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Changes in anatomical and physiological parameters of soybean under drought stress

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Abstract: Anatomical changes in the root, stem, and leaf of soybean (*Glycine max* (L.) Merr. cv. Nazlıcan) plants under drought stress were studied by light microscope and their significance was evaluated by numerical analysis. Some anatomical features related to parenchymatous cells, diameter of tracheas belonging to the root, stem, and leaf; stomata index; and size and average number of stomata and epidermal cells were found to be important characters varying in soybean plants under stress. These characters were also statistically significant. Leaf water potential (Ψ_{leaf}), root:shoot ratio, total chlorophyll content, and stomatal conductance were measured. Ψ_{leaf}, total chlorophyll content, and stomatal conductance were lower while root:shoot ratio was higher in stressed plants compared to the unstressed ones. It has been concluded that some anatomical and physiological changes occurred in soybean under drought stress.

Key words: Drought stress, *Glycine max*, anatomical structure, chlorophyll content, water potential

Kuraklık stresi altındaki soya fasülyesinin anatomik ve fizyolojik parametrelerindeki değişimler

Özet: Bu çalışmada, kuraklık stresi altındaki soya fasülyesi (*Glycine max* (L.) Merr. cv. Nazlıcan) bitkilerinin kök, gövde ve yaprağındaki anatomik değişiklikler ışık mikroskobuyla çalışıldı ve önemlilik dereceleri nümerikal analizle değerlendirildi. Parankima hücreleriyle ilgili bazı anatomik özelliklerin, kök, gövde ve yapraktaki trake çaplarının, stoma indeksinin, stoma ve epidermis hücrelerinin büyüklüğü ve ortalama sayılarının stres altındaki soya fasülyesi bitkilerinde değişen önemli karakterler olduğu bulundu. Ayrıca bu karakterlerin istatistik açıdan önemli olduğu sonucuna varıldı. Yaprak su potansiyeli (Ψ_{leaf}), kök:gövde oranı, toplam klorofil içeriği ve stoma iletkenliği ölçüldü. Kontrolle kıyaslandığında stresli bitkilerde kök:gövde oranı artarken, Ψ_{leaf}, toplam klorofil içeriği ve stoma iletkenliğinin azaldığı belirlendi. Sonuç olarak, kuraklık stresi altındaki soya fasülyesi bitkilerinde bazı anatomik ve fizyolojik değişikliklerin meydana geldiği bulundu.

Anahtar sözcükler: Kuraklık stresi, *Glycine max*, anatomik yapı, klorofil içeriği, su potansiyeli

Introduction

Plants are subjected to several harsh environmental stresses that adversely affect their growth, metabolism, and yield. Among the environmental stresses, drought stress is one of the most adverse factors for plant growth and productivity (Araus et al., 2002; Chaves, 2002; Reddy et al., 2003; Reddy et al., 2004). Plants experience drought stress either

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when the water supply to the roots becomes limited or when the transpiration rate becomes very high. These 2 conditions often coincide under arid and semi-arid climates.

The responses of plants to drought stress are highly complex, involving deleterious and/or adaptive changes. Drought stress affects water status in plants. Leaf water potential and relative water content are useful means for determining the physiological water status of plants (Gonzales & Gonzales-Vilar, 2001). It is well known that leaf water status always interacts with stomatal conductance and a good correlation between leaf water potential and stomatal conductance always exists, even under drought stress (Giorio et al., 1999). It is now clear that there is drought-induced root-to-leaf signalling, which is promoted by soil drying through the transpiration stream, resulting in stomatal closure, which is an important adaptation to limited water supply in the field.

Anatomical changes induced by water deficits in higher plants are better observed indicators; they can be directly applied to agriculture and handled (Shao et al., 2008). Plant tissues responses to water stress depend on the anatomic characteristics that regulate the transmission of the water stress effect to the cells (Matsuda & Rayan, 1990; Olmos et al., 2007). Tissues exposed to environments with low water availability have generally shown reduction in cell size, and increase in vascular tissue and cell wall thickness (Pitman et al., 1983; Guerfel et al., 2009). Multiple characteristics of vascular structure have been investigated, such as modifications to the wall architecture and alteration of xylem/phloem ratio, which are thought to be involved in the resistance of the plant to environmental stresses (Child et al., 2003). Anatomical alterations may occur in plants under water deficit to protect and adapt the species to this stress.

