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GERMINATION, STOMATAL AND PHYSIOLOGICAL RESPONSE OF ROCKET (*Eruca sativa* L.) TO SALINITY

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ABSTRACT

The response of rocket (*Eruca sativa* L.) to salinity stress was tested for several germination and physiological parameters during seedling development. Two rocket cultivars (Ilıca and Istanbul) and various salinity stresses of 0, 5, 10, 15 and 20 dS m^{-1} created by NaCl were used in the study. Germination percentage (GP), mean germination time (MGT), germination index (GI), germination stress tolerance index (GSTI), stomata morphology, chlorophyll content (SPAD value), leaf dry matter, relative water content (RWC), cellular injury (CI) and cell membrane stability (CMS) were evaluated. Results showed that high salinity stresses led to a decrease in GP, GI and GSTI index, while MGT increased. Salinity reduced stoma length and pore length. Under saline conditions, leaf dry matter, chlorophyll content and CMS increased, while RWC decreased. Also, CI was enhanced by salinities over 10 dS m^{-1}. It was concluded that lower CMS, CI and RWC, and greater dry matter and chlorophyll were considered as salinity tolerance at the seedling stage of the rocket, and Istanbul was more tolerant to salinity than Ilıca.

Keywords: *Eruca sativa* L., NaCl, germination, membrane stability, ion leakage

INTRODUCTION

Rocket (*Eruca sativa* Mill.) has been used alone or mixed with other salad greens and vegetables for a long time in traditional Mediterranean diets. Young leaves are commonly consumed, together with fish meal, in salads in a small quantity, but its consumption has been increasing in recent years because of its content of vitamin C and mineral salts [Urlic et al. 2014, Katsarou et al. 2016]. It is a high-quality vegetable from the *Brassicaceae* family of flowering plants, and the valuable oil extracted from its seeds has also been used for edible oil due to its antioxidant and antimicrobial properties [Khoobchandani et al. 2010].

Rocket cultivation has several advantages for leaf production due to its short life cycle, and it also allows crop rotation with other vegetables. Due to the high demand and its short cultivation period, it has been continuously planted in the same area up to five times under intensive irrigation and fertilization conditions in Turkey. This has resulted in the accumulation of salt in the soil due to excessive water application. The *Brassica* species have been categorized as moderately salt tolerant by Ashraf and McNeilly [2004], with a significant genotypic variation [Kaya et al. 2005]. However, plant development stages influence the response of plant species to salinity. Bianco and Boari [1996] reported that wild rocket is tolerant to salinity, but with low germination performance over electrical conductivity of 10 dS m^{-1}. Increased NaCl levels have led to reduced shoot fresh weight [Santos et al. 2012], and reduction in fresh and dry weight of the leaves of two rocket cultivars [Jesus et al. 2015], while the salt tolerant varieties produced higher dry weight than the sensitive *E. sativa* [Ashraf 1994]. These researchers also indicated that salt-tolerant plants accumulated

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considerably greater soluble sugars, proline and free amino acids in the leaves than the sensitive plants. Mbarki et al. [2018] has stated that wheat cultivars with high flavonoid and anthocyanin contents could maintain dry matter production under salt stress. In addition to the traits previously studied, several physiological parameters are considered to be a viable for ranking the plants according to respective abiotic stresses. Relative water content (RWC), leaf turgor, cell membrane stability (CMS), chlorophyll content and enzyme activity are useful indicators of the drought, salinity and chilling tolerance of plants [Farooq and Azam 2006, Fahad et al. 2017]. Under saline conditions, decreased relative water content in leaves has resulted in closing stomata; ultimately, this reduces the photosynthetic activity. This study aimed to explain the use of several germination parameters, chlorophyll content, stoma morphology, RWC and CMS in rocket under salinity stresses.

MATERIALS AND METHODS

Two rockets (*Eruca sativa* L.) cultivars for fresh leaf production, a registered cultivar Istanbul and a local cultivar Ilıca extensively preferred by farmers and consumers were used as material in the study. Various salinity stresses with the electrical conductivities of 5, 10, 15 and 20 dS m^{-1} were arranged by sodium chloride (NaCl). Distilled water served as control.

