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CHANGES IN STOMATAL PROPERTIES OF SAFFLOWER CULTIVARS UNDER SALINITY

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Abstract: The stomatal characteristics in the leaves play a key role to adapt to several abiotic stresses such as drought, heat, and salinity. This study was conducted at the Seed Science and Technology Laboratory, Eskişehir Osmangazi University in 2022 in order to examine the abaxial and adaxial stomatal properties of 9 safflower cultivars (Dinçer 5-18-1, Remzibey-05, Balcı, Yekta, Linas, Olas, Olein, Safir, and Zirkon) under salt stress (100 mM NaCl). The density, width, length, size, and index of the stomata were measured. The data was analyzed by a two-factor factorial in completely randomized design. The results showed that significant differences for all stomatal features of the safflower cultivars were determined. The stomata density changed with safflower cultivars between 143 and 57 number mm^{-2} and stomata size was observed as 510-698 µm^2. The number of abaxial stomata was higher than the adaxial part of leaves and the stomatal density on the abaxial part of six safflower cultivars (Remzibey-05, Balcı, Yekta, Olas, Olein, and Safir) was decreased by salinity. In addition, abnormal stomata were observed in salt-affected cultivars of Dinçer 5-18-1, Remzibey-05, Yekta, Olein, and Zirkon. The stomata density mainly depended on genetic factors, suggesting that it should be used for separating safflower cultivars, but they declined considerably by salinity. It was concluded that stomatal properties should be considered to clarify the salt tolerance of safflower genotypes.

Keywords: *Carthamus tinctorius* L., Stomata density, Genotype, NaCl

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1. Introduction

Safflower (*Carthamus tinctorius* L.), an annual oilseed crop, is generally produced for edible oil content in seeds in the world. Also, its flowers with different colors are used for natural dye sources and spice (Silva et al., 2022). Due to the wide adaptation ability, safflower can be grown in a wide range of ecological conditions. It is a moderately salt-tolerant plant and produces sufficient seed yield under drought and heat conditions. For this reason, it is especially preferred in arid, semi-arid, and salt-infected areas where the other oilseed crops could not be grown in Türkiye (Kaya et al., 2019).

Salinity has hazardous effects on crop plants at every stage of their life cycle (Kumar et al., 2022) by enhancing the osmotic pressure of water in the soil, and the toxic ion effect, which causes an ionic imbalance in the plant tissues due to excessive Na^+ and Cl^- (Bresler et al., 2012). It leads to retardation in germination and emergence, irregular seedling establishment, and reduction in seed yield resulting from morphological and physiological disorders (Ergin et al., 2021a). Under salt stress, hormonal balance is destroyed, and photosynthesis and protein synthesis are reduced by decreasing nitrate intake (Hasanuzzaman et al., 2021). Moreover, stomatal structures and functions can be changed by salinity (Hedrich and Shabala, 2018). Because the stomata regulate gas exchange between plants and the environment, they are very important in adaptation to different environmental conditions (Ergin et al., 2021b). In wet, dry, cold, or warm conditions, the optimal parameters of the stomatal apparatus are different (Babosha et al., 2022). Additionally, the relationship between the resistance of plants to various environmental conditions and the stomatal characteristics was identified by several researchers (Reynolds-Henne et al., 2010; Hamani et al., 2021; Pitaloka et al., 2022). Mohamed et al. (2020) found that salt-tolerant rapeseed cultivars had fewer stomata than susceptible cultivars. On the other hand, detailed information on stomata movements in safflower under salinity stress has not been found in the literature. The objective of the present study was to investigate the stomatal characteristics and behaviors of some safflower cultivars newly registered in Türkiye under salt stress.

2. Materials and Methods

This study was carried out at the Seed Science and Technology Laboratory at Eskişehir Osmangazi University, Türkiye in 2021. The old and newly registered nine safflower cultivars by the Transitional

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Zone Agricultural Research Institute-Eskişehir (Dinçer 5-18-1, Remzibey-05, Balcı and Yekta), Trakya Agricultural Research Institute-Edirne (Linas and Olas) and Isparta University of Applied Sciences, Faculty of Agriculture-Isparta (Olein, Safir and Zirkon) were used as material. The genotypic properties of the safflower cultivars were shown in Table 1.