Soybean is planted over a wide range of conditions, but information related to anatomical changes in the plant organs under drought stress is lacking. The aim of the present work was to examine the effects of drought on several anatomical and physiological parameters of soybean by experimentally withholding irrigation. Understanding the anatomical and physiological responses to drought is essential for a holistic perception of plant resistance mechanisms to water-limited conditions.

Materials and methods

Growth of the plants and stress applications

Soybean (*Glycine max* (L.) Merr. cv. Nazlıcan) seeds were provided by Çukurova Agricultural Research Institute, Adana, Turkey. The plants were grown for 21 days in plastic pots (16 cm high, 18 cm top and 12 cm bottom diameter) containing soil and peat (5:1), in a greenhouse (temperature: 25 ± 2 °C and relative humidity: 60 ± 5%). After 21 days, one group of plants was maintained well watered by daily irrigation and another group subjected to drought stress by withholding irrigation for 10 days. Leaf, stem, and root in all plants were harvested on the 31st day and the following analyses were performed.

Leaf water potential

Leaf water potential (Ψ_{leaf}) was measured with a C-52 thermocouple psychrometer (Wescor, Inc., Logan, UT, USA). Six discs about 6 mm in diameter were cut from the youngest fully expanded leaves of plants and sealed in the C-52 psychrometer chamber. Samples were equilibrated for 45 min before the readings were recorded by a Wescor PSYPRO water potential datalogger in psychrometric mode.

Stomatal conductance

Stomatal conductance (g_s) was monitored on randomly selected 6 leaves using a dynamic diffusion porometer (AP4, Delta-T Devices, Burwell, Cambridge, UK) after it had been calibrated with a standard calibration plate following the manufacturer's instructions.

Determination of root:shoot ratio

Shoots were weighed immediately after their excision and the roots were removed carefully, washed with tap water, and blotted with paper towels before weighing. Dry weights were determined after drying at 70-80 °C for 48 h.

Chlorophyll content

Total chlorophyll content was determined following the method of Arnon (1949). Fresh leaf samples were selected randomly and homogenized in a mortar in 80% acetone. The extract was centrifuged

at 5000 × *g* for 5 min. Absorbance of the supernatant was recorded at 663 and 645 nm by spectrophotometer (Nicolet evolution 100, Thermo Scientific, USA).

Anatomical studies

The materials for the anatomical studies were fixed in FAA (formaldehyde:acetic acid:alcohol) for 24 h and then preserved in 70% alcohol. All observations were performed on transverse sections of the root, the stem, and the leaves, and surface sections of leaves were taken by hand. All sections were stained with hematoxylin for 30 min and mounted with glycerine-gelatine in order to obtain permanent slides (Vardar, 1987). Then well-stained sections were photographed with an Olympus BX51 and all the measurements and observations were performed 10 times on different slides.

Statistical analysis

Physiological parameters and anatomical characters presented in Tables 1 and 2 were assessed by numerical analysis performed by t test using SPSS for Microsoft Windows (Ver. 10.0, SPSS Inc., USA).

Results

Leaf water potential

Leaf water potential of soybean significantly declined during drought stress in the leaves ($P < 0.001$). Water potentials of leaves (Ψ_{leaf}) fell from –0.88 MPa in unstressed leaves to –1.18 MPa in drought stressed leaves (Table 1).

Stomatal conductance, chlorophyll content, and root:shoot ratio

Stomatal conductance (g_s) decreased during the drought period in leaves. Exposure to drought stress resulted in decreasing in stomatal conductance of 42% in drought stressed leaves as compared to the unstressed leaves. Chlorophyll content was also affected by drought stress. There was a significant decrease in chlorophyll content under drought stress in soybean leaves. These decreases in stomatal conductance and chlorophyll content were statistically significant ($P < 0.001$). In contrast to pigment content, root:shoot ratio increased during the drought period, reaching 1.51 in the unstressed plants and 2.04 at the end of the drought period (Table 1).

Anatomical results

Anatomical features of the stressed and unstressed plants based on transverse sections of the root, the stem, and the leaf and surface preparation of the lamina were studied (Table 2).

