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Effect of levels of water stress on some physiological and chemical traits of wheat cultivars (*Triticum aestivum* L.)

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Abstract

A field experiment was carried out 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 and chemical responses of different bread wheat varieties to incomplete irrigation. The experiment was conducted utilising the randomised complete block design (RCBD) employing the split plot method, with three replications. The primary factors investigated in this study were related to irrigation deficiencies, which were represented by four different stages: D1 (control), D2 (tillering stage), D3 (elongation stage), and D4 (booting stage). The secondary factors examined in this research were different cultivars, denoted as V1 (Mawadah), V2 (Bohuth 22), V3 (Baraka), and V4 (Ibaa 99).

The statistical analysis revealed significant variations among the stages of water stress. The D2 treatment exhibited the highest proline content at 41.692 mg per 100g, while the D4 treatment demonstrated the greatest thickness of vascular bundles in the roots at 201.23 µm.

Regarding the cultivars under investigation, it was observed that the Baraka variety exhibited a higher chlorophyll content (54.24 SPAD units). In contrast, the Bohooth22 cultivar displayed a larger diameter of the vascular bundle in the roots (209.78 µm). Additionally, the Mawada variety demonstrated a greater thickness of the leaf vascular bundles (102.38 µm), while the cultivar IPA99 exhibited a higher content of Proline (35.481 mg per 100g). In terms of interference, treatment D3V1 exhibited a higher proline content of 48.607 mg/100g, indicating its superiority.

The results of histological analysis showed the superiority of the combination D2V1 in the diameter of the vascular bundles of the roots (247.33) micrometers, and the occurrence of rupture and damage in the cells of the basal tissue of the root tissue in (D2V1, D3V1, D3V2)

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and in (D2V1, D2V2, D3V2), in addition to the occurrence of rupture and damage in the upper and lower epidermal cells in the tissue of its leaves, while the combination D2V3 wrinkled and damaged mesophyll cells, and excelled in the thickness of the leaf vascular bundles (142.40) µm, as well as showed that detachment and rupture of the upper epidermis from the mesophyll occurred in D2V4. The results of histological cutting of root cells showed that bundle damage appeared in the root tissue of combination D3V3. Also, fracture and separation occurred in the region of the vascular bundle from the underlying tissue, and some wood vessels were also damaged in the root tissue of the treatment D4V1, and in D4V3 there was a rupture in the lower epidermis of the leaf tissue, and a break in the cells of the cortex and epidermis of the root tissue, while in D4V4, damage was observed in the bundle and cells of the cortex, in addition to the occurrence of a tear in the lower epidermis of the leaves.

Keywords: stomata, proline, chlorophyll, tissue, water stress

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Introduction

Wheat (Triticum aestivum L.) holds a prominent position among cereal crops worldwide due to its significance, extensive cultivation, and global production. It serves as a primary food source for over one-third of the global population (1).

In field settings, 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 alterations that impede plant growth and development, ultimately impacting productivity (3).

The impact of water stress on plants manifests in multiple dimensions and at various levels, with its detrimental effects frequently observed in phenotypic, physiological, and biochemical characteristics (4). Plants elicit a variety of anatomical and physiological alterations (5).

The drought tolerance trait is a quantitative trait influenced by a substantial number of genes that manifest as a tolerance trait. Additionally, there exist various chemical-biological, functional, anatomical, and morphological manifestations that are linked to a diverse combination of genes, enabling the plant to exhibit tolerance towards water stress (6).

The avoidance of stress is a physiological mechanism employed by plants to endure periods of stress, while concurrently managing water stress within their tissues. The effectiveness of this process relies on the mitigation of tissue stress, specifically by sustaining a heightened water stress within plant cells when faced with a restricted water supply. The optimisation and reduction of water release from leaves (7) is achieved through osmotic modulation, which involves the active accumulation of solutes in the cytoplasm. This process elevates the osmotic pressure within the cytoplasm, resulting in a decrease in water loss and mitigating the detrimental consequences of water stress (8). It is important to note that excessive water loss can cause damage to the cell membrane (9). Stomata are crucial in the regulation of water stress, as well as in facilitating gaseous exchange and transpiration. The size and density of stomata per unit leaf area directly influence the rate of transpiration and gaseous exchange. Specifically, a lower stomatal density and smaller stomata size can enhance the plant's ability to utilise available water resources more effectively (10).

The central objective in the majority of wheat breeding programmes is to enhance grain yield. Nonetheless, the task of breeding for heightened yield and water stress tolerance presents difficulties owing to the intricate genetic composition and the fluctuating environmental circumstances (6).

The objective of this study is to investigate the impact of water stress on

various chemical and physiological characteristics of four different bread wheat cultivars.

Materials and methods

A winter field experiment was conducted in mid-November of the 2022-2023 season at the second agricultural research and experiment station of the College of Agriculture - Al-Muthanna University. The experiment took place in the Al-Bandar region, located at a longitude of 45.30 and a latitude of 31.32. The objective of the experiment was to investigate the response of different wheat varieties. The chemical and physiological properties of bread are influenced by the incomplete irrigation process.

The variables considered in the experiment One of the primary factors to be considered is water stress. The initiation of incomplete irrigation treatment occurred 40 days post-planting, and this treatment was consistently implemented throughout the entire duration of the plant's growth cycle, following the four distinct stages of plant development as outlined by the Zadox scale (11).

1. **The control in the absence of stress.**
2. **The stage of tillering.**
3. **The stage of elongation.**
4. **The booting stage.**

The second factor pertains to the four specific varieties of bread wheat, namely Mawadah, bohuth22, Baraka, and Ibaa 99.

Experiment design

The land underwent preparation and division, with the coefficients being distributed based on the characteristics of the factors included in the study. This was accomplished through the utilisation of the randomised complete plot design (R.C.B.D) and the split plot design.

Agricultural operations

The land was prepared and subsequent field operations were conducted and organised based on the designated design. On November 15, 2021, wheat seeds were planted at a density of 2.4 grammes per line (12). Fertilisation procedures were conducted in accordance with the guidelines outlined in reference (13), while weeding activities were performed as required.

Studied traits:

Total Chlorophyll Content (SPAD): The chlorophyll content of the flag leaf at the stage of completion of flowering was assessed by employing the CCM200-Plus Leaf Chlorophyll Content Metre.

Proline content in leaves: The estimation of proline content was conducted in accordance with the method outlined in reference (14) following a three-month period of initiating the water stress treatment.