Table 1. Some varietal properties of the investigated safflower cultivars

Cultivar	Spininess	Flower color	Oleic/linoleic	Registration date
Dinçer 5-18-1	Spineless	Red	Linoleic	1983
Remzibey-05	Spiny	Yellow	Linoleic	2005
Balcı	Spiny	Yellow	Linoleic	2011
Linas	Spiny	Orange	Linoleic	2013
Olas	Spiny	Yellow	Oleic	2015
Yekta	Spiny	Yellow	Linoleic	2019
Olein	Spiny	Orange	Oleic	2019
Safir	Spiny	Orange	Linoleic	2019
Zirkon	Spiny	Orange	Oleic	2019

The seeds of safflower cultivars were pre-germinated in petri dishes at 25°C for 24 h and the seeds with radicle protrusion were transferred to the vials filled with peat:perlit:vermiculite (3:1:1 v:v:v) mixture. Thirty plants from each cultivar were grown in a growth chamber with temperatures of 20°C/15°C day/night, respectively, and relative humidity was set in the range of 60 to 70%. They were divided into two groups. Control group plants were irrigated with distilled water and salt-stressed plants were watered with 100 mM NaCl. Twenty days after sowing, the plants were in the four leaves stage, and five healthy leaves from the first true leaf from each treatment were selected for stomatal measurements. Both lower (abaxial) and upper (adaxial) epidermal surfaces of the leaf were peeled off. Extracted samples were first soaked in acetone, then washed with distilled water and stained with acetocarmine dye. Three-five places of each sample were randomly specified, and the stomata observations were performed by 40× objective lens and 10× eyepieces under a light microscope system Zeiss Axio Scope A.1. Stomata number was counted in a 0.083 mm^2 area. Besides, an ocular micrometer calibrated using a stage micrometer was used to measure the stomata width and length.

The leaf stomata density was expressed as the number of stomata per unit leaf area (number of stomata mm^{-2}). Stomata size was also computed according to the following formula (Equation 1).

$$\text{Stomata size } (\mu m^2)=\pi\left(\frac{\text{Stomata width}}{2}\times\frac{\text{Stomata length}}{2}\right) \qquad (1)$$

The stomata index was calculated as stomata length divided by stomata width (Çimen et al., 2016). Abnormal stomata were observed and classified by following the description of Mandal et al. (2012).

The experiment was analyzed by a two-factor completely randomized design with four replications using the MSTAT-C (Michigan State University, v. 2.10) statistical program. The means were separated by Duncan's multiple range test at $P<0.05$ level.

3. Results

Analysis of variance showed that significant differences among safflower cultivars were determined for stomata density. The number of abaxial stomata ranged from 143 to 72 number mm^{-2}, while the number of adaxial stomata varied from 135 to 57 number mm^{-2} (Table 2). Zirkon and Linas gave lower stomata numbers than the others and their stomata number did not decrease by salinity. Our results revealed that the stomata density was changed by safflower cultivars. Similar findings were reported by Ergin et al. (2021b) and Roudbari et al. (2012), who indicated that stomata density was a genetic characteristic and the genotypes possessed different stomata densities. Ergin et al. (2021b) observed higher stomata number per mm^{-2} in safflower cultivars Balcı, Dinçer 5-18-1, Linas, Olas, and Yekta than our results because they used the plants grown at later stages. Under salt stress, the stomata density on abaxial and adaxial leaf surfaces was significantly lower than in control plants. The highest decrease in stomata density on abaxial and adaxial leaves occurred in cv. Safir. Although the number of abaxial stomata of Dinçer 5-18-1 and Linas increased, both the abaxial and adaxial stomata number of cv. Zirkon increased when the salinity was applied.