The cross sections taken from the roots revealed the following elements (Figure 1). Entire surface of the epidermis is covered with hairs in stressed and unstressed roots. Exodermis consists of 2 layers of cells with thickened walls with suberin in unstressed plants and 3-layered in stressed ones (Figure 1). Root cortex consists of 10 or 13 layers of usually oval cells in both plants. Slender is surrounded by thin-walled endodermis and a single-layered pericycle. Phloem consists of thin-walled cells and surrounded with 1 or 2 layered xylem from the inside. Xylem arms are seen obviously. The centre of the root and the slender is filled with thin-walled parenchymatous cells. Measurements of the roots related to anatomical characters of stressed and unstressed plants are given in Table 2.

Transverse sections taken from the stem of both stressed and unstressed plants were analysed (Figure

Table 1. Changes in some physiological parameters of soybean under drought stress.

	Unstressed plants	Stressed plants
Leaf water potential (Ψ_{leaf})	–0.88 ± 0.025	–1.18 ± 0.015
Stomatal conductance (g_s)	16.76 ± 0.20	9.53 ± 0.30
Total chlorophyll	2.11 ± 0.092	1.52 ± 0.026
Root:shoot ratio	1.51 ± 0.010	2.04 ± 0.011

* Data correspond to the mean ± standard deviation of 5 independent measurements.

Table 2. Changes in some anatomical parameters of soybean under drought stress.

	Characters	Unstressed plants	Stressed plants
Root	Width of cortex (μm)	710.2 ± 32.09	866 ± 83.2
	Width of cortex (μm) / Width of vascular bundle (μm)	3.4 ± 0.34	4.07 ± 0.14
	Width of phloem (μm) / Width of xylem (μm)	1.99 ± 0.09	1.75 ± 0.1
	Diameter of trachea (μm)	47.8 ± 4.49	40 ± 5.71
	Diameter of slender (μm)	587.7 ± 18.35	714 ± 34.86
Stem	Width/length of epidermal cells (μm/μm)	0.76 ±0.11	0.83 ± 0.12
	Width/length of collenchyma cells (μm/μm)	0.72 ± 0.1	0.76 ± 0.11
	Average row number of collenchyma cells	1.4 ± 0.51	1.4 ± 0.51
	Width/length of cortex cells (μm/μm)	0.72 ± 0.07	0.75 ± 0.07
	Average row number of cortex cells	5.2 ± 0.78	4.5 ± 0.52
	Width of cortex (μm)	177.5 ± 34.61	171.2 ± 23.55
	Width of intravascular sclerenchyma (μm)	46.1 ± 8.5	40.3 ± 5.33
	Width of phloem sclerenchyma (μm)	46.9 ± 13.73	47.1 ± 8.27
	Average row number of phloem sclerenchyma cells	2.75 ± 0.71	2.5 ± 0.52
	Width of cortex / Width of vascular bundle (μm/μm)	0.57 ± 0.03	0.6 ± 0.02
	Width of phloem / Width of xylem (μm/μm)	0.73 ± 0.05	0.79 ± 0.05
	Diameter of trachea (μm)	57.3 ± 13.23	41.6 ± 8
	Average row number of trachea	4.5 ± 0.52	3.5 ± 0.52
	Diameter of pith cells (μm)	76.2 ± 7.52	62 ± 10.74
Leaf	Width/length of upper epidermal cells (μm/μm)	0.67 ± 0.13	0.75 ± 0.11
	Width/length of lower epidermal cells (μm/μm)	0.67 ±0.11	0.7 ± 0.07
	Diameter of parenchyma cells for midrib (μm)	48.8 ± 4.7	39.5 ± 6.86
	Diameter of trachea (μm)	31.3 ± 6.09	19.9 ± 6.02
	Width/length of vascular bundles (μm/μm)	0.74 ± 0.13	0.76 ± 0.08
	Length of outer palisade cells (μm)	39.5 ± 3.37	38.2 ± 1.93
	Length of inner palisade cells (μm)	33.3 ± 5.16	32.4 ± 2.63
	Width of palisade/ Width of spongy parenchyma (μm)	0.65 ± 0.09	0.71 ± 0.16
	Average row number of spongy parenchyma	6.4 ± 0.69	4 ± 0.81
Leaf lower surface	Width/length of epidermal cells (μm/μm)	0.43 ± 0.1	0.43 ± 0.05
	Width/length of stoma cells (μm/μm)	0.67 ± 0.03	0.64 ± 0.05
	Average number of epidermal cells (1 mm^2)	380.1 ± 21.27	352 ± 6.32
	Average number of stoma cells (1 mm^2)	93.6 ± 5.79	96 ± 6.32
	Stoma index	19.95 ± 0.4	21.31 ± 0.9
Leaf upper surface	Width/length of epidermal cells (μm/μm)	0.41 ± 0.06	0.42 ± 0.07
	Width/length of stoma cells (μm/μm)	0.74 ± 0.06	0.59 ± 0.04
	Average number of epidermal cells (1 mm^2)	403.5 ± 6.85	452.1 ± 18.07
	Average number of stoma cells (1 mm^2)	25 ± 2.3	38 ± 3.1
	Stoma index	5.80 ± 0.1	7.74 ± 0.28