Germination test. Four replications of 50 seeds from each cultivar were placed into three layers of filter papers with 21 mL of respective test solutions. After the papers were rolled, they were inserted into a sealed plastic bag in order to prevent moisture evaporation. The papers were renewed every 2 d to avoid the salt accumulation. Germination test was performed in an incubator at 20 ±1°C under dark conditions. A two-millimeter root protrusion was considered as germination criterion. The germinated seeds were counted every day for 10 d and germination speed was evaluated in terms of mean germination time (MGT) according to ISTA rules [2003].

$$MGT = \Sigma Dn / \Sigma n$$

where D is the number of newly germinated seeds on each day and n is day number on which the count took place.

Germination index (GI) was computed according to the mentioned following equation by Salehzade et al. [2009]. GI = Number of germinated seeds / day of first count + ….. + Number of germinated seeds / day of final count.

Germination stress tolerance index (GSTI) =

= [PI of stressed seeds / PI of control seeds] × 100,

where PI (promptness index) = $(nd_2 \times 1) + (nd_4 \times 0{,}75) + (nd_6 \times 0{,}50) + (nd_8 \times 0{,}25)$.

nd_2, nd_4, nd_6 and nd_8 denote the number of seeds germinated on day 2, 4, 6 and 8, respectively [Toscano et al. 2017].

Seedling growth experiment. The fifty seeds with four replicates were sown in pots with dimensions of 8 × 15 × 8 cm, filled with peat : vermiculite mixture (5:1 v/v). They were put into a growth chamber at a constant temperature of 20°C and relative humidity 60% under 16 h day / 8 h dark conditions. The irrigation was performed with deionized water until the seedling emergence completion. After first true leaves appearance, the plants were firstly thinned to five seedlings in each pot/replicate and then exposed to NaCl stresses used in the germination test. The plants were watered with the respective salt solutions for 30 days. After the stress period, the fresh leaves of five plants were collected from each NaCl stress, replicate and genotype.

Relative water content (RWC). Five fresh leaves from each replication were directly weighed to determine fresh weight (FW), and then immersed in falcon tubes filled with 50 mL distilled water for 24 h. The leaves were placed into the tubes after they were slightly rolled. After the incubation period, the leaves were gently surface dried with paper towels to drain excessive water and weighed to record turgid weight (TW). They were then dried in the oven at 80°C for 24 h to determine dry weight (DW). The RWC was calculated as described below.

$$RWC = [(FW - DW) / (TW - DW)] \times 100.$$

Cell membrane stability (CMS) and cellular injury (CI). Five fully expanded young leaves (3^{th} leaf) were chosen from each genotype, salt level and replicate. The leaves were immediately washed for 5 minutes with deionized water in order to remove solutes from

leaf surfaces. Ten disc segments (1 cm diameter) obtained from these leaves were directly placed into glass test tubes filled with distilled water. The tubes were incubated at 10°C for 24 h, and then transferred to an incubator at 25°C for 1 h before measuring the electrical conductivity (EC_1) of the contents. Afterwards, the tubes were autoclaved at 121°C for 15 min to determine the electrical conductivity of the dead leaves (EC_2). Ion leakage was measured with a conductivity meter (WTW 3.15i, Germany). Cellular injury (CI) at each NaCl level was calculated by the equation below.

$$CI\ (\%) = [(IL_s - IL_c) / (100 - IL_c)] \times 100,$$

where IL_s and IL_c are the percentage ion leakage for the salinity levels or control sample, respectively [Gulen and Eris 2003].

The cell membrane stability (CMS) was determined according to the formula;

$$CMS\ (\%) = [1 - (EC_1/EC_2] \times 100$$

Chlorophyll content. It was measured using a SPAD meter (Konica-Minolta SPAD 502Plus, Japan) and evaluated as SPAD value, which is index value displayed by Konica Minolta Chlorophyll meters, with highly correlated with chlorophyll density [Süß et al. 2015]. SPAD values were recorded using the same leaves (3th leaf from the upper) from the same plants prior to sampling. Five plants were used in each replicate, totally twenty SPAD measurements were performed for each treatment.

Stoma size and density. Stomatal observations were made at each level of NaCl at 30 days after true leaf senescence on randomly selected five leaves among twenty plants in a treatment. Approximately 1 cm^2 of the lower surface of fully developed leaves (excised from areas between the main veins) were taken from 3th leaf from top of the plants. Adaxal sheets were peeled and stomata number per unit area was counted by 40 × objective lens and 10 m× eyepieces under light microscope. Three samples on epidermal cells were randomly selected from different parts of each leaf and counting was replicated three times. Stomata density was determined by counting in a 1 mm^2 area; furthermore, the stomata width, length and porelength were also measured by an ocular micrometer calibrated using a stage micrometer.