Table 2. Changes in stomata density (number mm^{-2}) of safflower cultivars under saline (100 mM NaCl) and non-saline (control) conditions

Cultivars	Abaxial		Adaxial	
	Control	100 mM NaCl	Control	100 mM NaCl
Dinçer 5-18-1	102^{ef}	112^{de}	90^{cd}	89^{cd}†
Remzibey-05	119^{cd}	72^{g}	74^{fgh}	$63^{ıj}$
Balcı	120^{cd}	72^{g}	84^{de}	$64^{ıj}$
Yekta	105^{ef}	76^{g}	86^{cde}	$70^{ghı}$
Linas	99^{f}	101^{ef}	79^{ef}	57^{j}
Olas	143^{a}	100^{ef}	115^{b}	95^{c}
Olein	129^{bc}	93^{f}	87^{cde}	74^{fgh}
Safir	141^{ab}	74^{g}	135^{a}	$66^{hıj}$
Zirkon	95^{f}	98^{f}	$62^{ıj}$	75^{fg}
Mean	117^{a}	89^{b}	90^{a}	73^{b}
Analysis of Variance				
Salinity (A)	**		**	
Cultivar (B)	**		**	
A×B	**		**	

†Means followed by the same letter(s) are not significantly different at P<0.05. ** Significant at 1%.

The stomata images of the investigated safflower cultivars were displayed in Figure 1 and it can be easily understood that the number of abaxial stomata was reduced by salt stress. Also, we determined that the salinity caused a reduction in stomatal density on the abaxial part of 6 safflower cultivars. In previous studies, a decreased stomata density in salt-affected plants was reported by Hamani et al. (2021) in cotton, Çavusoğlu et al. (2007) in barley, El-Kady et al. (2021) in sugar beet, and Dikobe et al. (2021) in maize. As expected, the adaxial part of the leaves had a lower number of stomata than the abaxial surface.

The effect of salt stress on the width of the abaxial stomata was significant (Table 3). The stomata width on the adaxial part was not changed by salinity, while it was decreased in the abaxial. However, Remzibey-05, Yekta, and Safir had similar stomata width on the abaxial surface. Adaxial stomata width was narrowed in all cultivars except Dinçer 5-18-1, Remzibey-05, and Olein cultivars. To regulate water balance during salt stress, the plants reduce evaporation by closing the leaf stomata. Thus, variations in stomatal morphology and physiology can be considered the first defensive reactions or acclimation mechanisms against salinity (Kiani-Pouya et al., 2020; Yan et al., 2020). A decrease in stomatal width was recorded by Cavusoglu et al. (2007) in barley and Hamani et al. (2021) in cotton.

Under salt stress, the stomata length of safflower cultivars was significantly varied. The abaxial stomata length of cv. Yekta (33.6 µm) and the adaxial stomatal length of cv. Balcı (35.4 µm) were superior to the other cultivars (Table 4). Generally, the abaxial stomatal length was enhanced under salinity, while the adaxial stomatal length was decreased. Safflower cultivars Dinçer 5-18-1, Balcı, Olas, and Olein shortened their abaxial stomatal length under salt stress. However, the adaxial stomatal length of safflower cultivars was severely depressed by salt stress, while it was slightly increased in Remzibey-05, Olein, and Safir. These results are in agreement with the findings of Kiliç and Kahraman (2016), who determined a 24% reduction in stomata length of barley under salt stress. Roudbari et al. (2012) stated that there were limited significant changes in stomata length of 15 safflower genotypes, but the length of stomata was shortened by drought.

There was a significant difference in the interaction of salinity × cultivar for stomata size of abaxial and adaxial parts. Under salinity, Yekta showed a 14.8% increase in stomata size, while Dinçer 5-18-1 cultivar possessed 20.6% smaller stomata than the others (Table 5). The largest adaxial stomata size was measured in cv. Balcı (724 μm^2), but Yekta had the smallest stomata (470 μm^2). A clear negative correlation between the size of stomata and sensitivity to salinity in cotton by Munis et al. (2010) and in bean by Bray and Reid (2002) was reported. In our study, the negative and significant correlation between stomata density and size in control and salinity was calculated as r= -0.508** and r= -0.690**, respectively. This shows that increasing the stomata number caused a decrease in stomata size.

