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Morphological and anatomical characterization of leaves in *Aegilops triuncialis* L. specimens subjected to osmotic stress

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Abstract: The aim of this study was to assess the degree of variation between 5 accessions of barbed goat grass (*Aegilops triuncialis* L) in terms of some basic morphological and anatomical characteristics of the leaves during the seedling stage, under osmotic stress induced by 0.5 M and 1 M sucrose solutions. Significant variations were found in both osmotic stress variants with respect to length and width of stoma, length and width of epidermal cells, length and width of first and second leaves, specific leaf area of the first and second leaves. The greatest reduction in stomatal length and width, as well as epidermal cell width, was found in the 1 M solution of sucrose for 44365, while in terms of epidermal cell length for BGR43679. The highest relative changes caused by the highest osmotic stress for leaf length and width were recorded for BGR43307. PC-analysis grouped accessions according to similarity on the basis of investigated morphological and anatomical characteristics of leaves in two components in the factor plane.

Key words: *Aegilops triuncialis* L.; osmotic stress; anatomy of leaf; morphology of leaf; PC-analysis; variation

INTRODUCTION

Water availability is a critical environmental factor affecting plant growth, development, and productivity. Osmotic stress, arising from drought, salinity, or other factors that reduce water potential, triggers a cascade of physiological and biochemical responses in plants (Dos Santos et al., 2022). Understanding these responses is crucial for improving crop resilience in the face of increasing global water scarcity (Balasubramaniam et al., 2023).

Plants exhibit a variety of adaptations to mitigate the effects of osmotic stress, including changes in root architecture, osmotic adjustment, and stomatal regulation (Blum, 2010). At the cellular level, modifications in epidermal cells and stoma play a significant role in controlling water loss through transpiration. Epidermal cells, forming the outermost protective layer of the leaf, can influence water retention through changes in cell wall thickness, cuticular wax deposition, and cell size (Kerstiens, 1996). Stoma, specialized pores formed by guard cells, regulate gas exchange and transpiration (Zeng et al., 2010). Stomatal closure is a rapid response to water stress, reducing water loss but also limiting CO_2 uptake for photosynthesis (Falquetto-Gomes et al., 2024).

Aegilops is a genus of the *Poaceae* family and tribe *Tritaceae*. It consists of nearly 20 annual plant species. *Aegilops* species, are frequently utilized in hybridization programs with the objective of enhancing wheat's resilience to abiotic stresses (Itam et al., 2020; Kiani et al., 2021; Ayaz et al., 2025), due to their wide adaptability to many different climates, including drought/

heat environments, various disease hot spots, and nutrient-poor regions (Kishii et al., 2019). *Aegilops triuncialis* L is known for its adaptability to harsh environmental conditions. It is an allotetraploid, with a UUCC genomic formula (2n = 28) and with a basic chromosome number (x = n = 7), originating from the hybridization of *Aegilops umbellulata* Zhuk. and *Aegilops markgrafii* (Greuter) Hammer. In Europe *Ae. triuncialis* L. is one of the most widespread species together with the species *Ae. biuncialis* Vis., *Ae. cylindrica* Host, *Ae. geniculata* Roth, *Ae. neglecta* Req. ex Bertol. (Khodaee et al., 2021; Feldman and Levy, 2023). It demonstrates robust drought tolerance through physiological adaptations and genetic mechanisms, making it a promising candidate for enhancing drought resistance in wheat (Khodaee et al., 2021).