Statistical analysis. The experiment was established at two factors factorial in a completely randomized design with four replicates. Data for percentage were transformed to arcsine values before ANOVA was performed with MSTAT-C program (Michigan State University v. 2.10). The differences between the means were compared using Duncan's Multiple Range Test ($P < 0.05$). The relationship between the investigated traits was determined using Pearson's correlation coefficients.

RESULTS

The germination percentage of two rocket cultivars showed different responses to increasing salinity stresses. No significant differences were observed among NaCl treatments up to 20 dS m^{-1}, but the germination of Istanbul significantly reduced at 20 dS m^{-1} (Tab. 1). However, MGT considerably prolonged with each increase in NaCl stress in two cultivars. Higher salinity levels than 5 dS m^{-1} resulted in retardation in germination, and Ilıca germinated more rapidly than Istanbul under all salt stresses. GI was significantly reduced by increasing NaCl, while the cultivars showed different responses along with the superiority of Ilıca. Under non-saline conditions, GI was calculated at 44.8, but it dropped off 18.5 at 20 dS m^{-1}. The highest GSTI values of the cultivars were observed at control treatment; whereas, increasing NaCl levels diminished them significantly (Tab. 1). Ilıca gave higher GSTI at 20 dS m^{-1} than that of Istanbul.

Increased NaCl levels considerably influenced the stomata density, stoma length and pore length of the rocket cultivars (Tab. 2). The stoma density of the lower epidermis at different concentrations of NaCl was greater at 20 dS m^{-1} than the control. Conversely, the stoma and pore length decreased as salinity levels increased. Higher number and smaller stomata were obtained from the plants exposed to increased salinity. The stoma width of Istanbul gradually reduced due to salinity, but any significant changes were not observed.

Leaf dry matter of both cultivars was similar to each other at NaCl levels of 15 and 20 dS m^{-1}, while the differences were more prominent at lower doses of NaCl along with the superiority of Ilıca (Fig. 1a). This means that lower water and higher dry matter con-

Table 1. Germination parameters of two rocket cultivars affected by salinity levels

Factors	GP (%)	MGT day	GI	GSTI
Variety				
ILICA	95.1	1.60[b]	36.6[a]	93.5
ISTANBUL	93.5	1.67[a]	34.3[b]	92.6
Salinity levels (dS m^{-1})				
Control	97.5[a]	1.18[d]	44.8[a]	100.0[a]*
5	96.5[a]	1.20[d]	43.5[a]	99.1[a]
10	95.3[a]	1.34[c]	39.7[b]	97.7[a]
15	96.0[a]	1.76[b]	30.6[c]	97.5[a]
20	86.3[b]	2.69[a]	18.5[d]	71.1[b]
Interaction				
IL× Control	97.0[a]	1.12[f]	45.8	100.0[a]
IL × 5	95.5[a]	1.13[f]	44.8	98.1[a]
IL × 10	93.0[a]	1.29[de]	40.4	95.0[a]
IL × 15	95.5[a]	1.71[c]	31.1	97.9[a]
IL × 20	94.5[a]	2.76[a]	20.7	76.7[b]
IST × Control	98.0[a]	1.23[ef]	43.9	100.0[a]
IST × 5	97.5[a]	1.28[de]	42.2	100.1[a]
IST × 10	97.5[a]	1.40[d]	39.0	100.3[a]
IST × 15	96.5[a]	1.80[c]	30.0	97.2[a]
IST × 20	78.0[b]	2.61[b]	16.3	65.4[c]

* means followed by the same letter(s) in each column are not significant at level of 5%. GP – germination perncetage, MGT – mean germination time, GI – germination index, GSTI – germination stress tolerance index