No significant changes were determined in the abaxial stomata index, and it was measured between 1.34 and 1.12 (Table 6). However, salinity led to a reduction in the stomatal index of the adaxial layer. The highest decrease was calculated in cv. Olein with 13%. These results agree with the findings of Bray and Reid (2002), who reported significant differences in the stomata index of bean.

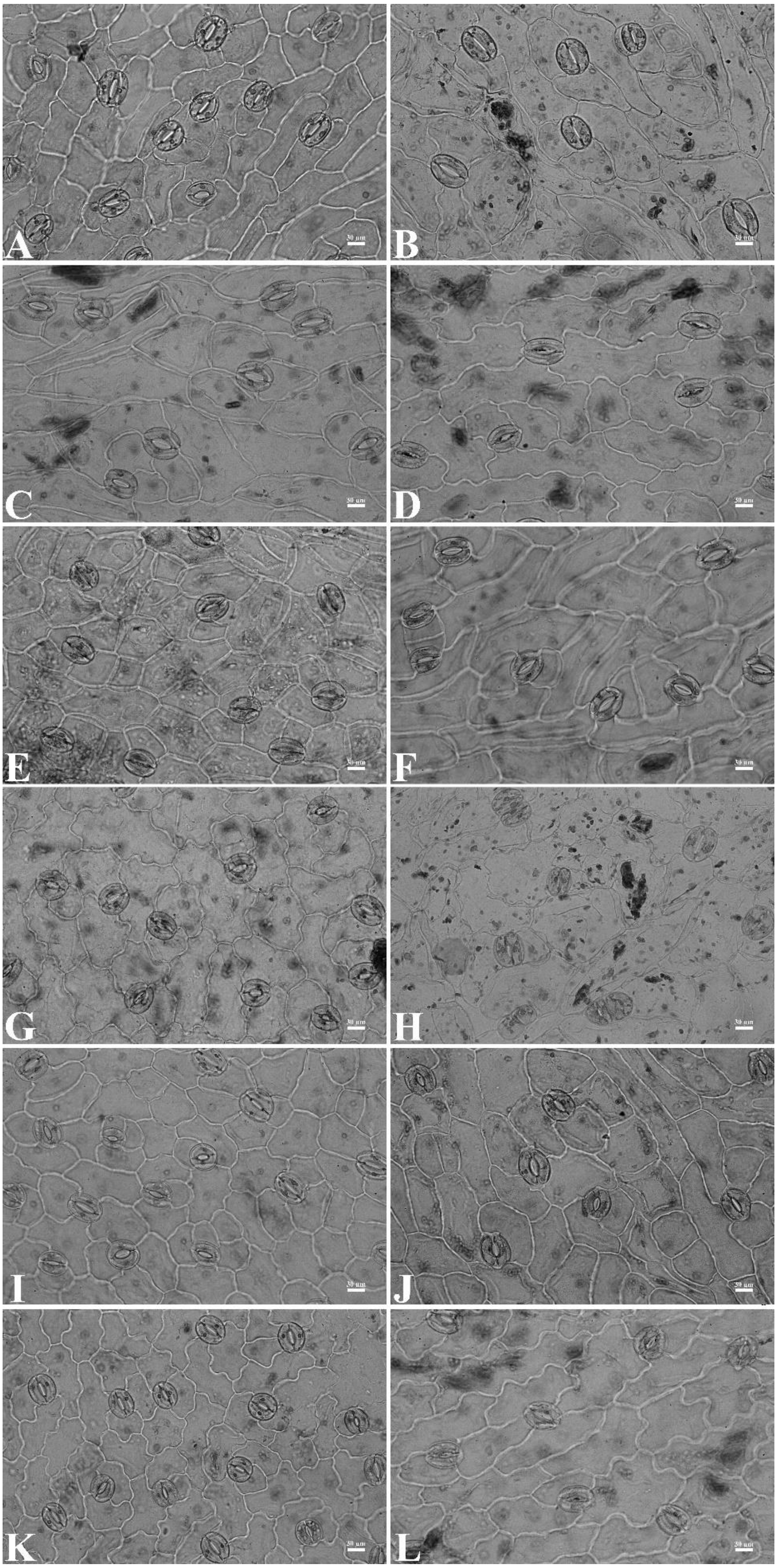

Figure 1. The abaxial stomata images of safflower cvs. Remzibey-05 (A), Balcı (C), Yekta (E), Olas (G), Olein (I), and Safir (K) in control on the left column, and salt-stressed cvs. Remzibey-05 (B), Balcı (D), Yekta (F), Olas (H), Olein (J), and Safir (L) on the right column.

Table 4. Changes in stomata length (µm) of safflower cultivars under saline (100 mM NaCl) and non-saline (control) conditions

Cultivars	Abaxial		Adaxial	
	Control	100 mM NaCl	Control	100 mM NaCl
Dinçer 5-18-1	32.4^{abc}	28.7^{f}	30.4^{def}	28.9^{fgh}†
Remzibey-05	30.9^{cde}	32.7^{ab}	$28.0^{hı}$	31.4^{bcd}
Balcı	32.5^{abc}	31.8^{b-e}	35.4^{a}	30.1^{def}
Yekta	30.6^{e}	33.6^{a}	31.3^{cd}	30.4^{de}
Linas	28.9^{f}	32.3^{a-d}	29.7^{efg}	29.2^{e-h}
Olas	28.9^{f}	28.4^{f}	32.6^{bc}	$28.6^{ghı}$