Aegilops triuncialis L., like other *Aegilops* species, undergoes significant morphological and anatomical changes under osmotic stress. These changes include variations in stomatal density (Kharazian, 2007), increased protective tissue dimensions (Zhang et al., 2023; Wasim et al., 2022), and enhanced mesophyll development (Terletskaya and Kurmanbayeva, 2017), all of which contribute to improved water conservation and photosynthetic efficiency. These adaptations are crucial for the plant's survival and productivity under adverse environmental conditions. Osmotic stress often leads to changes in leaf thickness and the structure of vascular bundles. For instance, in other grass species, increased leaf thickness and modifications in the vascular bundle area have been observed as adaptive responses to stress. Azmat et al. (2009) and Wasim et al. (2022) noted that the presence and density of trichomes (leaf hairs) can increase under stress conditions, which helps in reducing water loss and protecting against environmental stressors. Understanding the responses of *A. triuncialis* L. to osmotic stress is essential for developing strategies to mitigate its effects. Therefore, the aim of this study was to assess the degree of variation between five accessions of barbed goat grass (*Aegilops triuncialis* L) in terms of some basic morphological and anatomical characteristics of the leaves during the seedling stage, under osmotic stress induced by 0.5 M and 1 M sucrose solutions.

MATERIALS AND METHODS

Five populations of barbed goat grass (*Aegilops triuncialis* L.) originating from Bulgaria, maintained in the *ex-situ* collection of the National Seed Gene-bank at the Institute of Plant Genetic Resources "Konstantin Malkov"-Sadovo, Bulgaria, were evaluated for different morphs-anatomical characteristics under osmotic stress induced by sucrose during the early seedling stage. Seeds of all genotypes were sterilized and germinated on the moist filter paper in Petri dishes with 20 ml of distilled water in a thermostat for 72 hours, at 20ºC, in dark. After germination, seedlings of each genotype were sown in pots filled with soil and grown in a climate chamber under controlled conditions 25/20°C day/night temperature, 16 h photo period, and 60% relative humidity. Two-week-old seedlings were subjected to osmotic stress treatments using sucrose solutions. Moderate osmotic stress was induced with 0.5 M sucrose solution, which provoked osmotic stress with a pressure of 12.23 ATM, while the strong osmotic stress was induced with 1 M sucrose solution, which provoked osmotic stress with a pressure of 24.45 ATM. Control plants were grown in distilled water (0 M solution of sucrose). Plants were watered every second day with 40 ml of the respective solution for two weeks, distilled water was used in the control. The sucrose solutions were renewed every 2 days to maintain consistent osmotic potential. Then, 10 biometric measurements per replicate were performed on the length and width of the first and second leaves. Specific leaf area was calculated by the formula of Kerin at all. (1997), Chanda and Singh (2002) and Berova et al. (2004), A=k.l.b, where: k- coefficient, different for each genera (0.65); l - length of the leaf along the central vain; b - maximum leaf width.

The first leaves were immersed for 24 hours in ethyl alcohol before being used for microscopic observations of the epidermal cells. Epi-

dermal peels were gently removed from the ab axial (lower) surface of the leaves. The peels were mounted on microscope slides in distilled water and observed under light microscope Olympus CX22LED, with total magnification 400. The following characteristics of the first leaves were analyzed: length and width of stomata and length and width of epidermal cells.

The relative changes caused by osmotic stress on traits were calculated as follows:

RC = (Xww − Xws)/Xww ×100, where Xww and Xws are the means of a trait in a given accession under well-watered and osmotic stress treatments, respectively (Ahmadi et al., 2018).

The experiment was designed as a completely randomized design. Data were analyzed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for multiple comparisons. Statistical significance was determined at $p < 0.05$. PC-analysis was applied to group accessions according to similarity on the basis of morphological and anatomical characters in two components in the factor plane. Statistical analyses were performed using the statistical program SPSS 19.0.

RESULTS AND DISCUSSIONS

Osmotic stress, commonly caused by drought or high salinity, leads to water deficit within plant tissues, impacting physiological and structural traits. Leaves, being the primary sites for photosynthesis, exhibit morphological and anatomical changes that reflect the plant's response to such stress.