Tissue sectioning: The samples were prepared and analysed following the

procedures outlined in reference (15). Utilising an ophthalmic lens microscope, the samples were scrutinised, and measurements of the sections and their dimensions were recorded. Subsequently, the samples were photographed using the camera attached to the CH3 Olympus microscope.

Statistical analysis: The data were subjected to statistical analysis using the GenStat 12 statistical software. The means were compared using the least significant difference test (L.S.D) at a significance level of 0.05 to determine any statistical differences between the arithmetic means of the coefficients (16).

Results

Chlorophyll content in the leaf

According to the data presented in Table 1, the Baraka V3 variety exhibited the highest mean Spad Unit value of 54.24, whereas the Bohuth22 (V2) variety displayed the lowest mean Spad Unit value of 44.96. The findings of this study are consistent with the results reported by previous studies (17) and (18), which also concluded that there were no significant differences among the varieties in terms of chlorophyll content. Chlorophyll content serves as an indicator of the photosynthetic capacity of plant tissues, and it was observed that this capacity either decreased or remained unchanged under conditions of water stress caused by water deficiency (19).

Table 1 presents the impact of water stress, cultivars, and their interaction on the chlorophyll content in the flag leaf, measured in Spad Units.

water stress	cultivars				Average	
	V1	V2	V3	V4		
D1	49.45	43.35	56.23	46.49	48.88	
D2	47.88	46.68	51.79	44.28	47.66	
D3	48.98	45.48	54.71	47.51	49.17	
D4	49.65	44.32	54.21	46.35	48.63	
Average	48.99	44.96	54.24	46.16		
L.S.D (0.05)	stress D		cultivars V		DxV	Cv%
	N.S		2.361		N.S	5.8

Proline content (mg 100g-1)

The findings presented in Table 2 indicate that the water stress treatment applied

during the tillering stage (D2) resulted in the highest average proline content of 41.692 mg 100g-1. Conversely, the water stress treatment in the control group (D1) exhibited the lowest average proline content of 16.729 mg 100g-1. This disparity can potentially be attributed to The accumulation of proline in plant tissues is attributed to the inhibition of protein synthesis due to elevated levels of proteolytic enzymes, particularly proteinase. This enzymatic activity promotes the buildup of proline, an amino acid that functions to decrease the water potential of leaf cells. Consequently, water enters these cells, resulting in increased water content within the leaves. This process ultimately leads to the dilution of proline. One of the adverse consequences of water stress that the plant experiences (20).

The observed cultivars exhibited a notable impact on the proline content found in the leaves. Specifically, the cultivar Ipaa99 V4 demonstrated the highest average proline content of 35.481 mg per 100g, whereas the Baraka V3 cultivar displayed the lowest average proline content of 26.023 mg per 100g.

The study observed a significant impact of the interaction between water stress and cultivars on the proline content in the leaves. Specifically, the treatment D3V1 exhibited the highest average proline content of 48.607 mg 100g-1, while the treatment D1V1 had the lowest average of 8.560 mg 100g-1. The process of proline accumulation In plant tissues, the phenomenon of osmotic adjustment serves as an adaptive response to water stress. This process helps alleviate water stress in leaf cells by facilitating the entry of water into these cells, thereby increasing water content in the leaves. Consequently, osmotic adjustment plays a crucial role in mitigating the detrimental effects of water stress on plants (20).

Table 2 presents the impact of water stress, cultivars, and their interaction on the proline content in leaves (mg $100g^{-1}$).

water stress	cultivars				Average	
	V1	V2	V3	V4		
D1	8.560	26.477	4.400	27.480	16.729	
D2	39.320	40.487	44.590	42.370	41.692	
D3	48.607	14.520	39.647	25.540	32.078	
D4	38.600	42.217	15.453	46.533	35.701	
Average	33.772	30.925	26.023	35.481		
L.S.D (0.05)	stress D		cultivars V		DxV	Cv%

	0.3504	**0.4308**	**0.7950**	**1.6**

Conducting a vertical sectional analysis of cross-sectional leaf tissue.

Vascular bundle thickness in a leaf (µm)

The findings presented in Table 3 indicate that the treatment of water stress during the tillering stage, denoted as D2, exhibited the highest mean value of 117.14 (µm). The water stress treatment exhibited the lowest average (84.11) (µm) during the booting stage D4.

One possible explanation for this phenomenon is the occurrence of water stress, which results in a reduction of water content within the tissues. Consequently, the rupture pressure in the cells is diminished, impeding cell enlargement and division. As a consequence, plant growth is hindered (21).

The findings from the aforementioned table indicate a statistically significant influence of cultivars on the observed outcomes. Specifically, the cultivar Mawada V1 exhibited the highest mean value of 102.38 µm, whereas the cultivar Ipaa99 V4 displayed the lowest mean value of 88.02 µm. This discrepancy in measurements could potentially be attributed to genetic variations among the cultivars.

In terms of the observed overlap, the interaction effect of water stress levels and (D2V3) cultivars yielded the highest mean value of (142.40) (µm), whereas the (D3V4) treatment exhibited the lowest mean value of (66.87) (µm). The leaves of the plant under stress exhibit hormonal activity that hinders growth by impeding cell division and elongation, as well as suppressing the synthesis and production of nucleic acids.

Table 3 presents the impact of water stress and different cultivars, as well as their interaction, on the thickness of leaf vascular bundles (µm)

water stress	**cultivars**				**Average**
	V1	**V2**	**V3**	**V4**	
D1	**101.97**	**96.97**	**78.10**	**76.97**	**88.50**
D2	**123.03**	**86.83**	**142.40**	**116.30**	**117.14**
D3	**111.30**	**116.83**	**95.83**	**66.87**	**97.71**
D4	**73.23**	**87.70**	**83.53**	**91.97**	**84.11**

Average	**102.38 97.08 99.97 88.02**			
L.S.D (0.05)	**stress D**	**cultivars V**	**DxV**	**Cv%**
	2.068	**1.141**	**2.647**	**1.4**

Figure 1 illustrates the vertical cross-sections of a leaf, wherein the upper epidermis layer is coated with a cuticle. The epidermis comprises a single layer of oval-shaped cells, followed by the mesophyll layer consisting of typical parenchyma cells. The lower epidermis is composed of small oval-shaped cells. Additionally, the bundles are formed within the leaf structure. The arrangement of the vascular bundle occurs within the mesophyll, with each individual vascular bundle being safeguarded by an upper and lower layer of sclerenchyma tissue. The aforementioned layer exhibits connectivity with both the upper and lower epidermis of the leaf. In each vascular bundle, the outermost layer is composed of bark, followed by the subsequent layer of wood, and beneath it lies the innermost layer of wood. Within the innermost layer of wood is the central cavity. The formation of the schizogenous-lysigenous intercellular space occurs beneath it, while the bundle is enveloped externally by Bundle sheath fibres.