Olein	32.1^{a-e}	30.6^{e}	$28.3^{ghı}$	29.7^{efg}
Safir	28.7^{f}	31.0^{cde}	$27.5^{ı}$	29.7^{efg}
Zirkon	30.7^{de}	32.0^{a-e}	32.7^{b}	$28.0^{hı}$
Mean	30.6^{b}	31.2^{a}	30.6^{a}	29.6^{b}
Analysis of Variance				
Salinity (A)	**		**	
Cultivar (B)	**		**	
A×B	**		**	

†Means followed by the same letter (s) are not significantly different at P<0.05. **Significant at 1%.

Table 5. Changes in stomata size (µm^2) of safflower cultivars under saline (100 mM NaCl) and non-saline (control) conditions

Cultivars	Abaxial		Adaxial	
	Control	100 mM NaCl	Control	100 mM NaCl
Dinçer 5-18-1	698^{a}	$554^{ghı}$	558^{ef}	574^{de}†
Remzibey-05	615^{c-f}	654^{abc}	510^{g}	632^{bc}
Balcı	686^{ab}	634^{b-f}	724^{a}	571^{e}
Yekta	578^{e-h}	664^{abc}	581^{de}	564^{ef}
Linas	591^{d-h}	619^{c-f}	615^{bcd}	590^{de}
Olas	$543^{hı}$	$512^{ı}$	644^{b}	513^{g}
Olein	613^{c-g}	581^{e-h}	470^{h}	599^{cde}
Safir	572^{fgh}	629^{b-f}	517^{g}	562^{ef}
Zirkon	651^{a-d}	639^{a-e}	647^{b}	527^{fg}
Mean	616	610	585^{a}	570^{b}
Analysis of Variance				
Salinity (A)	ns		*	
Cultivar (B)	**		**	
A×B	**		**	

†Means followed by the same letter (s) are not significantly different at P<0.05. *, **Significant at 5% and 1%, respectively.ns= non-significant.