In Figure 1 are presented the results from the biometric analysis of the morphological characteristics of the leaves - length and width of first and second leaves (cm). Osmotic stress induced a significant reduction in leaf length and width compared to control plants.= However, the extent of reduction varied significantly among accessions. Length of the first leaf in the control variant varied between 10.26 cm for BGR43669 and 13.72 cm for BGR43665, while for the second leaf between 6.38 cm for BGR43679 and 11.00 cm for BGR43665. In the variant with moderate osmotic stress induced with 0.5 M sucrose solution the length of the first leaf ranged between 9.60 cm for BGR43669 and 13.27 cm for BGR43665, while the length of the second leaf varied from

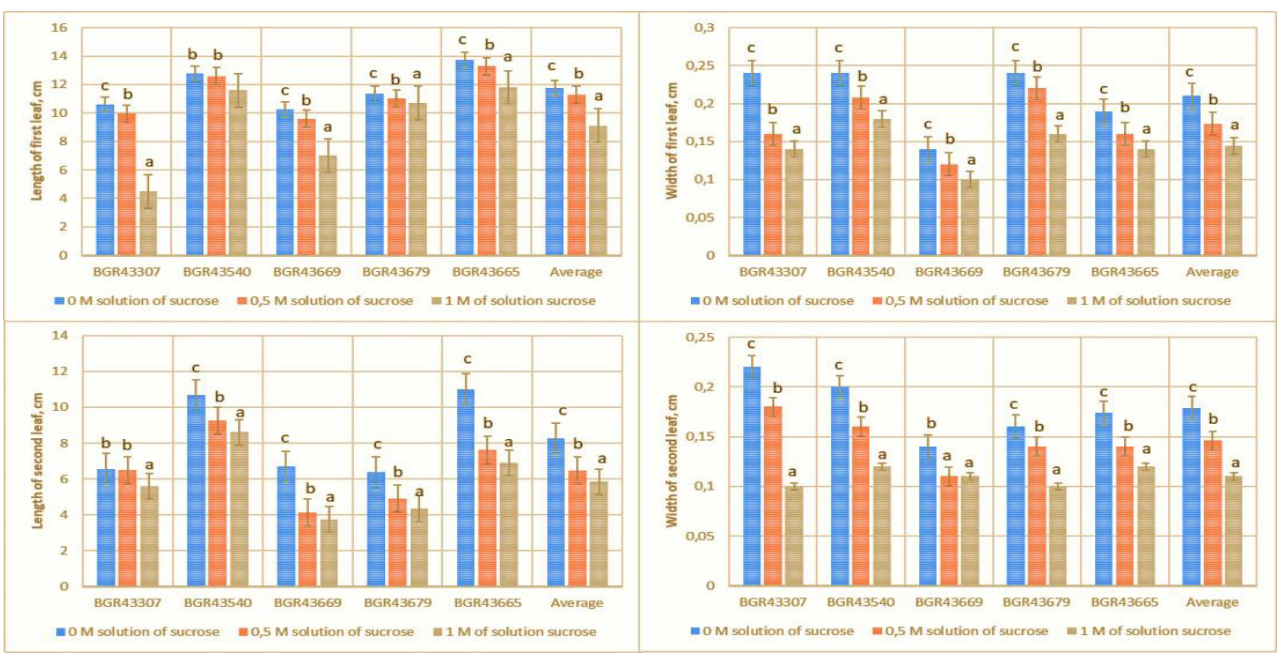


Figure 1. Variation in length and width of first and second leaves at different levels of osmotic stress. Bars (Mean value ± St. Error) with different letters in the group are significantly different from each other according to Duncan's Multiple Range Test at $p < 0.05$

4.14 cm for BGR43669 to 9.26 for BGR43540. In the trial with strong osmotic stress induced with 1 M sucrose solution the lengths varied respectively between 4.50 cm for BGR43307 and 11.80 cm for BGR43665 in the first leaves and between 0.10 cm and 0.12 cm for the second leaves. The recorded mean values of the width of the first and the second leaves were 0.21 cm and 0.18 cm in the variant with 0 M solution of sucrose, 0.17 cm and 0.15 cm in the variant with 0.5 M solution of sucrose and 0.14 cm and 0.11 cm in the variant with 1M solution of sucrose. The lowest width of the first leaf was found in the 1 M solution of sucrose for BGR43669, while for the second leaf for BGR43679. The largest value of width of the first leaf was recorded for BGR43307, BGR43540 and BGR43679 (0.24 cm), while for the second leaf for BGR43307 at 0 M of sucrose.