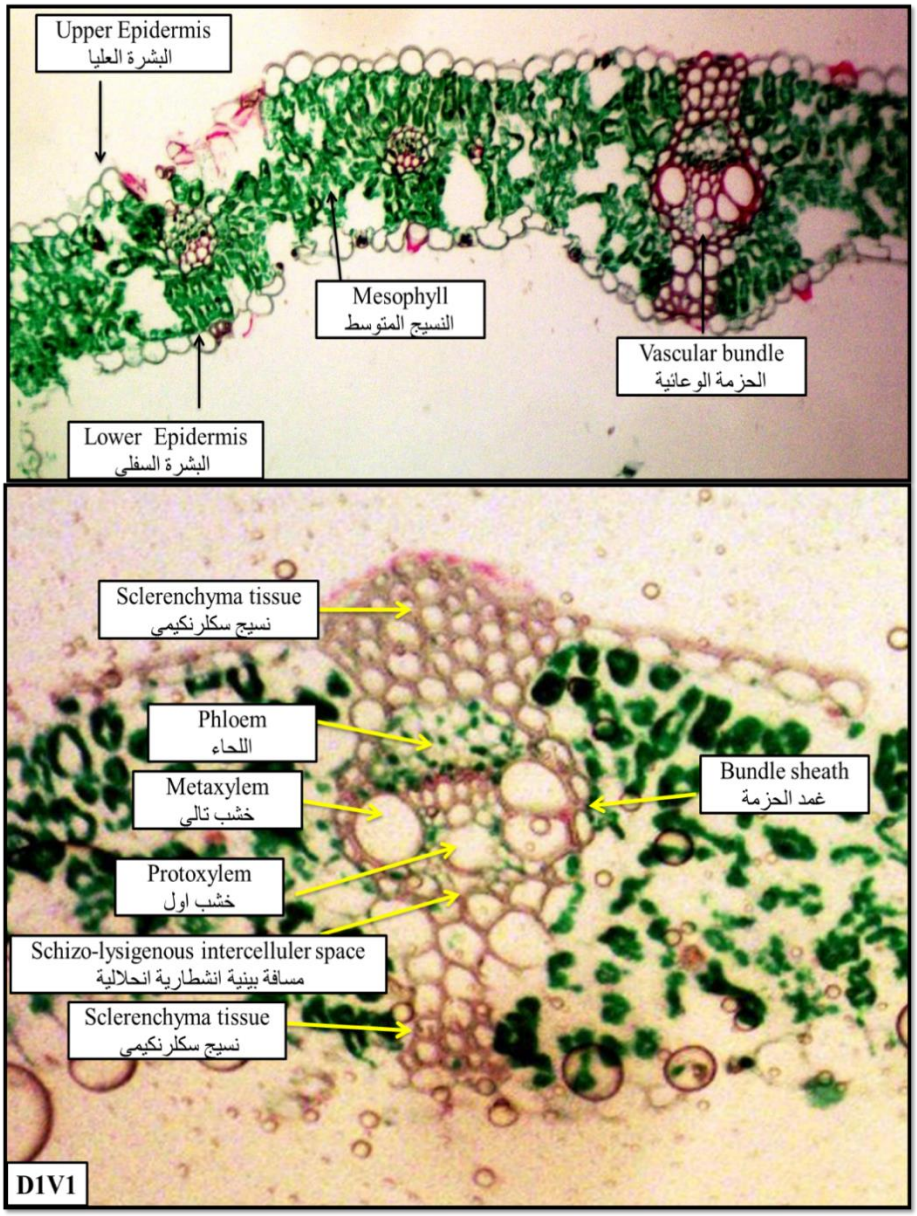


Figure 1 shows a wheat plant leaf cross-section showing the tissue types and treatments.

Figure 2 illustrates the observed phenotypic alterations in leaf morphology across various treatments. The findings indicate that in treatment D2V1, there was evident disruption and impairment of both upper and lower epidermal cells within the leaf tissue. Conversely, treatments D3V1 and D4V1 did not exhibit any discernible impact on the cellular structure.

Figure 2 shows a wheat plant leaf cross-section showing the tissue types and treatments.The occurrence of rupture and damage in the upper and lower epidermal cells in the leaf tissue was depicted in Figure 3 for the two treatments D3V2 and D2V2. Conversely, the treatment D4V2 did not exhibit any discernible effect on the cells.

