Some anatomical characteristics of stomata in four bread wheat cultivars under water stress influence

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Abstract

A field experiment was done at the second agricultural experiment station of the College of Agriculture - Al-Muthanna University, during the agricultural season of 2022-2023. The objective of the study was to investigate the physiological response of different bread wheat varieties to the impact of partial irrigation.

The study employed a randomized complete block design (R.C.B.D) with split plots arrangement and three replications. The main plots consisted of different levels of irrigation deficiency, denoted as D1 (control), D2 (tillering stage), D3 (elongation stage), and D4 (booting stage). The secondary plots included different cultivars, coded as V1 (Mawadah), V2 (Bohuth22), V3 (Baraka), and V4 (Ibaa99).

The statistical analysis yielded considerable disparities among the stages of water stress. The D2 treatment demonstrated superior results in terms of stomatal length on the upper surface (3.720 µm) and the width of stomatal opening on the upper surface (1.300 µm). Following this, the D4 treatment exhibited exceptional performance in terms of stomatal density on the upper surface (147.0 stomata mm^{-2}) and the width of the aperture on the lower surface (2.880 µm).

Regarding the cultivars, Bohuth22 demonstrated superior performance in various anatomical characteristics, including the length and opening of the stomata on the upper surface, as well as the length, width, and opening width of the stomata on the lower surface (measuring 3,760, 4,200, 3,540, 2,800, and 1,340 µm, respectively). On the other hand, the cultivar Mawadah exhibited higher density of stomas. The lower surface had a stomatal density of 115.9 stomata mm^{-2}, while the variety Ibaa99 demonstrated a higher stomatal density of 136.7 stomata mm^{-2} on the upper surface.

Regarding the observed overlap, a notable impact was observed across the majority of features, as evidenced by the superior performance of treatment D1V1 in terms of stomatal density on the lower surface (134.8 stomata mm^{-2}), while the combination D2V1 exhibited superiority in stomatal density on the lower surface (133.3 stomata mm^{-2}). The treatment D4V2 exhibited a significant increase in stomata density (4.240 stomata mm^{-2}) and stomata width (127.4 µm) on the lower surface. Conversely, the combination D4V3 demonstrated superior stomata length (4.480 µm) on the lower surface. Additionally, the treatment D4V4 displayed superiority in stomata density (167.4 stomata mm^{-2}) and stomata length (4.560 µm) on the upper surface, as well as stomata density (125.2 stomata mm^{-2}) on the lower surface.

Based on the aforementioned information, it can be inferred that water stress exerts a detrimental influence on physiological characteristics. This phenomenon is seen as a plant's adaptive response to withstand stress by diminishing transpiration.

Keywords: cultivars, deficient irrigation, Stomata, physiological characteristics, stress.

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Introduction:

The crop commonly referred to as wheat, with its scientific name *Triticum aestivum* L., holds a prominent position among grain crops worldwide due to its great importance, broad cultivation, and large output. Wheat, as a crucial staple food for almost one-third of the global population, occupies the first place in terms of cultivated land area and is ranked second in terms of global output. Global Grains, in adherence to the agricultural tradition of planting yellow corn, (1) In field conditions, the wheat crop frequently encounters a range of biotic and abiotic stresses that exert detrimental effects on the plant. (2) One such stress is water scarcity, which induces a cascade of physiological and biochemical changes that impede plant growth, development, and ultimately, productivity. (3) Water assumes a crucial role in enhancing nutrient absorption and availability, facilitating cell elongation and division, and regulating carbon metabolism. It acts as a solvent and medium for transporting these essential materials to different plant parts. (4) Consequently, the occurrence of water scarcity in the soil, coupled with the resultant decrease in water availability within plant tissues, leads to... Physiological impairment in plants results in diminished growth and productivity (5). This impairment is primarily caused by a decline in the rate of cell division and elongation, as well as reduced enzymatic efficiency (6). The stomata, which control water stress and facilitate gas exchange and transpiration, are crucial in this regard. The size and density of stomatal openings on the leaf surface influence the rate of transpiration and water utilization efficiency. Specifically, a lower stomatal density and smaller stomatal size can enhance the plant's ability to effectively utilize the available water resources (7). The density and size of stomata play a crucial role in determining leaf conductance, which in turn has significant implications for carbon absorption, transpiration, plant water use efficiency, and productivity (8).