Table 2. Stomatal morphology of two rocket cultivars under salinity stresses

Factors	Stomata density (number mm^{-2})	Stomata length (µm)	Stomata width (µm)	Pore length (µm)
Variety				
Ilıca	169	23.3	19.2	11.4
Istanbul	172	23.0	18.6	11.7
Salinity levels				
Control	146[c]	25.3[a]	19.7	13.9[a]*
10 dS m^{-1}	168[b]	23.1[b]	19.1	10.9[b]
20 dS m^{-1}	196[a]	21.1[c]	18.0	9.8[b]
Interaction				
IL× Control	147	24.2	19.3	13.3
IL × 10	169	23.7	19.3	10.6
IL × 20	189	21.9	19.1	10.2
IST × Control	143	26.4	20.1	14.5
IST × 10	168	22.4	18.8	11.1
IST × 20	205	20.3	16.8	9.4

* means followed by the same letter(s) are not significant at level of 5%

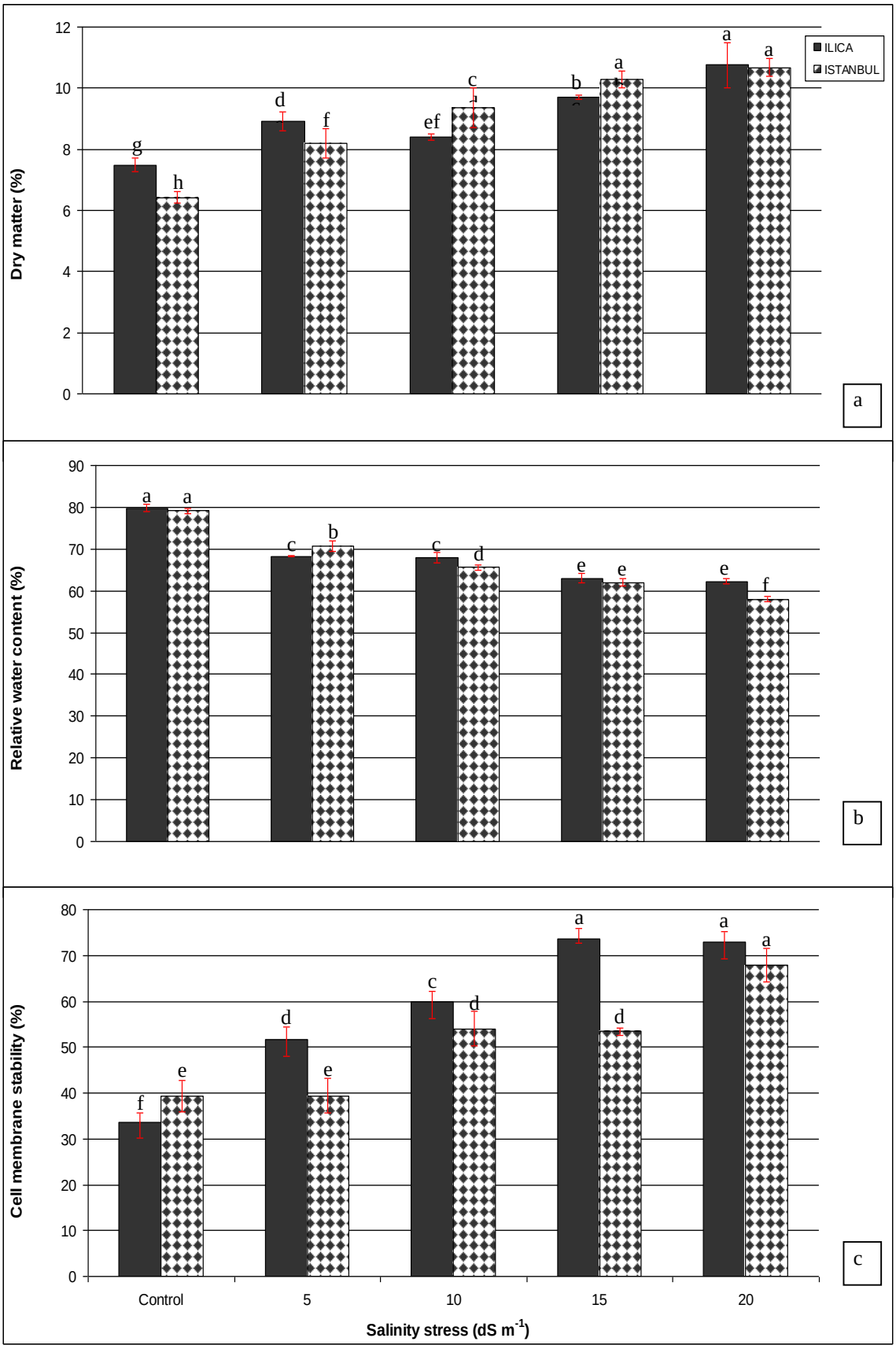

Fig. 1. Changes in dry matter (a), relative water content (b) and cell membrane stability (c) of two rocket cultivars subjected to increasing salinity stresses