As shown in Figure 2, significant differences in the reduction of leaf length and width were observed in the treatments with different sucrose doses compared to the control variant of the studied genotypes. The highest relative changes in the studied leaf characteristics, were observed in the trial with strong osmotic stress (1M solution of sucrose). At the highest osmotic stress, the reduction of leaf length varied between 1.41% (BGR43540) to 5.86% (BGR43307) for the first leaf and between 0.91% (BGR43307) and 38.02% (BGR43669) for the second leaf. Similar to leaf length, osmotic stress significantly reduced leaf width in all *Aegilops* accessions.= Again, significant variations were observed among accessions in their response to osmotic stress. Tolerant accessions maintained a relatively larger leaf width under osmotic stress compared to sensitive accessions.= At the highest osmotic stress, the reduction of leaf width varied between 25.00% (BGR43540) to 41.67% (BGR43307) for the first leaf and between 21.43% (BGR43669) and 54.55% (BGR43307) for the second leaf.

A maximum change in leaf area of the first leaves recorded under moderate stress and strong stress was 38.16% and 75.11% in BGR43307, while the minimum reduction change for the same character was observed in BGR43665, which was 8.86 under moderate stress and in BGR43540, which was 32.04% under strong stress. The highest relative changes in the leaf area of the second leaf were found in BGR43679 under 0.5 M sucrose solution stress (52.09%) and in BGR43665 and BGR43307 under 1 M sucrose solution stress (61.75% and 61.17%) (Figure 3).

The changes in leaf morphology under osmotic stress are consistent with previous findings on plant responses to water deficit (Xu et al., 2009;

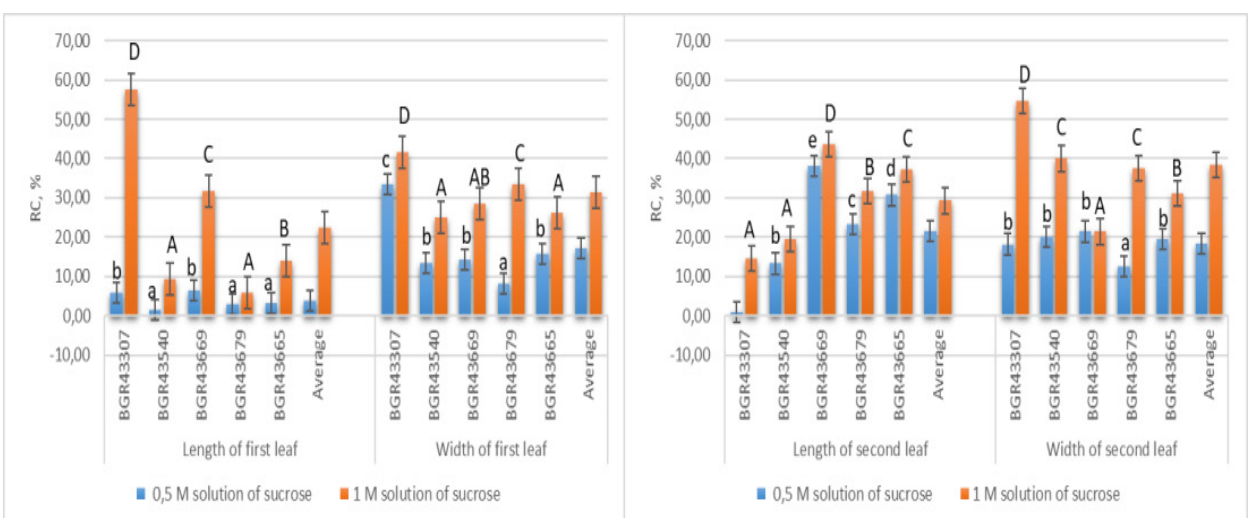


Figure 2. Variation in the relative change index (RC, %) of length and width of the leaves at different levels of osmotic stress. Bars (Mean value ± St. Error) with different letters in the osmotic variant are significantly different from each other according to Duncan's Multiple Range Test at $p < 0.05$