* Data correspond to the mean ± standard deviation of 10 independent measurements.

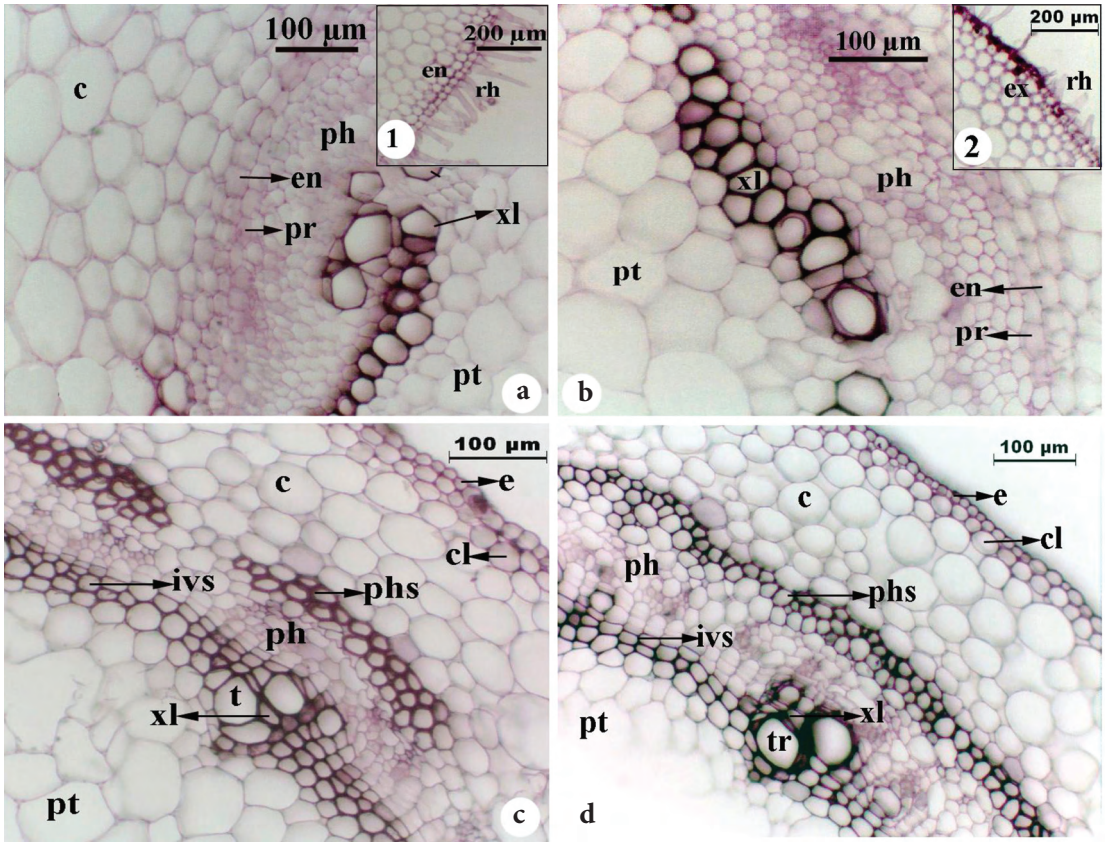


Figure 1. Anatomical features of stressed and unstressed soybean root and stem. a- Cross sections of root (unstressed), b- Cross sections of stressed root, c- Cross section of stem (unstressed), d- Cross section of stressed stem. rh: root hairs, ex: exodermis, c: cortex, ph: phloem, xl: xylem, pt: pith, pr: pericycle, en: endodermis, e: epidermis, cl: collenchyma, phs: phloem sclerenchyma, ivs: intravascular sclerenchyma, t: trachea, tr: tracheid.