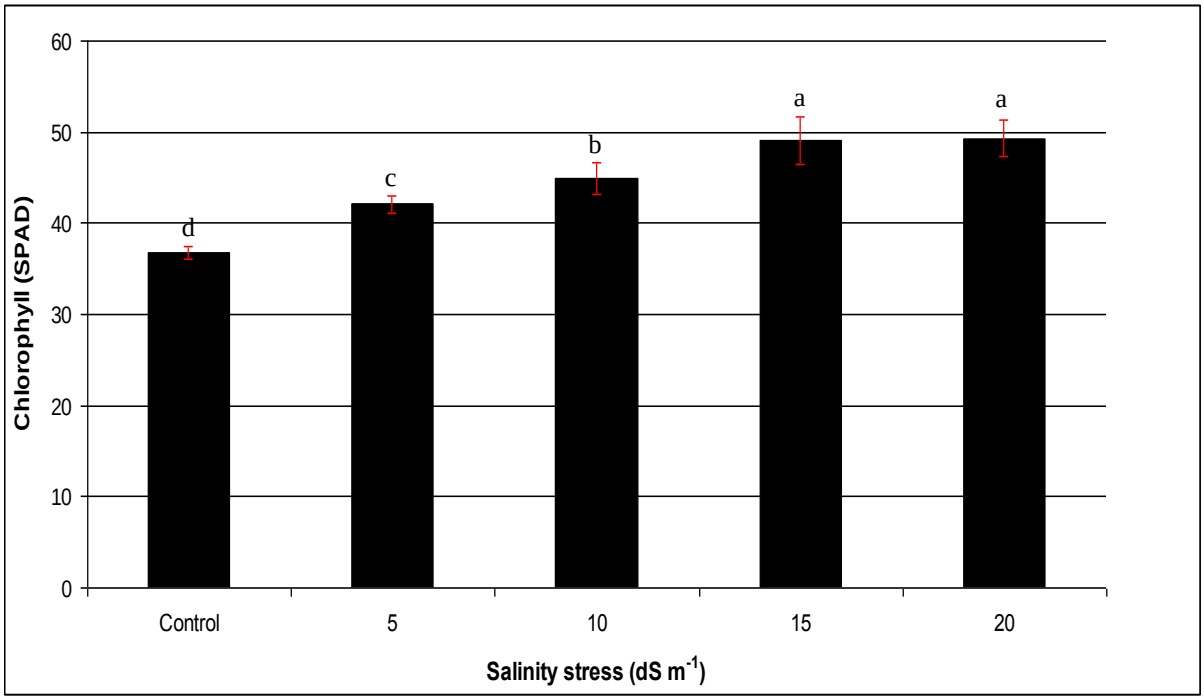

Fig. 2. Changes in chlorophyll content (SPAD value) due to salinity

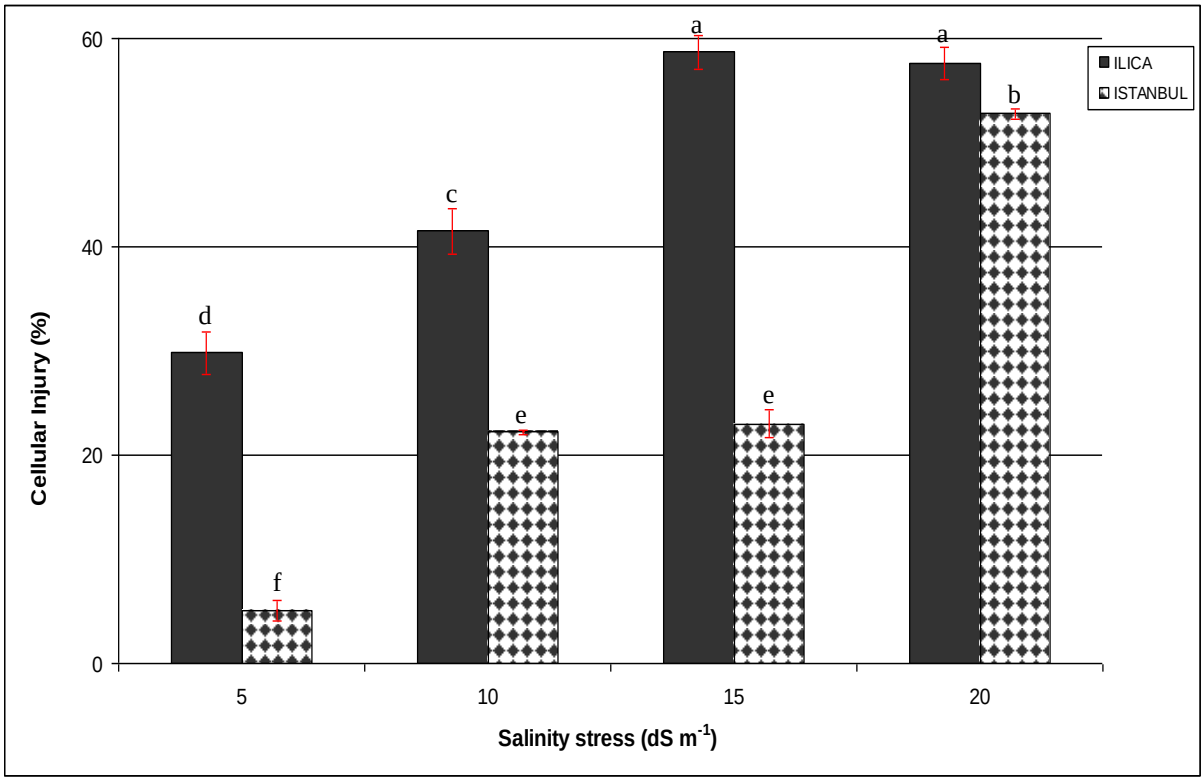

Fig. 3. Cellular injury of two rocket cultivars under salinity stresses

Table 3. Pearson's correlation coefficients between germination and physiological parameters

	GP	MGT	GI	GSTI	RWC	DM	Chl
MGT	–0.549**	–	–	–	–	–	–
GI	0.642**	–0.968**	–	–	–	–	–
GSTI	0.842**	–0.869**	0.856**	–	–	–	–
RWC	0.510**	–0.723**	0.810**	0.604**	–	–	–
DM	–0.431**	0.751**	–0.801**	–0.605**	–0.918**	–	–
Chl	–0.432**	0.668**	–0.764**	–0.525**	–0.921**	0.899**	–
CMS	–0.373*	0.715**	–0.757**	–0.563**	–0.808**	0.741**	0.784**

*, ** significant at 5% and 1% levels of probability, respectively. GP – germination percentage, MGT – mean germination time, GI – germination index, GSTI – germination stress tolerance index, RWC – relative water content, DM – dry matter, Chl – chlorophyll content, CMS – cell membrane stability

tent in rocket leaves were determined under salinity. Leaf relative water content (RWC) was significantly changed between rocket cultivars subjected to increasing salinity levels. Under non-saline conditions, it was approximately 80% for two cultivars (Fig. 1b). Salinity resulted in a remarkable reduction in RWC, but the reduction tendency was different in the cultivars. Local cultivar Ilıca showed the lowest reduction in RWC at the same salinity level. The RWC of Ilıca dropped to 62.1% at 20 dS m^{-1} of NaCl, while it was recorded as 58% in Istanbul. A significant increase in cell membrane stability due to increasing salinity at seedling growth stage was observed (Fig. 1c). The highest amount of CMS was measured in the plants watered with 15 and 20 dS m^{-1} as compared to lower salinities and control. Ilıca had higher CMS than Istanbul at all levels of NaCl, except for control. Accordingly, chlorophyll content promoted with increasing salinity, while the cultivars did not show significant variation. Chlorophyll content was measured at 36.7 SPAD value at non-saline conditions, and it reached up to 49.2 SPAD value at 20 dS m^{-1} (Fig. 2). Cellular injury increased significantly in both cultivars when salinity levels were increased, but the magnitude of increase was more apparent in Ilıca than Istanbul (Fig. 3). At salinity level of 20 dS m^{-1}, the difference between the cultivars reached the minimum level.

