

EFFECT OF ECOLOGICAL CONDITIONS CREATED BY OVER-IRRIGATION AND LIMITED IRRIGATION ON THE ANATOMY OF MAIZE

TUYLU, G. İ.

*Department of Agricultural Structures and Irrigation, Faculty of Agriculture, University of Harran, Şanlıurfa, Turkey
(e-mail: gokhantuylu@harran.edu.tr)*

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Abstract. In the study carried out in Turkey in 2015, maize (*Zea mays* L. *indentata*), was cultivated by applying the drip irrigation system, with the aim of examining the effect of different irrigation applications on some anatomical features of the plant in detail. A period of 6 days was selected for irrigation interval and C₂S₁ quality irrigation water was used. Optimum condition (100%) and ecological conditions identified as $\pm 25\%$ of the condition were created based on the Class A Pan method. For anatomical examination, the samples of maize were put in epon 812 and semithin sections were stained with toluidin blue. The semithin sections were examined with light microscopy and photographed. Ultrathins were examined with transmission electron microscopy (TEM) after staining with uranyl acetate and lead citrate, and their micrographs were taken. The vascular bundles surrounded the pith like a ring in the root. Very few stomata were observed in the stem. The pith was paranchymatic. The leaf was unifacial. The lower and the upper epidermis were amphistomatic and contained graminea stomate type. Numerous bullate cells with different sizes were observed in the epidermis. The mesophyll was formed by irregular cells due to stress under over-irrigation and limited irrigation conditions. The small vascular bundles were embedded in the mesophyll. The results were compared for two different ecological conditions by performing biometric measurements in some tissues in the root, stem and leaf. As a result, it was determined that maize cultivated under over-irrigation and limited irrigation conditions showed anatomical changes due to stress effect, adapted to the conditions and did not show abnormal structures compared to maize cultivated under optimum condition. Ecological condition created by reducing irrigation water by 25% in the cultivation of maize studied is anatomically convenient. The results obtained are significant for water saving in irrigation. However; morphological, physiological, etc. parameters of the plant should also be considered for an accurate suggestion.

Keywords: *plant histology, plant cytology, drip irrigation, stress effect, water*

Introduction

Irrigation is one of the most important factors of plant production. Water, as an abiotic ecological factor to create ecological conditions, is effective in the development of plant organs. The fact that more or less irrigation water is applied than the plant needs causes morphological and anatomical changes in some tissues of root, stem and leaf. The plant adapts to its ecological condition as a result of the changes identified as stress effect. Plants undergo changes in terms of quality of stress factor and genetical and ontogenetic features of plants. Thus, plants adapt to factors of their environment by developing stress reactions (Korkmaz and Durmaz, 2017). In this case, not only morphological reactions of plants but also their physiological and anatomical reactions, etc., should be considered as a whole.

Use of drip irrigation systems has become common, depending on technological developments in the world and in Turkey. Drip irrigation is one of the systems, which applies irrigation under control and can irrigate plant with water as needed in contrast to applying too much or too little irrigation. It has been effective where there is a shortage

of water resource. Drip irrigation systems have been commonly used in conditions in which water resources are particularly limited in Turkey. The fact that there is a shortage of irrigation water in Mazıdağı, Mardin, requires the use of pressurized irrigation methods such as drip or sprinkler irrigation systems. *Zea mays* L. *indentata*, which is one of the plant patterns belonging to the province, has been cultivated using drip irrigation system commonly.

Maize is an important grain in the second place with respect to production and in the third place after wheat and paddy rice in terms of cultivation area. Maize is the grain having the highest yield of all and is cultivated in both cool and hot climate conditions. It is cultivated in 1 848 009 690 da, 1 037 791 518 ton is produced and mean yield is 561.6 kg da⁻¹ in the world. Economic value and fields of use of maize, which is an important plant in Turkey, are increasing. The cultivation area of grain maize is 6 800 192 da, the production is 6 400 000 ton, and the yield is 942 kg da⁻¹ in Turkey (Öktem et al., 2017). The cultivation area of maize, cultivated as the second crop in Mardin province in 2015, is 580200 da, the production is 670000 ton, and the yield is 1154 kg da⁻¹ (Anonymous, 2018).