1). Epidermis consists of monolayer of thin-walled and rectangular cells. Cortex is surrounded by a single layer of collenchyma consisting of 4-5 rows, usually oval cells with intercellular spaces in both plants. Phloem contains 2-3 rows and grouped sclerenchyma fibres in unstressed plants but sclerenchyma fibres are arranged continuously in phloem tissue of the stressed plants. The other phloem members are seen obviously. Xylem is composed of grouped tracheal elements and 3-5 rows of intravascular syclerenchyma binding it. Stem centre has a large cavity surrounded by 4-5 rows and thin-walled parenchymatous cells having intercellular spaces in both plants. All the detailed measurements related to the stem for both stressed and unstressed plants are given in Table 2.

Transverse sections of the midrib and lamina (Figure 2), and surface preparations of the leaves (Figure 3) were also analyzed. Midrib is semi-circular and has a monolayer epidermis. Arc-shaped vascular bundle is surrounded by orbicular and thin-walled parenchymatous cells. Diameter of parenchyma cells for midrib is statistically significant. Phloem and xylem are seen obviously. Phloem has some differentiated cells with thickened walls. It is observed that tracheas having a statistically crucial trait are shrinkage in stressed plants. Mesophyll consists of 2-3 layers of elongated palisade cells and 3-4 layers of isodiametric spongy parenchymatic cells with large intercellular cavities. Statistical results show that mesophyll properties such as average row number of spongy parenchyma and width of palisade are significant. Bifacial leaf has anomocytic stomata on both surfaces (Figure 3). Stoma index varies in lower and upper surfaces in stressed and unstressed plants.