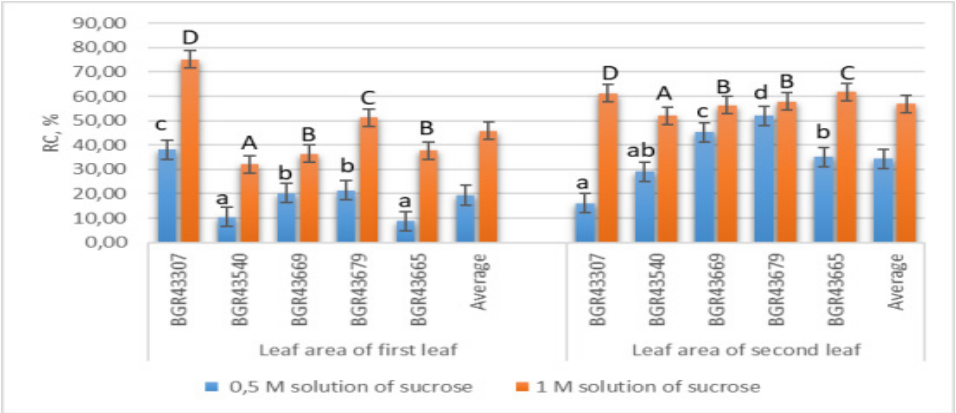


Figure 3. Variation in the relative change index (RC, %) of specific leaf area at different levels of osmotic stress. Bars (Mean value ± St. Error) with different letters in the osmotic variant are significantly different from each other according to Duncan's Multiple Range Test at $p < 0.05$

Galmés et al., 2007). The decrease in leaf area and leaf length is often observed as a mechanism to reduce transpiration surface and minimize water loss. The increase in specific leaf area in some accessions may reflect a shift towards thinner leaves, which can enhance photosynthetic efficiency under stress conditions (Yavas et al., 2024).

Osmotic stress significantly affected epidermal cell length and width, and stoma length and width in *A. triuncialis* leaves (Table 1, Figure 4). Ab axial epidermal cell length and width decreased with increasing osmotic stress. The greatest reduction was recorded in the highest osmotic stress treatment. Significant variation between accessions was also observed.= The average epidermal cell length in control seedlings was 281.90 µm, while in treated seedlings it was 230.67 µm and 197.73 µm, respectively. Similarly, the average epidermal cell width in control seedlings was 28.83 µm, while in treated seedlings it was 23.95 µm and 19.83 µm, respectively.=In addition, osmotic stress significantly decreased the length and width of stoma.= The average stoma length in control seedlings was 61.56 µm, while in treated seedlings it was 56.93 µm and 51.96 µm, respectively. Similarly, the average stoma width in control seedlings was 8.53 µm, while in treated seedlings it was 7.60 µm and 6.91 µm, respectively (Table 1).

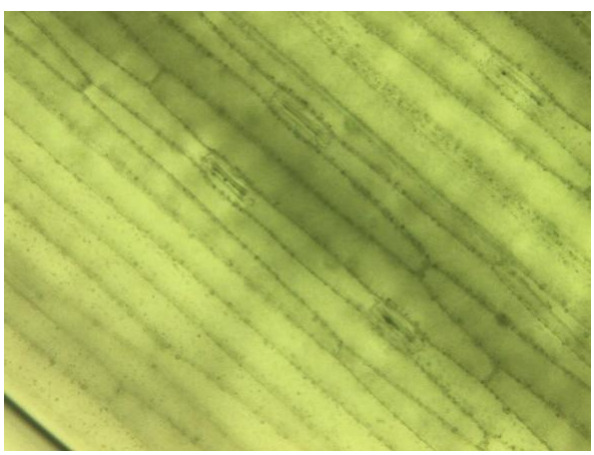


Figure 4. Epidermal cells and stomatal complex in *Aegilops triuncialis* L. (BGR43307) at 1 M sucrose solution