Hence, the principal objective of our study is to investigate the physiological responses of cultivars when subjected to the impact of water stress.

Materials and methods

In mid-November of the winter season (2022-2023), a field experiment was conducted at the second agricultural research and experiment station of the College of Agriculture - Al-Muthanna University, located at longitude (45.30) and latitude (31.32). The objective of the experiment was to investigate the physiological response of different bread wheat varieties to the impact of irrigation deficit.

Soil samples were obtained from the field prior to planting, as well as from nine distinct spots on the ground, at a depth ranging from 0 to 30 centimeters. A composite sample was obtained from the subjects and subsequently examined for its physical and chemical properties at the Soil Physics Laboratory within the Department of Soil and Water Sciences, College of Agriculture, University of Al-Muthanna. The results of these analyses are presented in Table (1).

The area was prepared and divided, and the parameters were distributed in a split plot design using a randomized complete block design (RCBD), depending on the data and the nature of the elements included in the study. The planting event occurred on November 1, 2022, with a seed supply of 120 kg per hectare. The fertilization procedures were conducted in accordance with the prescribed guidelines. The study conducted by (9) involved the implementation of irrigation and weeding activities as required.

The study incorporated two variables, namely water stress. The initiation of partial irrigation

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treatment was implemented 40 days post-planting, following the Zadoks scale (10) which includes the control group (without induced stress), as well as the stages of tillering, elongation, and booting.

The second component pertains to the four distinct varieties of bread wheat, namely Mawadah, Bohuth 22, Baraka, and Ibaa99.

Studied traits :In order to determine the stomatal density on both the upper and lower leaf surfaces, We conducted a physiological analysis in the Graduate Studies Laboratory of the Department of Field Crops at Al-Muthanna University's College of Agriculture. The analysis followed a method outlined by a previous study (11) and involved measuring the stomata. The following steps were undertaken for the analysis:

In this study, a number of samples were chosen from a collection of previously conserved specimens. The technique of peeling was employed for further analysis.

The samples that had been prepared were moved to a sterile glass Petri dish that contained a solution of sodium hypochloride with a concentration of 5.1%. This transfer was allowed to occur for a duration of five minutes.

Subsequently, the excised skin samples were carefully positioned onto a specifically prepared glass slide. A droplet of glycerin was then applied, gently spread around the sample, and finally covered with a second glass slide.

The samples underwent examination using an Olympus compound light microscope equipped with two lenses: one with a magnification power of X40 and another with a magnification power of X7. These lenses were calibrated using micrometer slides that measured 0.1 mm.

The study involved the collection of data pertaining to stomatal density (mm2), stomatal length (µm), stomata width (µm), stomata aperture length (µm), and stomata aperture width (µm). These measurements were obtained using an ocular micrometer, and the samples were photographed using a microscope-mounted camera equipped with Vividai software "Ablescope"

Statistical analysis: GenStat 12 was used for statistical analysis, and the Least Significant Difference (L.S.D) test was performed at a significance level of 0.05 to determine whether there were statistically significant differences between the means of the treatments (12).

Table 1 Experimental soil chemical and physical parameters before planting

chemical qualities	) PH(	8.2	—
	) EC(	12.4	Desi Siemens M-1
	N	23.8	mlg kg-1
	P	19.6	
	K	149.1	
physical traits	Sand	62.5	gm kg-1 soil
	Silt	12.5	
	Clay	25	
	soil texture	Clay sandy	

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Results and Discussion

the upper surface of the leaf

1-Density of stomata (stomata mm^{-2})

The findings presented in Table 2 indicate that the water stress treatment during the lining stage (D4) exhibited the highest average stomatal density of 147.0 stomata mm-2. Conversely, the water stress treatment for control (D1) had the lowest average stomatal density of 118.3 stomata mm-2.