Table 6. Changes in stomata index of safflower cultivars under saline (100 mM NaCl) and non-saline (control) conditions

Cultivars	Abaxial		Adaxial	
	Control	100 mM NaCl	Control	100 mM NaCl
Dinçer 5-18-1	1.18	1.17	1.30^{a-d}	1.15^{gh}†
Remzibey-05	1.22	1.29	1.21^{d-h}	1.23^{c-g}
Balcı	1.21	1.26	1.36^{a}	1.25^{b-f}
Yekta	1.28	1.34	1.33^{ab}	1.29^{a-d}
Linas	1.12	1.21	1.12^{h}	1.14^{h}
Olas	1.21	1.24	1.30^{a-d}	1.25^{b-e}
Olein	1.33	1.28	1.34^{a}	1.16^{fgh}
Safir	1.13	1.20	1.15^{gh}	1.23^{c-g}
Zirkon	1.14	1.26	1.31^{abc}	1.17^{e-h}
Mean	1.20^{b}	1.25^{a}	1.27^{a}	1.21^{b}
Analysis of Variance				
Salinity (A)	**		**	
Cultivar (B)	**		**	
A×B	ns		**	

†Means followed by the same letter (s) are not significantly different at P<0.05. **Significant at 1%. ns= non-significant.

In our study, abnormal stomata were monitored and it was determined that some cultivars had contiguous (twin) stomata under salt stress, while abnormal stomata in non-saline conditions were detected in 3 of 9 cultivars (Figure 2A-P). Dinçer 5-18-1, Remzibey-05, Yekta, Olein, and Zirkon cultivars had contiguous stomata only in the presence of saline stress. The contiguous stomata were observed in both control and salt-stressed plants of Linas, Olas, and Safir cultivars. As seen in Figure 2G, Linas had abnormal stomata in control plants. On the other hand, no contiguous stomata were determined in cv. Balcı, indicating that abnormal stomata were mainly determined by genetic factors and were secondarily affected by salinity. Abnormal stomatal patterning has been recorded in *Brassicaceae*, *Asteraceae, Crassulaceae*, *Iridaceae*, *Leguminosae*, *Sonneratiaceae*, and *Moracea*e (Gan et al., 2010; Khan et al., 2018; Choi et al., 2022). Abnormal stomata have been found in many plants grown in arid, salty, or otherwise adverse environments, similar abnormality was also detected in a halophyte, *Sonneratia alba* J. Smith (Gan et al., 2010; Khan et al., 2018).