The lowest calculated relative changes in cell length in the highest osmotic stress experiment were observed for BGR43665 (4.79%), while the highest for BGR43679 was (38.98%), with statistical differences from the other samples in the experiment being proven at $p \leq 0.05$, The reduction in cell width was the lowest for BGR43307 (22.01%) and the highest for BGR43669 (38.75%) (Figure 5).= According to Turner (1979) smaller cells might be more resistant to cell wall collapse under drought conditions. This is consistent with findings in other plant species where smaller epidermal cells were associated with increased

drought tolerance (Lu et al., 2022; Gunnula et al., 2023). The relative changes in stomatal size varied between 9.14% (BGR43669) and 23.56% (BGR43665) for stoma length and from 13.05% (BGR43679) to 27.29% (BGR43669) for stoma width (Figure 5).

The results of the study showed that osmotic stress negatively affects the epidermal cell length and width and stomatal size in the investigated accessions of *Aegilops triuncialis* L. The reduction in epidermal cell length and width may be due to the decreased turgor pressure caused by osmotic stress, which leads to a decrease in cell expansion. Similarly, the reduction in stomatal size may be due to the decreased turgor pressure, which leads to a decrease in stomatal opening and closure. Our results are consistent with those of Wyka et al. (2019) and Ayaz et al. (2025).

The decrease in epidermal cell length and width and stomatal size may have negative implications for plant growth and development. Smaller cells and stomata may result in reduced photosynthesis, transpiration, and water use efficiency. Additionally, smaller stomata may be more susceptible to damage from environmental stresses such as high wind and temperature. (Ayaz et al., 2025; Li et al., 2021; Caine et al., 2019)

Principal Component Analysis (PCA) is a powerful multivariate statistical technique used to reduce the dimensionality of data and identify the principal components that explain most of the variance in a data set (Jolliffe and Cadima, 2016). In plant breeding research, PCA is widely used to analyze morphological and physiological data to understand the relationships among different plant traits and classify accessions based on their similarity (Jackson, 1991). PCA can effectively reveal the key traits that contribute most to the observed variation and help in identifying superior genotypes for breeding programs.

In this study the PCA revealed that the first two principal components (PC1 and PC2) ex-

Table 1. Variation in the length and width of stoma and length and width of epidermal cell of first leaf at different levels of osmotic stress

Accession number	Length of stomata, µm			Accession number	Width of stomata, µm		
	0 M solution of sucrose	0,5 M solution of sucrose	1 M solution of sucrose		0 M solution of sucrose	0,5 M solution of sucrose	1 M solution of sucrose
BGR43307	57,33a	47,00a	45,66a	BGR43307	8,66a	8,33bc	7,20bc
BGR43540	71,33c	70,33c	60,50c	BGR43540	10,33b	9,66c	8,66c
BGR43669	63,83b	62,33b	58,00c	BGR43669	7,33a	5,33a	5,33a
BGR43679	57,33a	55,33ab	51,33b	BGR43679	7,66a	7,33b	6,66b
BGR43665	58,00a	49,66a	44,33a	BGR43665	8,66a	7,33b	6,66b
Average	61,56	56,93	51,96	Average	8,53	7,60	6,91
Accession number	Length of epidermal cell, µm			Accession number	Width of epidermal cell, µm		
	0 M solution of sucrose	0,5 M solution of sucrose	1 M solution of sucrose		0 M solution of sucrose	0,5 M solution of sucrose	1 M solution of sucrose
BGR43307	329,50d	254,66c	212,33b	BGR43307	26,50a	23,40a	20,66a
BGR43540	305,66c	286,00d	204,00a	BGR43540	24,00a	20,66a	18,16a
BGR43669	252,00b	224,33b	189,33a	BGR43669	26,66a	23,33a	16,33a
BGR43679	334,33d	205,66a	204,00a	BGR43679	41,33b	33,33b	28,00b
BGR43665	188,00a	182,66a	179,00a	BGR43665	25,66a	19,00a	16,00a
Average	281,90	230,67	197,73	Average	28,83	23,95	19,83