The potential explanation for this phenomenon can be attributed to the elevation in proline content in response to heightened water tension. This increase in proline content enables the plant to modulate cellular osmosis (13), thereby promoting cell plumpness and expansion, as well as an augmented stomatal density (14), which aligns with previous findings.

The findings from the aforementioned table indicate a statistically significant impact of the different cultivars on stomatal density. Specifically, the cultivar Ibaa99 V4 exhibited the highest average stomatal density of 136.7 stomata mm-2, whereas the cultivar Buhooth22 V2 displayed the lowest average stomatal density of 121.5 stomata mm-2.

One possible explanation for this phenomenon could be attributed to the finding that Ibaa99 exhibited the highest mean relative water content, resulting in increased leaf plumpness. This, in turn, may have contributed to a higher density of stomata in the leaf.

In terms of the overlap, the interaction between water stress levels and (D4V4) cultivars exhibited the highest mean value of (167.4) stomata mm-2, whilst the (D2V2) treatment demonstrated the lowest mean value of (89.6) stomata mm-2.

Table 2: Effect of water stress and cultivars, as well as their interaction, on Density of stomata (stomata mm^{-2}).

stages of water stress	Cultivars				Average	
	V1	V2	V3	V4		
D1	121.5	94.1	143.0	114.8	118.3	
D2	159.3	89.6	123.0	126.7	124.6	
D3	91.1	147.4	160.0	137.8	134.1	
D4	157.8	154.8	108.1	167.4	147.0	
Average	132.4	121.5	133.5	136.7		
L.S.D (0.05)	D		V		DxV	C.v%
	11.44		10.54		20.43	9.5

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2- stomata length (µm)

The findings presented in Table 3 indicate that the water stress treatment at the ripening stage D2 exhibited the highest mean value of 3.720 µm, whereas the water stress level D1 (used as a control) demonstrated the lowest mean value of 3.020 µm. The potential cause for this disparity can be ascribed to a reaction in response to decreased transpiration. In order to maintain the moisture levels within the leaf, it is necessary to consider the impact of direct solar exposure on the upper surface of the leaf. This exposure leads to increased water loss, exacerbating the existing water stress experienced by the leaf. This observation aligns with the findings presented in reference (15).

The observed cultivars exhibited a notable impact, with Bohuth 22 V2 displaying the greatest mean value of 3.760 µm, while the cultivar Ibaa99 V4 demonstrated the lowest mean value of 3.140 µm.

Table 3: Effect of water stress and cultivars, as well as their interaction, on stomata length (µm)

stages of water stress	cultivars				Average	
	V1	V2	V3	V4		
D1	2.880	3.680	2.720	2.800	3.020	
D2	3.680	4.400	3.440	3.360	3.720	
D3	3.440	3.360	3.440	3.040	3.320	
D4	3.200	3.600	3.280	3.360	3.360	
Average	3.300	3.760	3.220	3.140		
L.S.D (0.05)	D		V		DxV	Cv%
	0.199		0.264		N.S	9.3

3- Stomata width (µm)

The results presented in Table 4indicate that the cultivar Bohuth22 V2 exhibited the highest average of 2,600 µm, whereas the cultivar Mawada V1 displayed the lowest average of 2,200 µm. This discrepancy in average size can potentially be attributed to genetic variations between the two cultivars, which aligns with the findings reported in study (16).

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Table 4: Effect of water stress and cultivars, as well as their interaction, on Stomata width (µm)

stages of water stress	Cultivars				Average	
	V1	V2	V3	V4		
D1	2.400	2.560	2.400	2.240	2.400	
D2	2.080	2.800	2.160	2.400	2.360	
D3	2.400	2.640	2.640	1.840	2.380	
D4	1.920	2.400	2.400	2.400	2.280	
Average	2.200	2.600	2.400	2.220		
L.S.D (0.05)	D		V		DxV	Cv%
	N.S		0.240		N.S	12.1

4- stomata aperture length (µm)

The findings presented in Table 5 indicate that the cultivar Bohuth22 V2 exhibited the highest mean value of 4,200 µm, whereas the cultivar Mawada V1 displayed the lowest mean value of 3,780 µm. This discrepancy in measurements could potentially be attributed to genetic variations between the two cultivars, which aligns with the findings reported by reference 1)7(.