The study aimed to anatomically examine the root, stem and leaf of maize to histologically and cytologically specify their adaptation to over-irrigation and limited irrigation conditions. In this way, supporting morphological results by examining structures of cell, tissue and organ will assist the producers and researchers to comment on the effect of different amount of irrigation water on plant development more efficiently.

Materials and methods

Mazıdağı is located at 1030–1090 m above sea level, 47 km northwest of Mardin province in the South Eastern Anatolia Region, Turkey. Features of terrestrial climate are efficient in the province. It is hot and dry in summers but cold and rainy in winters. The average rainfall has been 667.4 mm and July has been the hottest month for years (Anonymous, 2017). Some climatic data belonging to cultivation area and period (July 15 – November 15) is presented in *Table 1*. The soil in Mazıdağı is medium-textured and contains sand, silt and clay (*Table 2*).

Offspring F1 included in the group of Ventoruli Italian hybrid FAO 600 (the 120-day) of *Zea mays* L. *indentata* selected as research material is economical and cultivated as the second crop in Mazıdağı. The time of seed planting is July 15 on average. In the study area, the irrigated parcels were arranged in completely randomized experimental design and each irrigation treatment was replicated three times. Each parcel was 5 sequenced and had 12 plants m⁻². There were 13 plants in each sequence and two sequences were separated as border effect in the evaluation (*Fig. 1*). Irrigation water was applied by drip irrigation systems. Irrigation interval was selected as 6 days concerning application of farmers in the province. C₂S₁ class underground water was used as irrigation water.

Conditions were designed according to Class A Pan method (Demirok and Tuylu, 2017; Demirok, 2017; Orhangazi, 2017). Class A Pan Coefficient (kcp) was obtained from the different irrigation applications in the preliminary tests (kcp = 1). Optimum condition (I₁₀₀) was the issue of irrigation which the amount of irrigation water was as much as the amount of water evaporated from Class A Pan. The plants cultivated under optimum condition were defined as control group in the study.

While over-irrigation condition (I₁₂₅) was created by increasing the amount of optimum irrigation water by 25%, limited irrigation condition (I₇₅) was created by reducing the amount of optimum irrigation water by 25%. In the study, base fertilizer, N and P₂O₅ were evenly applied to the parcels for each condition (5 kg da⁻¹). Fighting against diseases was not required and the weeds were cleared by hand.



Figure 1. *Zea mays L. indentata*

Table 1. Some climatic data of the study area in 2015 (Anonymous, 2017)

