could be caused by stomatal, nonstomatal or both factors (Saibo et al. 2009). A very high correlation $r = 0.9107$ ($P \leq 0.05$) was established between stomatal conductance and photosynthetic rate (Table 2). Increased levels of Na$^+$ and Cl$^-$ in the leaf tissue that can significantly affect the metabolic processes of photosynthesis could be considered as nonstomatal factors of photosynthetic limitation (Biswal et al. 2011). Xu et al. (2008) also stated that the salt-induced, osmotic effect can adversely affect the activities of a number of stroma enzymes involved in CO$_2$ reduction.

**Chlorophyll fluorescence.** The values of chlorophyll fluorescence are shown in Table 1. The values of minimum fluorescence ($F_s$) were not significantly different at concentration 50, 100, 200 mmol/L NaCl and control plants. Minimum fluorescence significantly increased ($P \leq 0.001$) only at the concentration of 300 mmol/L NaCl. Maximum fluorescence ($F_m$) was significantly lower in comparison with the control plants at the concentration of 100 mmol/L NaCl and higher. The maximum fluorescence values were not significantly different with the further increase of salt stress at the concentration 100, 200 and 300 mmol/L NaCl. The increase of $F_o$ and decrease of $F_m$ with rising salinity corresponds to results from other researchers, for example Li et al. (2010). This difference in the stomatal conductance was between concentrations of 200 and 300 mmol/L NaCl.

Stomata closure in response to salinity stress generally occurs due to decreased leaf turgor and atmospheric vapour pressure, along with root-generated chemical signals (Chaves et al. 2009). Many authors, e.g. Dodd (2003), Buckley and Mott (2013) and Matthews et al. (2017) stated that the stomata regulate the exchange of CO$_2$ and water vapour between the leaf and the atmosphere. Control of stomatal conductance is essential both to resource CO$_2$ acquisition and to prevent desiccation. Significantly ($P \leq 0.001$) lower values were recorded in the substomatal CO$_2$ concentration at 200 and 300 mmol/L NaCl. Transpiration rate was significantly lower ($P \leq 0.001$) in a concentration as low as 50 mmol/L NaCl. The greatest decrease of transpiration in comparison to the control treatment took place in the concentrations of 200 and 300 mmol/L NaCl, by 65% on average. The decrease of transpiration rate in cases of salt stress is affected by the reduced stomatal conductance for H$_2$O, which also corresponds to the very high correlation ($r = 0.9806; P \leq 0.01$) between stomatal conductance and transpiration rates (Table 2).

**Table 2. Correlation coefficients ($r$) between the monitored parameters of water management and gas exchange parameters**

<table>
<thead>
<tr>
<th></th>
<th>$\psi_n$</th>
<th>$E$</th>
<th>$P_n$</th>
<th>$g_s$</th>
</tr>
</thead>
<tbody>
<tr>
<td>RWC</td>
<td>0.9533*</td>
<td>0.9062*</td>
<td>0.8022</td>
<td>0.9544*</td>
</tr>
<tr>
<td>$\psi_n$</td>
<td>-</td>
<td>0.9236*</td>
<td>0.8626</td>
<td>0.9597**</td>
</tr>
<tr>
<td>$E$</td>
<td>-</td>
<td>-</td>
<td>0.8569</td>
<td>0.9806**</td>
</tr>
<tr>
<td>$P_n$</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.9107**</td>
</tr>
</tbody>
</table>

$**P \leq 0.01; \,*P \leq 0.05; RWC – relative water content; \psi – osmotic potential; E – transpiration; P_n – net photosynthetic rate; g_s – stomatal conductance$
condition indicated the impairment of the light-harvesting complex in PSII, which finally reduced \( F_v \). Reduction of \( F_v \) results in decreasing PSII quantum yield (Fernandez et al. 1997).

The maximum quantum efficiency of PSII photochemistry (\( F_v/F_m \)) in the salt-stressed treatments was from 0.77 to 0.62. The significant decrease of \( F_v/F_m \) in comparison with the control took place at the concentrations of 100, 200 and 300 mmol/L NaCl. These results correspond with Al Gehani and Ismail (2016), who stated that the influence of 40 mmol/L NaCl concentration on rocket chlorophyll fluorescence is not significant. According to Maxwell and Johnson (2000), the \( F_v/F_m \) ratio in the range of 0.79 to 0.84 is the approximate optimal value for many plant species. Lower values indicate that a proportion of the PSII reaction centres is damaged or inactivated, a phenomenon commonly observed in plants under stress (Baker and Rosenqvist 2004).

Salt stress in rocket affected all monitored parameters. With increasing salt stress, water management was regulated by closing stomata. Stomata closure became particularly evident through limited transpiration rates, occurring at levels of 50 mmol/L NaCl and higher. Osmotic adjustment is also very important for sustaining tissue hydration. The decrease of osmotic potential enabled sustaining tissue hydration in treatments with lower salt concentrations (50 and 100 mmol/L NaCl), comparable with the level of the control plants. Therefore, the decrease of RWC in rocket leaves took place only in treatments with high concentrations of salt (200 and 300 mmol/L NaCl). Photosynthetic assimilation levels decreased at the concentration of 100 mmol/L NaCl, but a further increase of salt stress did not affect photosynthetic rates. The maximum quantum efficiency of PSII photochemistry (\( F_v/F_m \)) decreased with rising salinity. Its lowest value was measured at the concentration of 300 mmol/L NaCl. As stated above, the decrease of \( F_v/F_m \) provides evidence of the inhibiting effect of salinity on PSII activity. It is, however, a subject for discussion to what degree the reaction centres of PSII were damaged, given the stable level of photosynthetic assimilation of \( \text{CO}_2 \) at high salt concentrations. The results obtained therefore proved the tolerance of *Eruca sativa* to salt stress. However, given that the testing was performed on a single genotype of this species, and in regard to findings by other authors, broader research is recommended.
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