Different letters within each column indicate significant differences ($p < 0.05$).according to Duncan's Multiple Range Test

plained a substantial proportion of the total variance (PC1: 44.70%, PC2: 27.41%, Cumulative: 72.11%) (Table 2). This suggests that these two components capture the major patterns of variation in leaf morphological and anatomical traits among the *Aegilops triuncialis* genotype,s under osmotic stress. First factor had an important role to justify alteration of length and width of stoma, length of the first leaf, length and width of the second leaf and leaf area of the first and the second leaves, while second factor was in positive correlation with length and width of epidermal cell and width of the first leaf. The two factors had an almost equal role on the manifestation of the width of the second leaf.

The biplot generated from the PCA effectively distinguished the *Aegilops triuncialis* genotypes based on their scores on PC1 and PC2. Accessions with similar leaf morphological and anatomical characteristics under osmotic stress clustered together in the bi-plot, indicating that they share similar adaptation strategies to water deficit. The accessions grouped in the upper left quadrants had positive values for PC2 and negative values for PC1. The samples classified in the upper right quadrants had positives values for both factors (PC1 and PC2). Accessions in the lower left quadrants had respectively negative values for both factors. The samples in the lower right quadrants are characterized with negative values for PC2 and positive values for PC1. PC analysis identify BGR43540, which exhibited

Table 2. Weighted factors (PC1 and PC2) of descriptive characteristics on the rotated matrix with two factors

Characteristics	Component	
	PC1	PC2
Length of stoma	**0,66**	0,22
Width of stoma	**0,76**	0,42
Length of epidermal cell	0,33	**0,81**
Width of epidermal cell	-0,21	**0,87**
Length of the first leaf	**0,66**	0,16
Width of the first leaf	0,42	**0,82**
Length of the second leaf	**0,87**	0,05
Width of the second leaf	**0,66**	**0,58**
Leaf area of the first leaf	**0,91**	-0,06
Leaf area of the second leaf	**0,81**	0,24
Eigen values	4,47	2,74
Proportional variance, %	44,70	27,41
Cumulative variance, %	44,70	72,11