| Climatic data | | Maximum temperature (°C) | Minimum temperature (°C) | Evaporation (mm) | Relative moisture (%) | Wind speed (m sn ⁻¹) | Sunshine (hours) | Rainfall (mm) |
|---------------|---------|--------------------------|--------------------------|------------------|-----------------------|----------------------------------|------------------|---------------|
| Months | Decades | | | | | | | |
| June | 1 | 30.4 | 16.5 | 22.5 | 32.8 | 1.9 | 10.6 | 2.0 |
| | 2 | 31.4 | 16.5 | 23.8 | 33.2 | 1.9 | 13.0 | 0.0 |
| | 3 | 32.6 | 17.8 | 27.8 | 32.0 | 1.9 | 12.5 | 0.0 |
| July | 1 | 37.0 | 20.5 | 17.3 | 23.9 | 1.8 | 12.4 | 0.0 |
| | 2 | 38.0 | 23.5 | 8.7 | 22.7 | 2.4 | 12.0 | 0.0 |
| | 3 | 38.5 | 22.9 | 14.2 | 18.0 | 1.6 | 12.0 | 0.0 |
| August | 1 | 37.8 | 22.2 | 19.2 | 24.0 | 1.8 | 11.7 | 0.0 |
| | 2 | 37.0 | 21.7 | 24.5 | 29.9 | 1.8 | 12.2 | 0.0 |
| | 3 | 34.2 | 20.3 | 32.3 | 27.3 | 1.8 | 11.3 | 0.0 |
| September | 1 | 35.0 | 20.3 | 19.3 | 24.4 | 1.5 | 9.8 | 0.0 |
| | 2 | 34.6 | 20.1 | 27.2 | 21.0 | 1.4 | 10.1 | 0.0 |
| | 3 | 30.6 | 17.2 | 26.7 | 33.1 | 1.6 | 10.8 | 0.0 |
| October | 1 | 25.2 | 13.6 | 28.4 | 56.4 | 1.4 | 7.9 | 11.4 |
| | 2 | 25.2 | 12.0 | 33.0 | 45.8 | 1.2 | 9.5 | 0.3 |
| | 3 | 19.3 | 11.1 | 25.7 | 84.9 | 1.1 | 4.2 | 63.6 |
| November | 1 | 16.3 | 5.8 | 24.4 | 67.2 | 1.1 | 6.9 | 13.8 |
| | 2 | 14.0 | 4.9 | 21.7 | 59.6 | 1.4 | 7.4 | 22.8 |
| | 3 | 14.7 | 3.6 | 26.5 | 64.2 | 1.0 | 6.2 | 0.7 |
| Year | 2015 | 21.2 | 9.7 | 821.1 | 55.9 | 1.6 | 94.3 | 431.9 |

Table 2. Soil properties of the study area

| Soil depth (cm) | Field capacity (%) | Wilting point (%) | Bulk density (g cm ⁻³) | Infiltration (mm h ⁻¹) | pH | Texture | | |
|-----------------|--------------------|-------------------|------------------------------------|------------------------------------|-----|----------|-----------|----------|
| | | | | | | Sand (%) | Silty (%) | Clay (%) |
| 0-90 | 36.2 | 18.5 | 1.285 | 3.3 | 7.6 | 34.4 | 30.6 | 35.0 |

For anatomical studies, samples of root, stem and leaf were protected in ethanol (70%). The tiny pieces of the samples which were taken from lower, middle and upper parts of the roots, stems and leaves were fixed at + 4 °C by using 3% glutaraldehyde (with 0.1 M Na-P buffer solution) and 1% osmium tetroxide (with 0.1 M Na-P buffer solution), respectively. After dehydration and saturation, the samples were embedded in epoxy resin (Luft, 1961; Tuylu, 2015). The blocks were kept in an incubator at 30 °C for 1 night, at 45 °C for 1 day and at 60 °C for 1 night to be polymerized. Semithins and ultrathins were taken in cross-section from the blocks trimmed with ultra microtome (Reichert OMU-4). The thickness of semithins was 0.5-1 µm and the thickness of ultrathins was 60–100 nm. The semithins prepared were stained with toluidin blue and they were examined with light microscope (Leica 1000). The results were photographed with digital camera (Leica EC3), and biometric measurements were performed in some tissues with Las v4.3 program. In the anatomical study, the total number of cross-sections taken from the plants and examined was 90 on 9 preparats for 3 plants in each replication and a total of 270 values of measurement were obtained by taking 3 from each section. The arithmetic mean of the values of measurements and mean standard error were calculated by using basic statistical methods in Excel spreadsheet, and the results were anatomically expressed. On the other hand, after the ultrathins were stained with uranyl acetate and lead citrate (Stempak and Ward, 1964; Tuylu, 2015), they were examined with transmission electron microscopy (TEM) (JEOL CX-100) and micrographed. The total number of ultrathins examined in each replication was 27 in 9 grid for cytological evaluations.