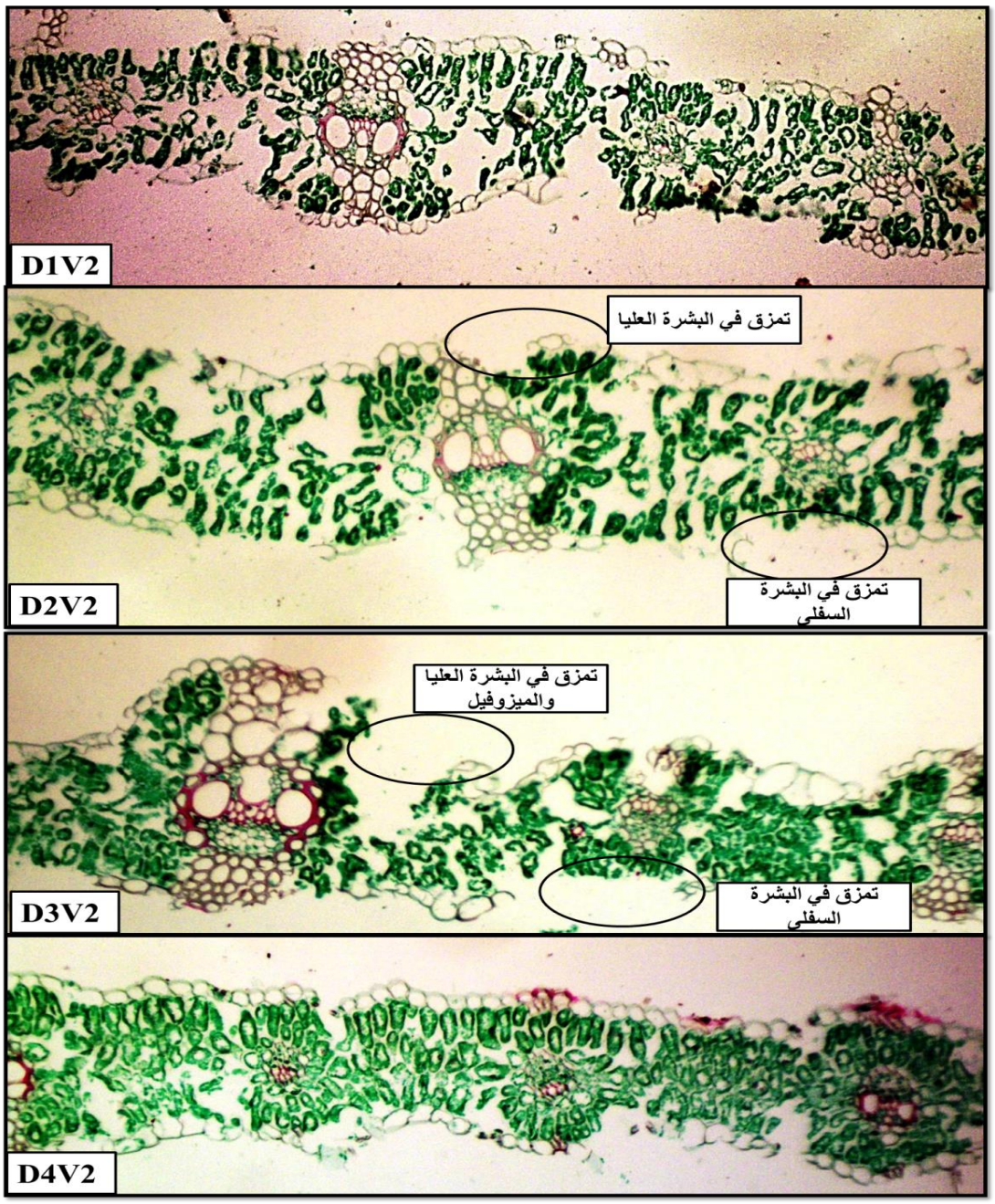


Figure 3 shows a wheat plant leaf cross-section showing the tissue types and treatments.

The results presented in Figure 4 indicate that the D2V3 treatment induced wrinkling and damage to the mesophyll cells in the leaves as a consequence of water stress. Conversely, no discernible impact on the cells was observed in the D3V3 treatment. Additionally, the D4V3 treatment resulted in the observation of a tear in the lower epidermis of the leaf tissue.

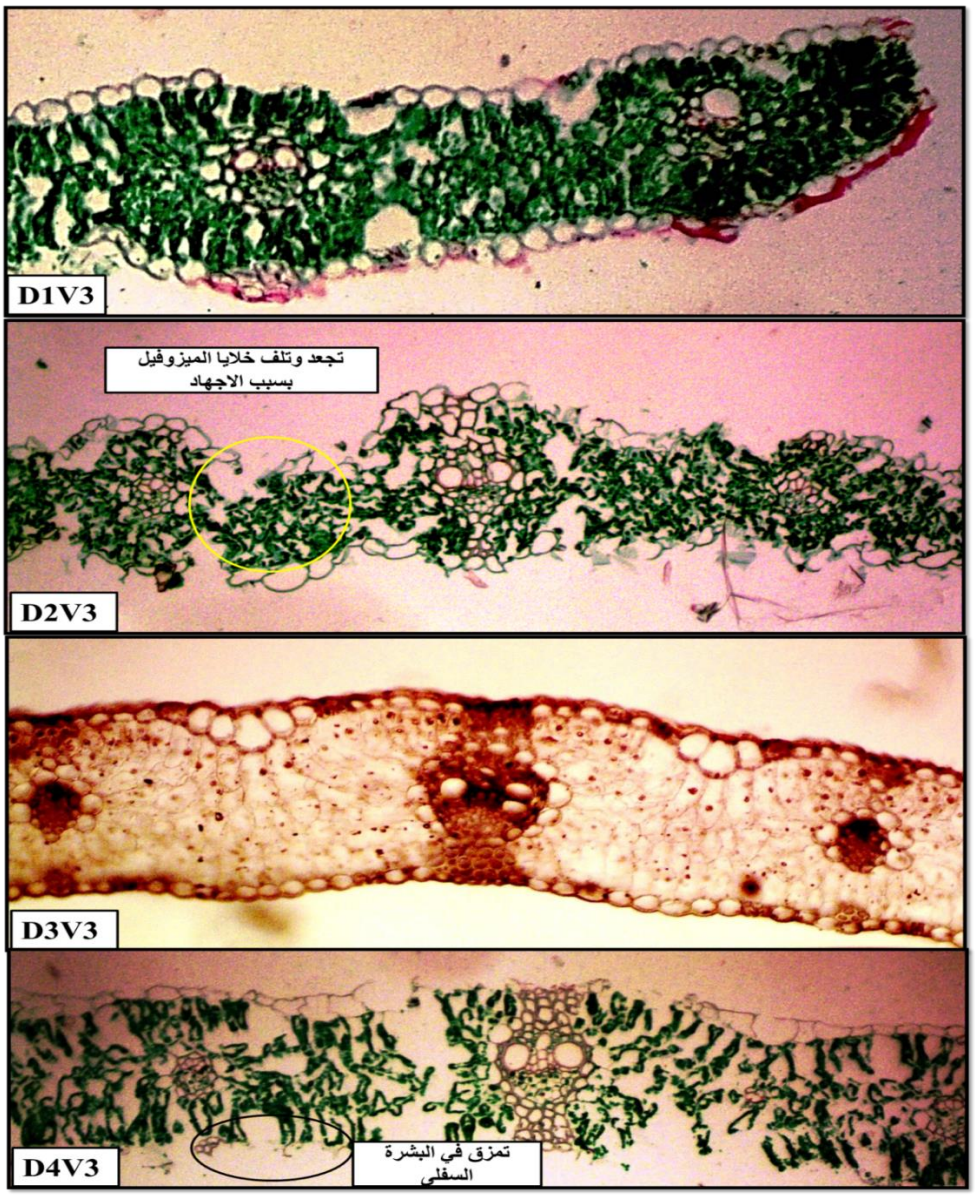


Figure 4 shows a wheat plant leaf cross-section showing the tissue types and treatments.

Figure 5 illustrates that the application of D2V4 treatment resulted in the separation and rupture of the upper epidermis from the mesophyll in multiple regions. Conversely, D3V4 treatment did not exhibit any discernible impact on the cells. Notably, D4V4 treatment demonstrated a rupture specifically in the lower epidermis.