The treatment known as D4V4 demonstrated the greatest average measurement of 4.560 µm, whereas the treatment referred to as D1V1 exhibited the lowest average measurement of 3.680 µm.

Table 5: Effect of water stress and cultivars, as well as their interaction, on stomata aperture length (µm)

stages of water stress	Cultivars				Average	
	V1	V2	V3	V4		
D1	3.680	4.240	3.760	3.680	3.840	
D2	3.760	4.560	4.560	4.000	4.220	
D3	3.760	3.760	3.840	3.840	3.800	
D4	3.920	4.240	3.920	4.560	4.160	
Average	3.780	4.200	4.020	4.020		
L.S.D (0.05)	D		V		DxV	Cv%
	N.S		0.217		0.495	6.5

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5- stomata aperture Width (μm)

The findings from Table 6 demonstrate that the water stress treatment during the tillering stage (D2) yielded the greatest average measurement of 1,300 μm, whereas the control treatment (D1) resulted in the lowest average measurement of 1,140 μm. The observed discrepancy can perhaps be explained by the presence of High proline under stress, which functions to decrease the water potential of leaf cells. This mechanism facilitates the entry of water into the cells, leading to an increase in water content within the leaves. Consequently, this process naturally results in the opening of stomata (18).

Table 6: Effect of water stress and cultivars, as well as their interaction, on stomata aperture Width (μm)

stages of water stress	Cultivars				Average	
	V1	V2	V3	V4		
D1	1.040	1.200	1.120	1.200	1.140	
D2	1.120	1.440	1.360	1.280	1.300	
D3	1.120	1.040	1.040	1.360	1.140	
D4	1.280	1.360	1.360	1.200	1.300	
Average	1.140	1.260	1.220	1.260		
L.S.D (0.05)	D		V		DxV	Cv%
	0.072		N.S		N.S	11.1

bottom surface of the leaf

1-Density of stomata (stomata mm^{-2})

The data presented in Table 7 indicates that the Mawadah V1 variety exhibited the highest mean value of stomata density at 115.9 stomata mm-2, whilst the Baraka V3 variety displayed the lowest mean value at 88.9 stomata mm-2. The observed discrepancy can be attributed to the Mawadah variety having the smallest leafy space, whereas the Baraka cultivar exhibits the largest leafy area. Consequently, a decrease in leafy area corresponds to an increase in stomatal density, likely caused by the absence of interstitial cells between the stomata. Alternatively, the dissimilarity in genetic composition between the cultivars may also account for this variation, which aligns with previous findings (17).

Regarding the phenomenon of interference, it was observed that the interference treatment (D1V1) exhibited the highest mean value of stomatal density, measuring at 134.8 stomata mm-2. Conversely, the treatment (D4V3) shown the lowest mean value of stomatal density, measuring at 83.0 stomata mm-2.

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Table 7: Effect of water stress and cultivars, as well as their interaction, on Density of stomata (stomata mm^{-2})

stages of water stress	Cultivars				Average	
	V1	V2	V3	V4		
D1	134.8	85.9	89.6	92.6	100.7	
D2	133.3	94.8	90.4	117.0	108.9	
D3	103.0	121.5	92.6	116.3	108.3	
D4	92.6	127.4	83.0	125.2	107.0	
Average	115.9	107.4	88.9	112.8		
L.S.D (0.05)	D		V		DxV	Cv%
	N.S		10.37		20.95	11.6

2- Stomata length (µm)

The data presented in Table8 indicates that the cultivar Bohuth 22 V2 exhibited the highest mean value of 3.540 µm, whereas the cultivar IPA 99 V4 displayed the lowest mean value of 3.180 µm. The potential cause for this disparity may be attributed to the inherent characteristics of the genotype, which aligns with the findings reported by (19).

The treatment known as D2V2 demonstrated the highest average of 4,000 µm, whereas the treatment labeled as D3V4 exhibited the lowest average of 2,800 µm. This discrepancy in measurements can perhaps be attributed to variations in the physiological responses of the cultivars to stress phases (20).