Results

Roots

The vascular bundles surrounded the pith like a ring in the roots cultivated under each of the three conditions. Air spaces in the cortex under I₁₂₅ and I₇₅ conditions were more than the ones under I₁₀₀ condition (Figs. 2A, 3A, 3B, and 4A, 4B). The bundle was surrounded by one layered pericycle and one layered endodermis under I₁₀₀ (Figure 2B). The endodermis was 1–2 layered and the pericycle was one layered under I₁₂₅ (Figure 3A and 3B). One layered large-celled endodermis and one layered pericycle formed by some degradation were observed under I₇₅ (Figure 4A and 4B). Starch grains were clearly observed under each of the three conditions (Figure 2D, 3D and 4F). Lipid distribution observed in the root cultivated under optimum condition was dense (Fig. 2C). Lipids were rarely observed in the roots under I₁₂₅ and I₇₅. When the roots were cytologically examined, it can be expressed that lipid molecules are more electron-dense than starch molecules under I₁₀₀ (Fig. 2C, 2D). The cortex formed by thin-walled paranchyma cells and thin-walled xylem vessels were observed under I₁₀₀. Electron density was also low in these cells (Fig. 3C, 3D). While electron density was low in some paranchyma and xylem

cells under I₁₂₅ (Fig. 3C, 3D, 3E), the ones under I₇₅ were more electron-dense than the others (Fig. 4C, 4D, 4E). The wall thickness of paranchyma and xylem cells under I₁₂₅ and I₇₅ was relatively thicker than the ones under I₁₀₀.

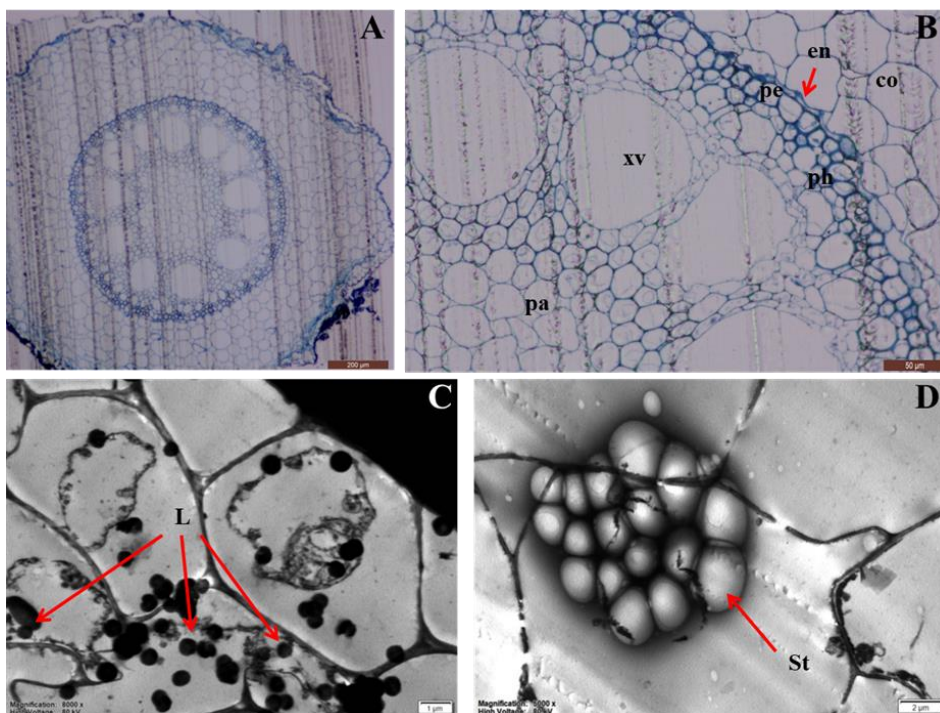


Figure 2. Histological and cytological structure of root in *Zea mays L. indentata* under I₁₀₀ condition (cross-section). A. general shape of root, bar = 200 µm, B. cortex (co)ndodermis (en), pericycle (pe), phloem (ph), xylem vessel (xv), paranchyma (pa)ar = 50 µm, C. lipid (L), bar = 1 µm (micrograph), D. starch (St), bar = 2 µm (micrograph)