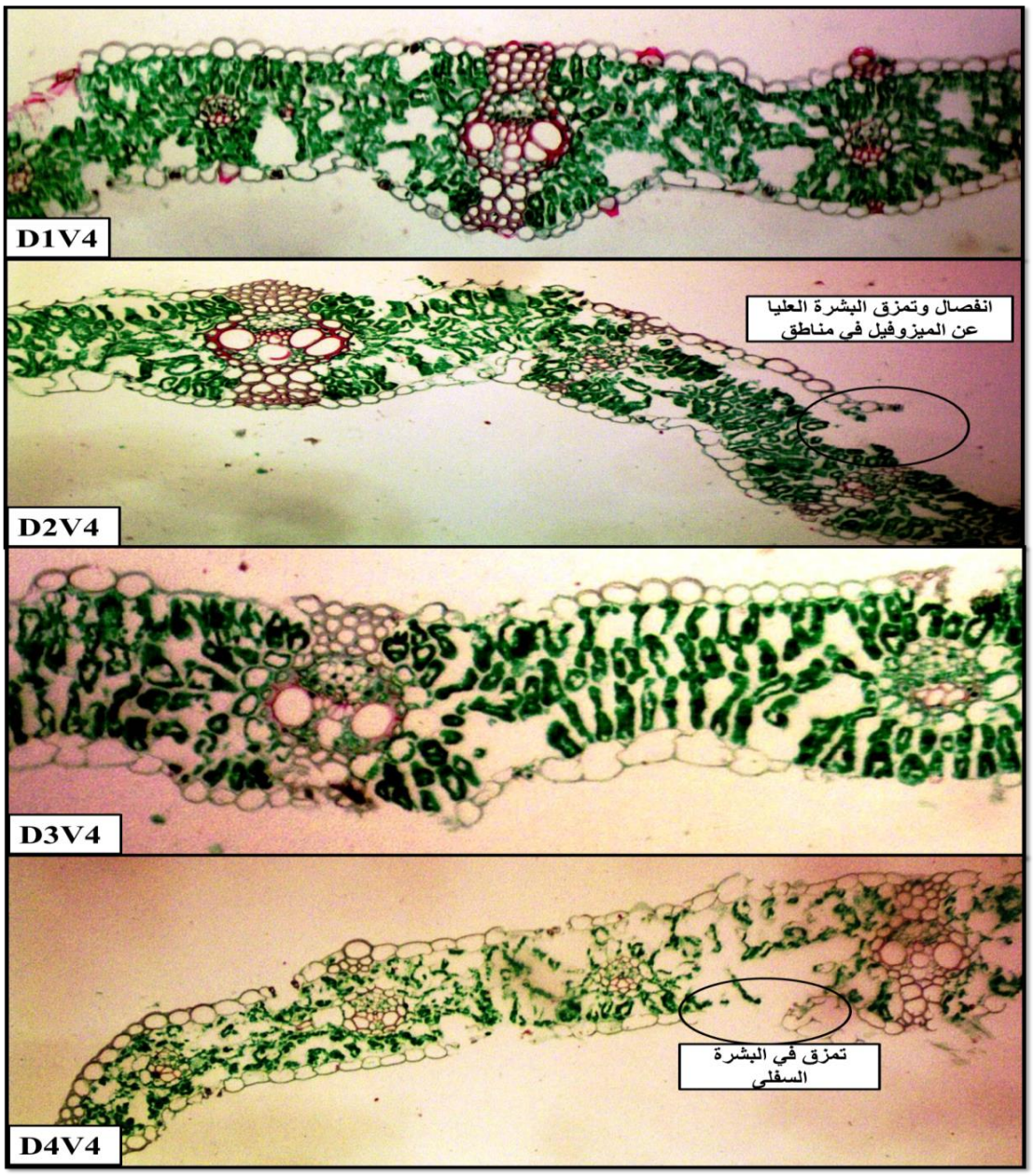


Figure 5 shows a wheat plant leaf cross-section showing the tissue types and treatments.

Vascular bundle thickness in a root (µm)

The findings presented in Table 4 indicate that the treatment involving water stress during the booting stage (D4) exhibited the highest average value of 201.23 µm. Conversely, the treatment involving water stress during the tillering stage (D2) exhibited the lowest average value of 181.51 µm. This discrepancy in results can be attributed to the detrimental impact of water stress during these stages, which hampers the plant's ability to absorb nutrients through the roots and subsequently transport them to other parts of the plant. This limitation can be attributed to reduced transpiration rates,

weakened active transport mechanisms, and impaired membrane permeability. Additionally, the decrease in soil moisture resulting from water stress leads to a decline in the diffusion rate of nutrients in the soil, thereby reducing their availability for absorption by the roots (22).

The table also indicates a noteworthy impact of the cultivars on the observed phenomenon. Specifically, the cultivar Bohuth22 V2 exhibited the highest mean value of (209.78) µm, whereas the cultivar Mawada V1 displayed the lowest mean value of (171.94) µm. This discrepancy can be attributed to the distinct physiological responses of the cultivars to various stages of water stress.

In terms of overlapping, the treatment (D1V2) exhibited the highest mean value of (247.33) µm, whereas the treatment (D2V1) demonstrated the lowest mean value of (145.97) µm.

Table 4: Impact of water stress, cultivars, and their interaction on the diameter of root vascular bundles (µm)

water stress	cultivars				Average	
	V1	V2	V3	V4		
D1	158.20	247.33	152.07	187.60	186.30	
D2	145.97	191.70	214.13	174.23	181.51	
D3	201.67	186.47	212.73	186.97	196.96	
D4	181.93	213.63	207.47	201.87	201.23	
Average	171.94	209.78	196.60	187.67		
L.S.D (0.05)	stress D		cultivars V		DxV	Cv%
	1.673		1.161		2.442	0.7

The circular shape of the root in cross-section is observed in Figure (6). The initial stratum is referred to as the epidermal layer, which comprises a single row of oval-shaped cells that are externally adorned with root hairs. This is then succeeded by the basal tissue region, which is composed of regular parenchyma cells. The vascular bundle is situated within the central region of the root, specifically categorised as the central vascular bundle. In this particular type, the bark envelops the wood, resulting in an amphivasal arrangement. The wood, in turn, is positioned peripherally to the phloem, forming a concentric configuration