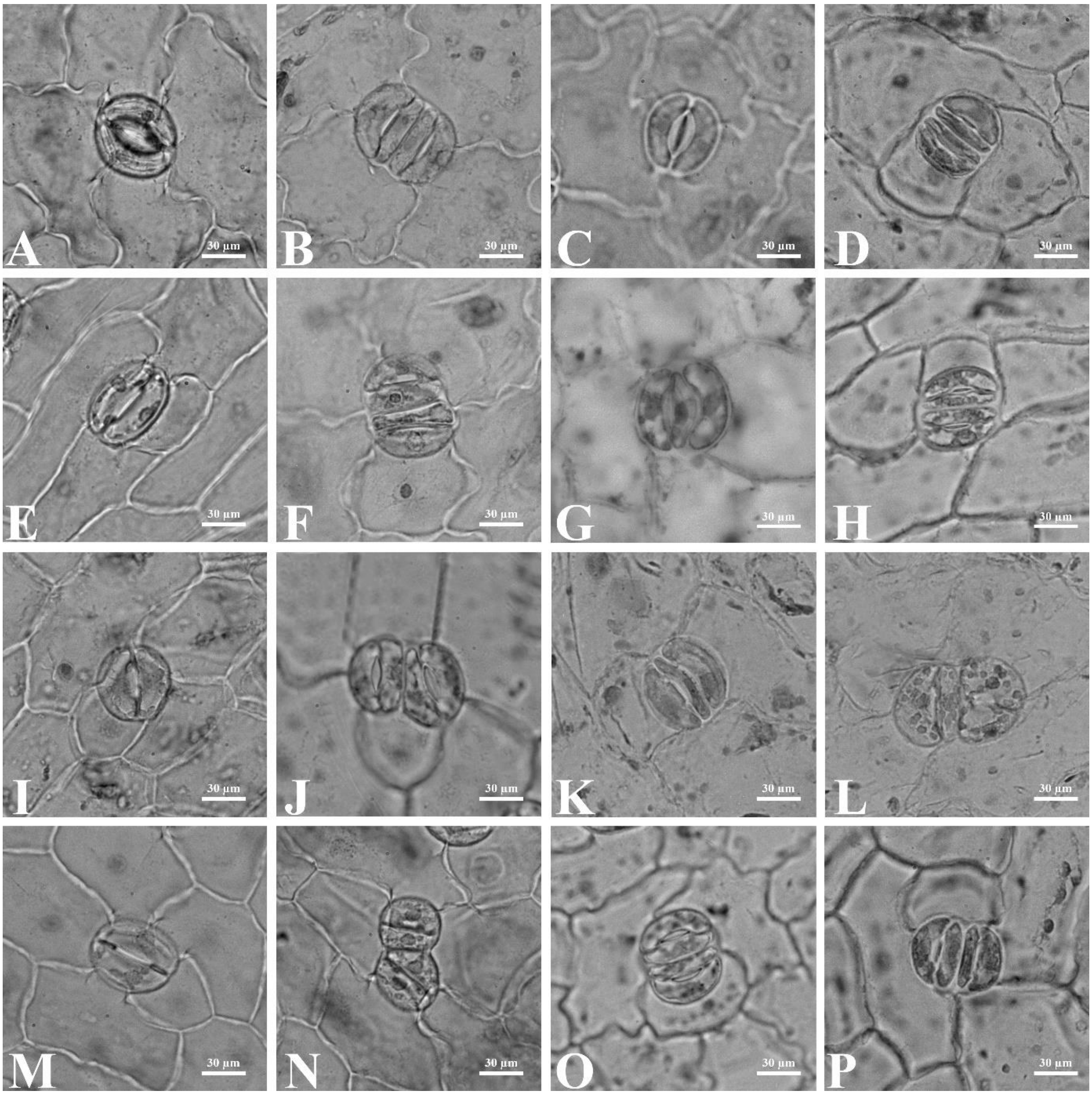

Figure 2. The abaxial contiguous stomata images of cvs. Dinçer 5-18-1 (A), Remzibey-05 (E), Yekta (I), Olein (M), Zirkon (C), Linas (G), Olas (K), and Safir (O) in control on first and third columns, and salt-stressed cvs. Dinçer 5-18-1 (B), Remzibey-05 (F), Yekta (J), Olein (N), Zirkon (D), Linas (H), Olas (L), and Safir (P) on the second and fourth columns.

4. Conclusion

The stomatal characteristics of the plants may vary with genetic and environmental factors. Therefore, determining the stomatal characteristics is very important to classify the adaptation level of plant varieties to adverse soil and climatic conditions. This study showed significant differences in stomata number and size of safflower cultivars; moreover, the stomata densities in cvs. Remzibey-05, Balcı, Yekta, Olas, Olein, and Safir were reduced by salinity. The number of stomata of safflower cultivars with low stomata density in control did not significantly change by salinity, considering that low stomata number is a hopeful indicator for tolerance to salinity in safflower. Salt stress altered the stomata size with significant reductions in cvs. Dinçer 5-18-1, Balcı, Olas, Olein, and Zirkon. However, abnormal stomata were observed in five cultivars exposed to salt stress. This study provides evidence that stomata anatomy may give useful clues for the salinity tolerance of safflower cultivars, so the stomata observations should be evaluated in further studies on salinity.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	N.E.	M.D.K.
C	50	50
D	50	50
S	30	70
DCP	80	20
DAI	70	30
L	50	50
W	50	50
CR	30	70
SR	80	20
PM	50	50
FA	20	80

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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