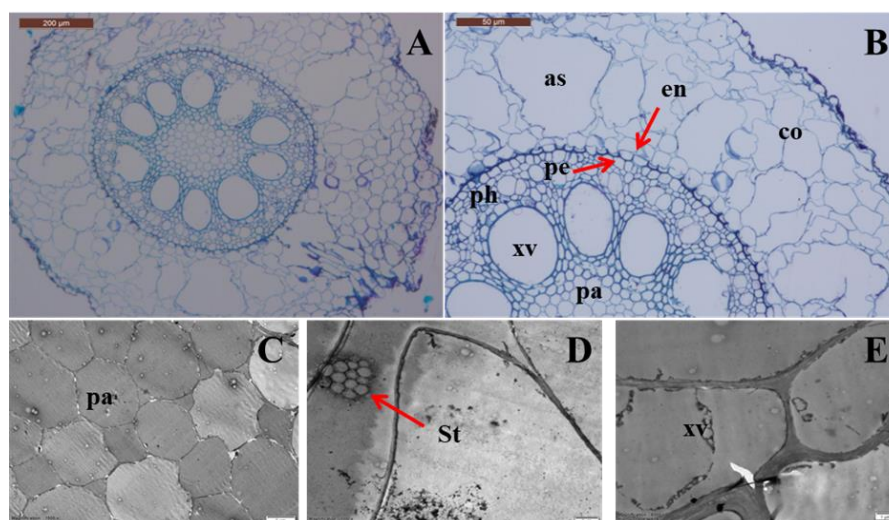


Figure 3. Histological and cytological structure of root in *Zea mays L. indentata* under I₁₂₅ condition (cross-section). A. general shape of root, bar = 200 µm, B. cortex (co), air space (as), endodermis (en), pericycle (pe), phloem (ph), xylem vessel (xv), paranchyma (pa), bar = 50 µm, C. paranchyma (pa), bar = 5 µm (micrograph), D. starch (St), bar = 5 µm (micrograph), E. xylem vessel (xv), bar = 1 µm (micrograph)