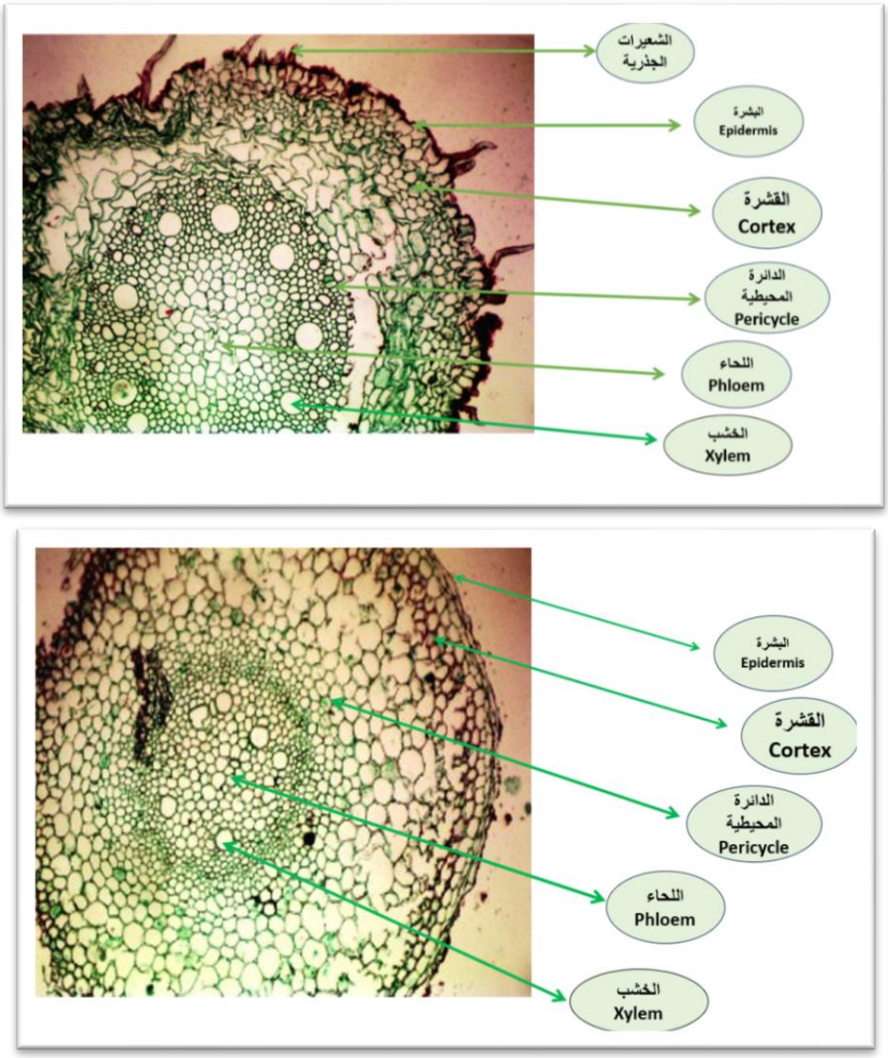
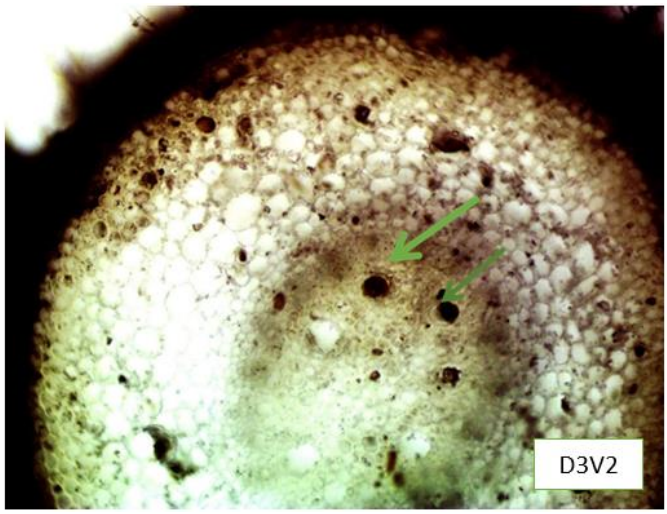


Figure 6 shows a wheat plant root cross-section showing the tissue types and treatments.

Figure 7 illustrates that in treatment D2V2, fracture and separation were observed in the region of the vascular bundle from the underlying tissue, along with damage to certain wood vessels. Similarly, in treatment D3V2, damage was observed in the wood vessels and the underlying tissue of the root tissue. Conversely, treatment D4V2 did not result in any damage to the tissue.



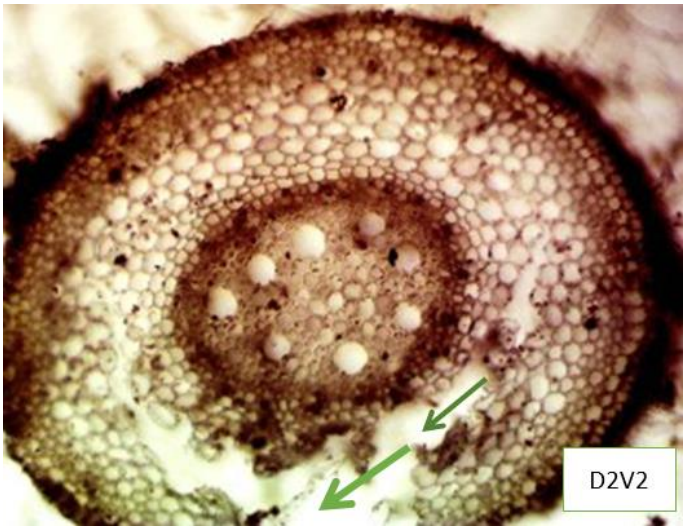
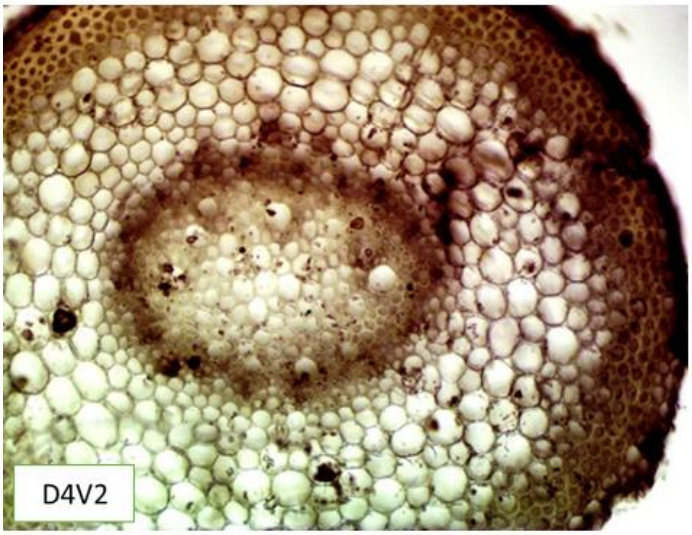


Figure 7 shows a wheat plant root cross-section showing the tissue types and treatments.