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Table 8: Effect of water stress and cultivars, as well as their interaction, on Stomata length (µm)

stages of water stress	Cultivars				Average	
	V1	V2	V3	V4		
D1	3.280	3.600	3.360	3.040	3.320	
D2	2.880	4.000	3.200	3.600	3.420	
D3	3.440	3.520	3.360	2.800	3.280	
D4	3.680	3.040	3.520	3.280	3.380	
Average	3.320	3.540	3.360	3.180		
L.S.D (0.05)	D		V		DxV	Cv%
	N.S		0.1994		0.4267	7.1

3- Stomata width (µm)

The findings presented in Table 9 indicate that the water stress treatment applied during the Booting stage D4 resulted in the highest average stomatal aperture of 2.880 µm. Conversely, the control treatment (D1) exhibited the lowest average stomatal aperture of 2.340 µm. This suggests that stomata on the lower leaf surface are less responsive to environmental factors (21). Furthermore, the relative water content was not significantly influenced by the different stress levels, indicating that the leaf remains intact and unaffected by changes in stomata width.

The cultivar Bohuth 22 V2 had the highest average measurement of 2.800 µm, whilst the cultivar Ibaa99 V4 demonstrated the lowest average measurement of 2.340 µm.

In terms of interference, the treatment labeled as D4V2 exhibited the highest average measurement of 4.240 µm, whereas the treatment labeled as D3V2 exhibited the lowest average measurement of 2.080 µm.

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Table 9: Effect of water stress and cultivars, as well as their interaction, on Stomata width (µm)

stages of water stress	Cultivars				Average	
	V1	V2	V3	V4		
D1	2.400	2.400	2.400	2.160	2.340	
D2	2.400	2.480	2.240	2.560	2.420	
D3	2.400	2.080	2.880	2.160	2.380	
D4	2.480	4.240	2.320	2.480	2.880	
Average	2.420	2.800	2.460	2.340		
L.S.D (0.05)	D		V		DxV	Cv%
	0.137		0.1238		0.2411	5.9

4- stomata aperture length (µm)

The findings shown in Table 10 indicate that the overlap treatment (D4V3) exhibited the highest average measurement of 4.480 µm. Conversely, the treatments D2V1 and D3V1 had the lowest average measurement of 3.760 µm. This discrepancy can be attributed to the inherent genetic variations among the different cultivars. The stomatal response of the plant to stress levels has been investigated (19).

Table 10: Effect of water stress and cultivars, as well as their interaction, on stomata aperture length (µm)

stages of water stress	cultivars				Average	
	V1	V2	V3	V4		
D1	4.080	4.160	4.000	4.240	4.120	
D2	3.760	4.320	4.080	4.000	4.040	
D3	3.760	3.840	3.920	4.320	3.960	
D4	4.240	3.440	4.480	4.080	4.060	
Average	3.960	3.940	4.120	4.160		
L.S.D (0.05)	D		V		DxV	Cv%
	N.S		N.S		0.4627	7.1

5- stomata aperture Width (µm)

According to the data shown in Table 11, it can be observed that the Bohuth22 V2 variety exhibited the greatest mean value of 1.340 µm, whereas the Mawada V1 variety displayed the lowest mean value of 1.160 µm. The potential cause for this observation can be ascribed to the 22 variety of the studied plant, which exhibited the highest average width of stomata as seen in Table 9. Consequently, this variety is likely to promote an increase in stomata width.

Table 11: Effect of water stress and cultivars, as well as their interaction, on stomata aperture Width (µm)

stages of water stress	cultivars				Average	
	V1	V2	V3	V4		
D1	1.200	1.280	1.120	1.200	1.200	
D2	1.200	1.520	1.360	1.200	1.320	
D3	0.960	1.280	1.200	1.280	1.180	
D4	1.280	1.280	1.280	1.120	1.240	
Average	1.160	1.340	1.240	1.200		
L.S.D (0.05)	D		V		DxV	Cv%
	N.S		0.1079		N.S	10.4

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