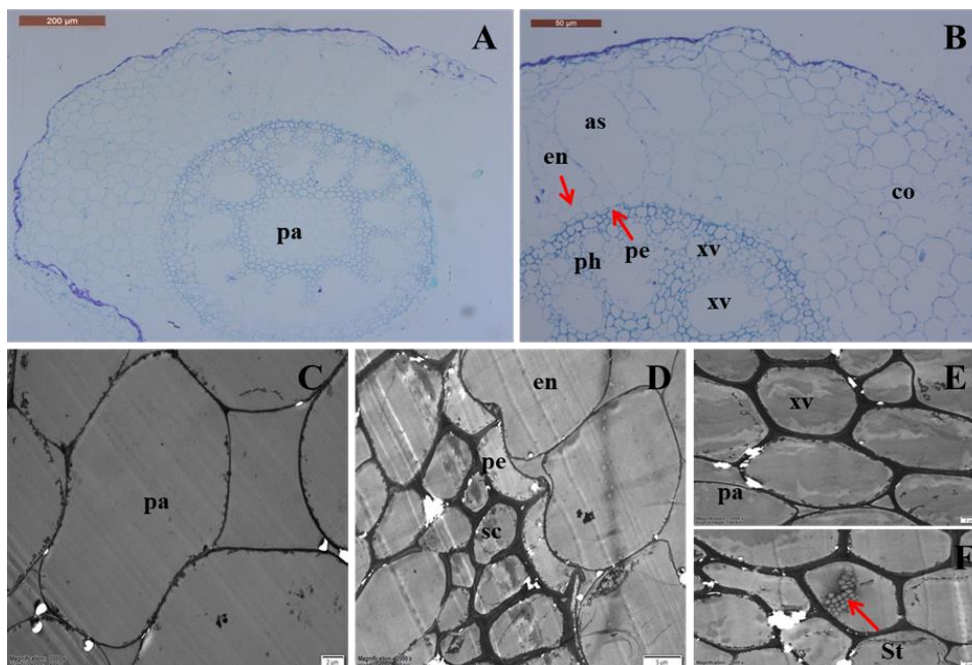


Figure 4. Histological and cytological structure of root in *Zea mays L. indentata* under I_{75} condition (cross-section). A. general shape of root, paranchyma (pa), bar = 200 μm , B. cortex (co), air space (as), endodermis (en), pericycle (pe), phloem (ph), xylem vessel (xv), bar = 50 μm , C. paranchyma (pa), bar = 2 μm (micrograph), D. endodermis (en), pericycle (pe), sclerenchyma (sc), bar = 5 μm (micrograph), E. xylem vessel (xv), paranchyma (pa), bar = 2 μm (micrograph), F. starch (St), bar = 2 μm (micrograph)

Biometric measurements were performed in some tissues in the roots. According to the results, it was determined that the thickness of cortex increased ($p < 0.05$) when the plant was irrigated with more or less water than its optimum water need. As long as the amount of the irrigation water applied reduced, it was observed that the diameter of xylem vessel narrowed ($p < 0.01$) (Fig. 5).

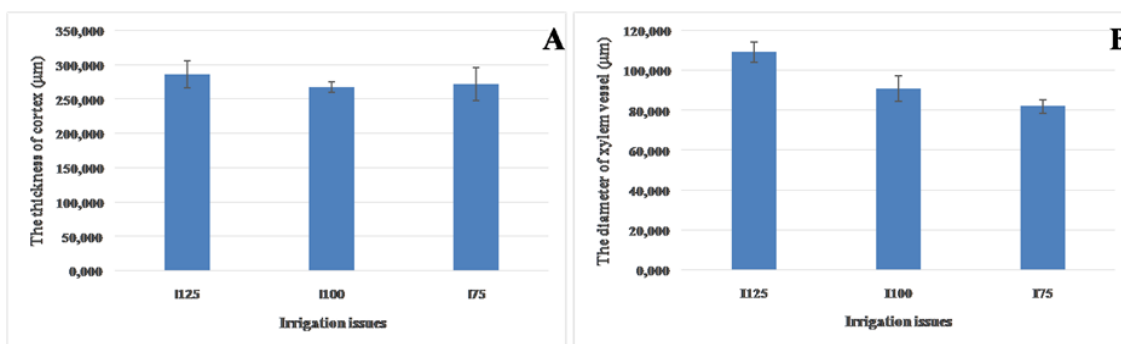


Figure 5. The relationship between the amount of irrigation water and measurements of some tissues in roots of *Zea mays L. indentata* under different irrigation conditions. A. The relationship between the amount of irrigation water and the thickness of cortex, B. The relationship between the amount of irrigation water and the diameter of xylem vessel. $p < 0.01$: highly significant, $p < 0.05$: significant, $p > 0.05$: no significant

Stems

The stems cultivated under each of the three irrigation systems were surrounded by one layered epidermis. Stomate was rarely observed (Figs. 6A, 6B, 7A, and 8A, 8B).