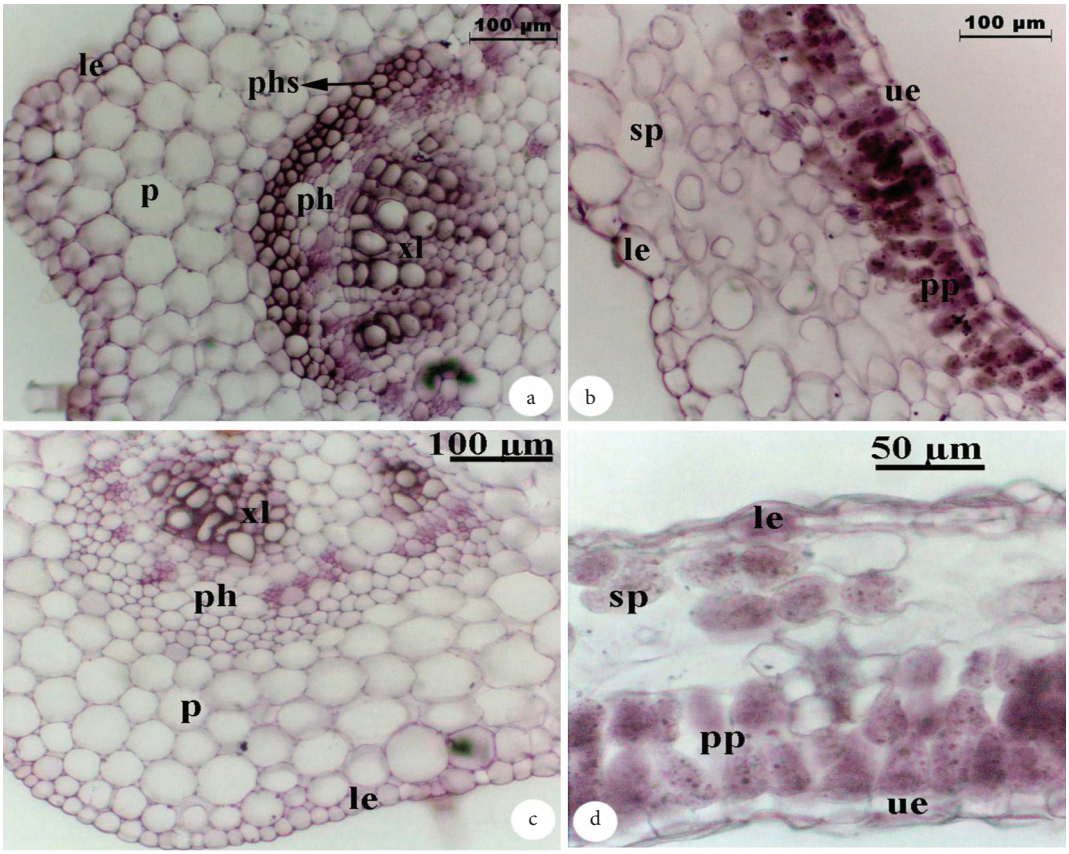


Figure 2. Anatomical features of stressed and unstressed soybean leaf**.** a and b- Cross sections of leaf (unstressed), c and d- Cross sections of stressed leaf. ph: phloem, phs: phloem sclerenchyma, xl: xylem, le: lower epiderma, ue: upper epiderma, p: parenchyma, pp: palisade parenchyma, sp: spongy parenchyma.

Entire surface of the leaf is covered with simple hairs. Surface features such as stomata index, stomata and epidermis numbers and their size are variable in stressed and unstressed plants (Table 2).

Discussion

In the present study, anatomical structure and some physiological parameters of soybean under drought stress were examined. This is the first anatomical and physiological note on the soybean under drought stress.

Ψ_{leaf} decreased while root:shoot ratio increased in response to drought stress. Similarly, most researchers showed that Ψ_{leaf} decreased under drought stress (Pennypacker et al., 1990; Siddique et al., 2000; Fu & Huang, 2001; Shaw et al., 2002). When water availability is limited, the root:shoot ratio of plants increases because roots are less sensitive than shoots to growth inhibition by low water potentials (Wu & Cosgrove, 2000). Moreover, drought stress caused a significant decline in total chlorophyll content of soybean. It was also reported in other species and this pigment was sensitive to increasing environmental stress (Ladjal et al., 2000; Younis et al., 2000; Terzi et al., 2010). The decrease in total chlorophyll content may have resulted from a decrease in leaf water status in the soybean.

Stomatal control is a major physiological factor for optimising the use of water in drought conditions. In the present study, stomatal conductance decreased in accordance with the decrease in Ψ_{leaf}. Giorio et al. (1999) reported that good positive relationships were found between stomatal conductance and both Ψ_{leaf} and soil moisture. This is commonly found in many species and may indicate a control of stomatal conductance through a hydraulic feed-back mechanism (Jones, 1998). On the other hand,