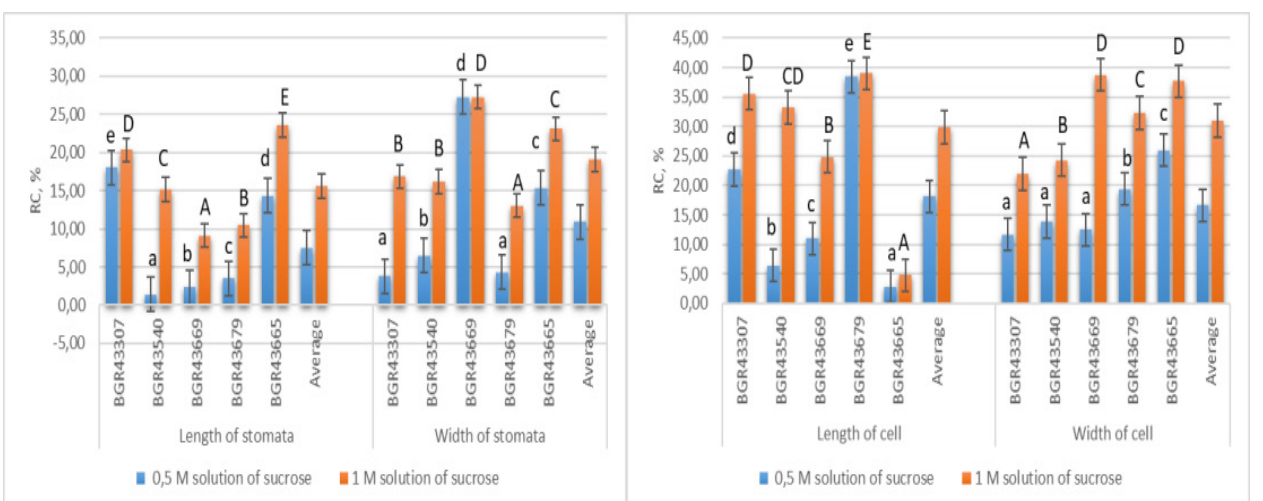


Figure 5. Variation in the relative change index (RC, %) of length and width of stoma and length and width of ab axial epidermal cell at different levels of osmotic stress. Bars (Mean value ± St. Error) with different letters in the osmotic variant are significantly different from each other according to Duncan's Multiple Range Test at $p < 0.05$

high specific leaf area, stomatal length and width, and relatively reduced epidermal cell size under moderate and strong drought stress, may be particularly promising donors for improving drought tolerance in wheat (Figure 6).

CONCLUSION

The results of this study demonstrate that osmotic stress significantly reduces leaf length and width, specific leaf area, length and width of epidermal cells and stoma length and width in *Aegilops triuncialis* accessions, confirming the sensitivity of leaf development to water deficit. However, the observed variation in the extent of reduction among accessions highlights the genetic diversity within *Aegilops* for osmotic stress tolerance. This study also demonstrates the usefulness of PCA for analyzing leaf morphological and anatomical traits of *Aegilops* accessions under osmotic stress. The PCA effectively grouped the accessions based on their adaptation strategies to water deficit. Future research should focus on validating these findings under field conditions and identifying the genes underlying the observed morphological and anatomical adaptations.

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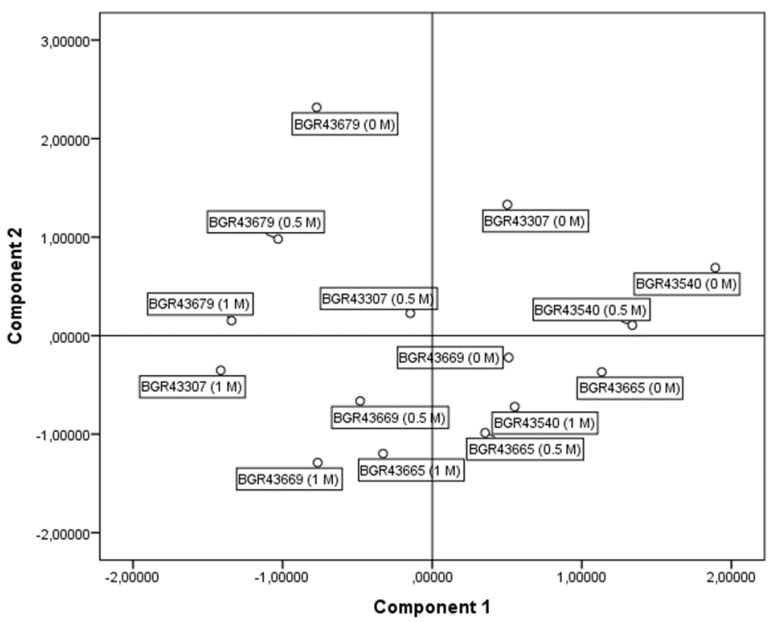


Figure 6. Distribution of evaluated accessions within the factor plane according to similarity of traits: length and width of first and second leaves, leaf area of first and second leaves, length and width of epidermal cells and length and width of stoma under osmotic stress induced by 0 M, 0.5 M and 1 M sucrose solutions

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