Figure 8 illustrates the outcomes of different treatments on the targeted area, as indicated by the arrows. In treatment D2V1, cellular rupture and damage were observed in the underlying tissue. Treatment D3V1 resulted in damage specifically to the wood vessels. In treatment D4V1, fracture and separation occurred in the region of the vascular bundle from the primary tissue, accompanied by



damage to certain wood vessels.

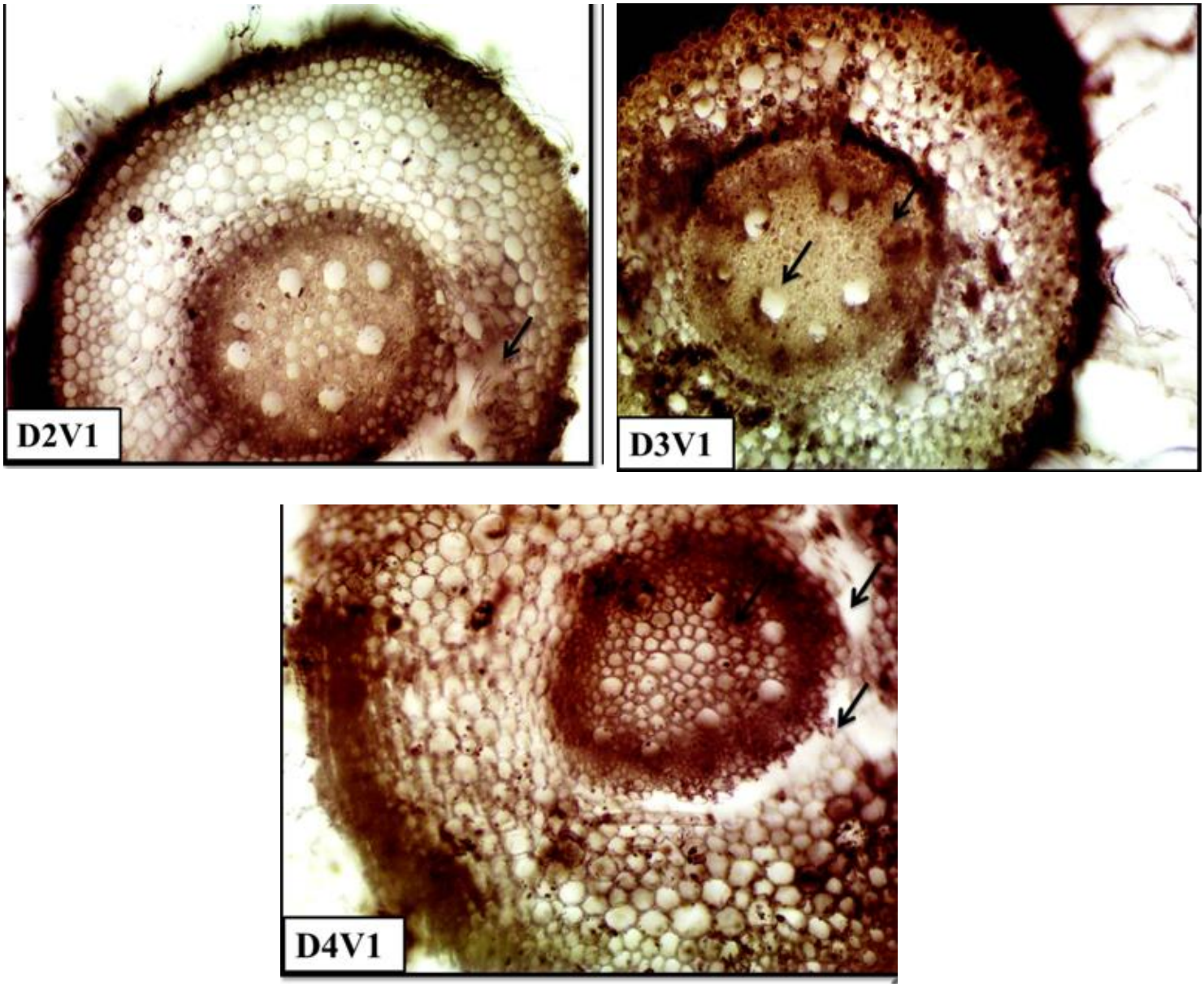


Figure 8 shows a wheat plant root cross-section showing the tissue types and treatments.

The results presented in Figure 9 indicate that treatment D2V3 resulted in preserved tissue integrity. Conversely, treatment D3V3 exhibited evidence of bundle damage within the root tissue, while treatment D4V3 displayed a disruption in the cells of the cortex and epidermis within