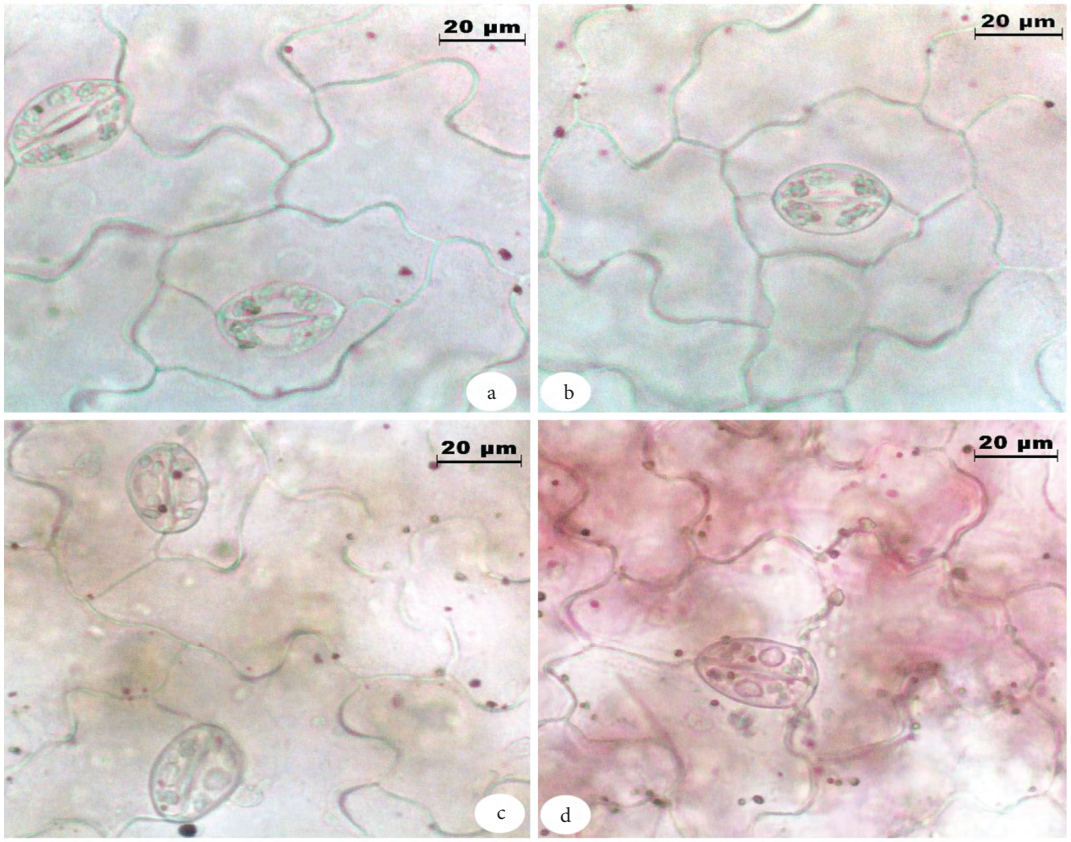


Figure 3. Anatomical features of stressed and unstressed soybean leaf. a and b- Superficial section of lower and upper epiderma (unstressed), c and d- Superficial section of lower and upper epiderma (stressed).

it was shown that, in anisohydric plants subjected to soil drying, a direct effect of the soil water status on stomata is compatible with a positive relation between stomatal conductance and Ψ_{leaf} (Tardieu et al., 1996). Thus, stomatal movements are very dynamic, involving complex regulation by several environmental factors. Larcher (1995) reported that leaves that developed during drought usually have smaller stomata than leaves under well watered conditions. We determined that width/length ratio of stoma cells decreased in stressed plants. Differences in terms of stomata index were also observed. While the stomata index varies from species to species, it is also well known that this trait is among the environmentally influenced anatomical characters (Özörgücü et al., 1991). Similarly, Güvenç and Duman (2010) reported that ecological factors affect some anatomical characters such as pubescence, organisation of the mesophyll, and distribution of the supporting tissue elements.

The anatomical structure varies greatly and is of significant value in many plants (Lersten & Curtis, 2001; Makbul et al., 2006; Makbul et al., 2008). Similarly, we found that the distribution of sclerenchymatic cells on phloem and diameter of parenchymatous cells of stem and leaf were variable in unstressed and stressed plants. Parenchymatous cells of the stem and leaves of unstressed plants are smaller than those of the stressed ones. It is thought that the parenchymatous cells with thin walls are affected by drought conditions. The distribution of sclerenchymatic tissue in the stem cortex and phloem is of considerable taxonomic value (Canne-Hilliker & Kampny, 1990). While the fibres spread as a continuous layer between cortex and phloem in stressed plants, they occur as grouped layers in unstressed plants. This means that their numbers increase under drought stress. Yentür (2003) indicated that sclerenchyma tissue provides an advantage against the loss of water.

Vessel diameters in stressed root, stem, and leaf were smaller than those in the unstressed plants. Ristic and Cass (1991) reported that the vascular tissue area was decreased by low soil moisture. Similar results were also presented by Kutlu et al. (2009). This difference may be conceived as an adaptation to stress and related to loss of water. We also found that width of cortex/width of vascular bundle rate belonging to the root and stem varies between the stress and unstressed plants. This character is also statistically significant ($P < 0.001$ for root; $P < 0.05$ for stem).

Guerfel et al. (2009) reported that olive leaves reveal an increase in palisade parenchyma thickness under low water conditions. In our study, similarly, some mesophyll tissue differences such as the width of palisade and spongy parenchyma and different surface characters including the average number and the size of upper and lower epidermal cells were determined between the stressed and unstressed plants (Table 2). Özörgücü et al. (1991) showed that the anatomical characters are influenced by the environmental conditions.

Abrams (1994) and Karabourniotis and Bornman (1999) also reported that leaf pubescence increased under drought stress. In contrast, our results showed that leaf pubescent did not increase under the drought conditions. The varying pubescence can provide an advantage in some plants growing in xeric environments but it was seen that pubescence is not a valuable character for the plant used in this study.

In conclusion, traits of parenchymatous cells; diameter of tracheas belonging to the root, stem, and leaves; stomata index; and size and average number of stomata and epidermal cells vary in soybean under drought stress. In addition, physiological changes are in accordance with the anatomical changes.

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