Simple correlation coefficients calculated among the characteristics showed that all of them were significantly correlated with each other (Tab. 3). The cell membrane stability gave the highest negative correlation coefficient with relative water content. The relationship between RWC and chlorophyll content was negative (r = –0.921**). Also, there was a significant positive relation between dry matter and chlorophyll.

DISCUSSION

Salinity is an important problem for crop production because of the reduction and delay of germination due to osmotic barrier or ion toxicity constituted by specific salts. In this study, both germination rate and time to germination of rocket cultivars were adversely affected by increasing salinity. Decreased and retarded germination at 20 dS m^{-1} was very clear. These types of inhibitions on seed germination were observed by other *Brassicaceae* species like canola, turnip rape and cabbage [Kaya et al. 2005]. However, several germination indices have been used for classification of the species or varieties against a specific stress. Both GI and GSTI have been extensively implemented in many plant species in order to classify the species for a particular stress [Aflaki et al. 2017, Bagum et al. 2017]. In our study, NaCl led to decreasing GI and GSTI of both cultivars. GI was significantly reduced at 10 dS m^{-1}, while GSTI was 20 dS m^{-1}. Similar findings were reported by Aflaki et al. [2017] and Bagum et al. [2017], who found that these indices were suitable for ranking the genotypes respective stresses.

The germination test indicated that the performance of the local variety Ilıca under salt stress was better than Istanbul's.

Stoma number and morphology were clearly influenced by salinity stresses. When salinity increased, the stoma number was enhanced considerably, while stoma length and pore length were shortened. Increased stoma number and reduced stoma length have been previously described in several plants by De Villieres et al. [1996] in *Atriplex semibaccata* and by Solmaz et al. [2011] in melons, and they also reported that salinity resulted in an increase in stoma number without changing in dimension.

Our results exhibited that the cellular injury increased and, while RWC decreased due to increasing salinity levels. This was consistent with the previous research results reported by Bajji et al. [2001], as well as Farooq and Azam [2006] in wheat, Ashraf and Ali [2008] in canola, Ziaf et al. [2009] in peppers, Orsinia et al. [2012] in strawberries and Saeed et al. [2014] in okra. All found that a progressive reduction in RWC and increase in cellular injury could be evaluated as indicators of salinity tolerance; indicating that the tolerant varieties possessed the lower cellular injury and the higher relative water content. Hnilickova et al. [2017] observed that the RWC of rocket was only significantly decreased at the highest salinity levels. In addition, the rocket cultivars produced higher dry matter and chlorophyll content under salinity stresses. Similarly, Urlic et al. [2017] and Schiattonea et al. [2017] found that the leaf dry matter and chlorophyll content of rocket increased at NaCl levels of 40 mM. An increase in dry matter due to salinity has also been reported by Eker et al. [2006] in maize. In similar studies of various species, chlorophyll content was linearly depressed in the presence of NaCl in rice [Lutts et al. 1996], in the common bean [Taïbi et al. 2016], and in radishes [Jungklang 2018], while Jaleel et al. [2008] found a significant reduction in the chlorophyll content of *Catharanthus roseus* under high salinity conditions. However, our study showed that increasing salinity up to 15 dS m^{-1} resulted in increasing chlorophyll content and that a similar value was obtained at 20 dS m^{-1}. In addition, the leaves were even dark green, while necrosis was commonly observed at the edges of leaves at NaCl levels of 15 and 20 dS m^{-1}. This may have resulted from the species, exposure duration, development stage or sensitivity to NaCl. Lutts et al. [1996] demonstrated that chlorophyll content was severely decreased in salt-sensitive cultivars when the duration of salt stress exposure was extended. For these reasons, Saleh [2012] supported the findings that chlorophyll SPAD value can be used to discriminate the cotton cultivars as salt sensitive or salt tolerant.

In conclusion, germination and seedling growth of rocket cultivars were severely inhibited by increasing salinity. Also, the germination indices were decreased under salt conditions. The cell membrane stability, cellular injury, chlorophyll content and dry matter should be considered for impressive separators of salt tolerance of rocket cultivars. Further research needs to be conducted with more rocket cultivars to clarify the relationship between the germination and progressive stages. However, the germination indices were related with these physiological characteristics of the subsequent development stages.

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