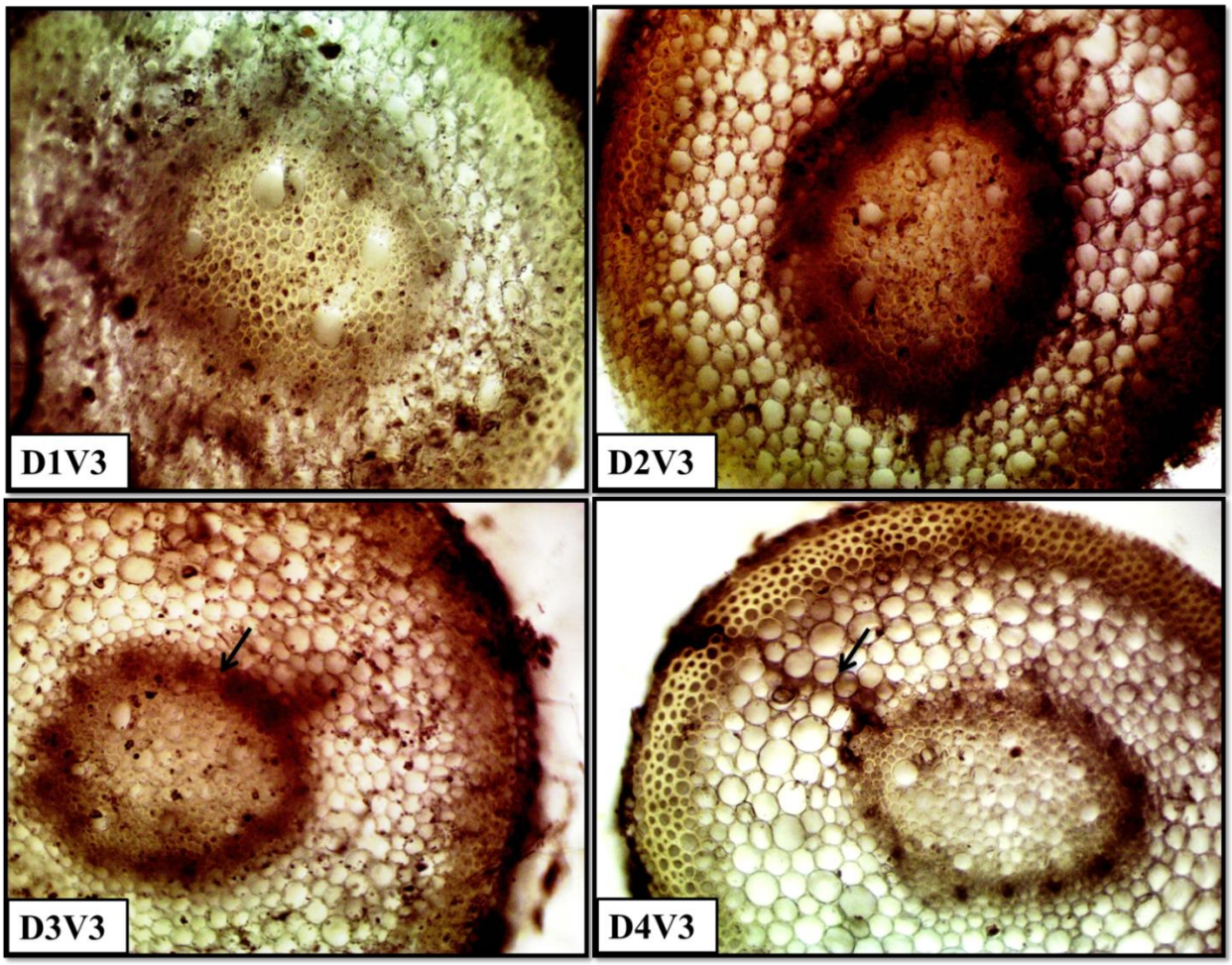


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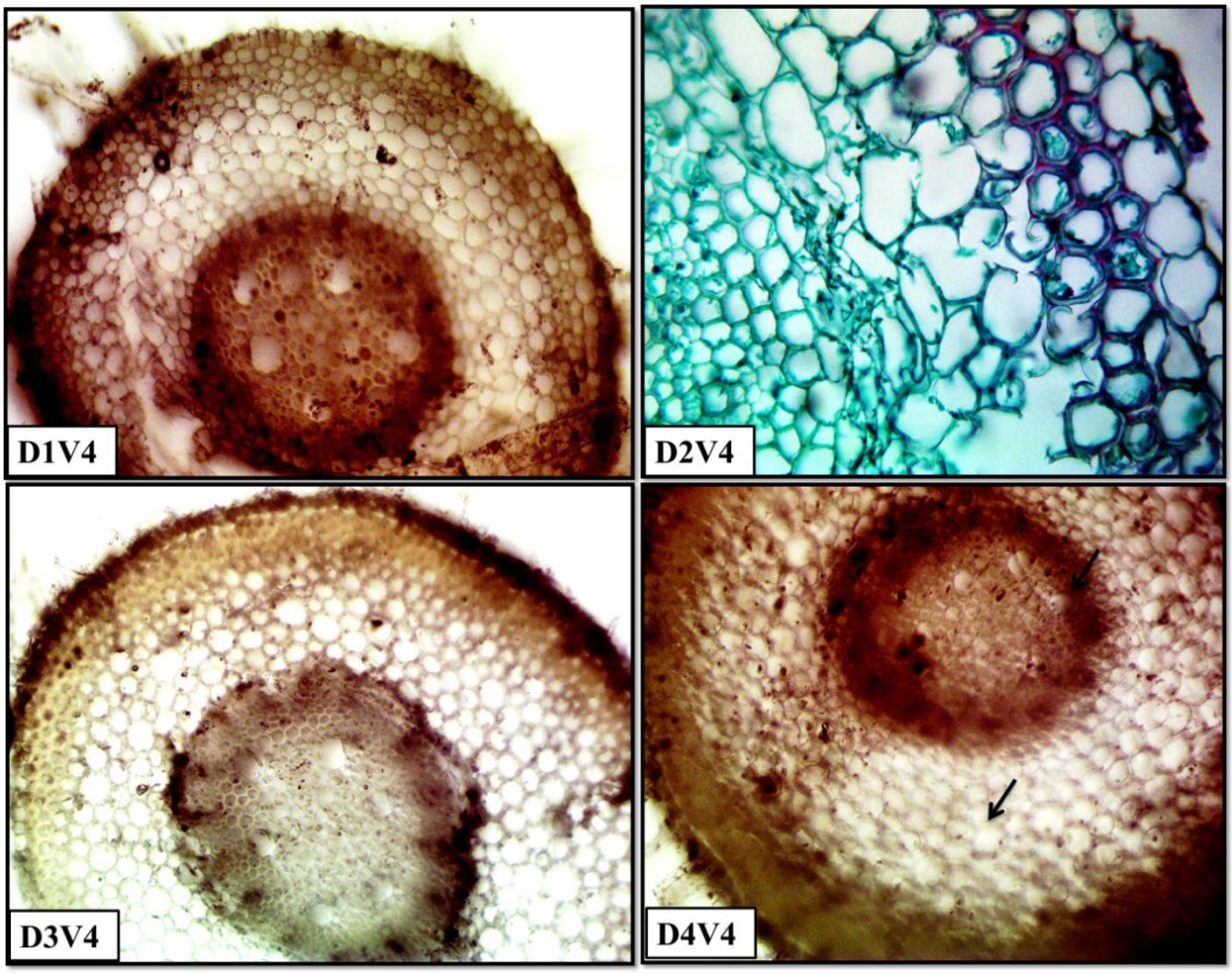
Figure 9 shows a wheat plant root cross-section showing the tissue types and treatments.

The results depicted in Figure 10 indicate that treatment D3V4 and D2V4 exhibited healthy tissue, whereas treatment D4V4 displayed damage to both the bundle and

cortex cells of the root tissue.

Figure 10 shows a wheat plant root cross-section showing the tissue types and treatments.



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