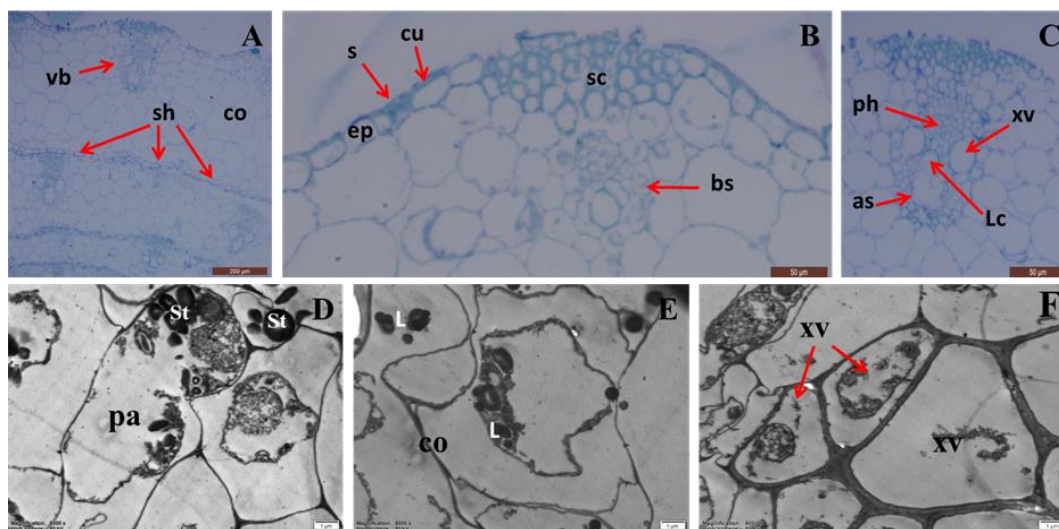


Figure 6. Histological and cytological structure of stem in *Zea mays L. indentata* under I_{100} condition (cross-section). A. vascular bundle (vb), starch sheath (sh), cortex (co), bar = 200 μm , B. cuticle (cu), stomate (s), epidermis (ep), sclerenchyma (sc), bundle sheath (bs), bar = 50 μm , C. phloem (ph), xylem vessel (xy), lacuna (Lc), air space (as), bar = 50 μm , D. paranchyma (pa), starch (St), bar = 1 μm (micrograph), E. cortex (co), lipid (L), bar = 1 μm (micrograph), F. xylem vessel (xy), bar = 1 μm (micrograph)

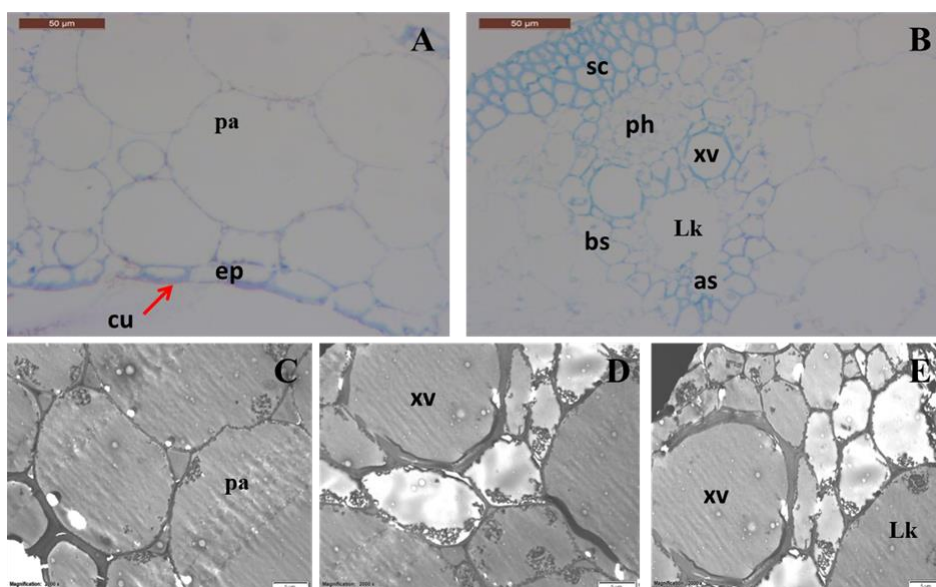


Figure 7. Histological and cytological structure of stem in *Zea mays L. indentata* under I_{125} condition (cross-section). A. cuticle (cu), epidermis (ep), paranchyma (pa), bar = 50 μm , B. sclerenchyma (sc), bundle sheath (bs), phloem (ph), xylem vessel (xy), lacuna (Lc), air space (as), bar = 50 μm , C. paranchyma (pa), bar = 5 μm (micrograph), D. xylem vessel (xy), bar = 5 μm (micrograph), E. xylem vessel (xy), lacuna (Lc), bar = 5 μm (micrograph)

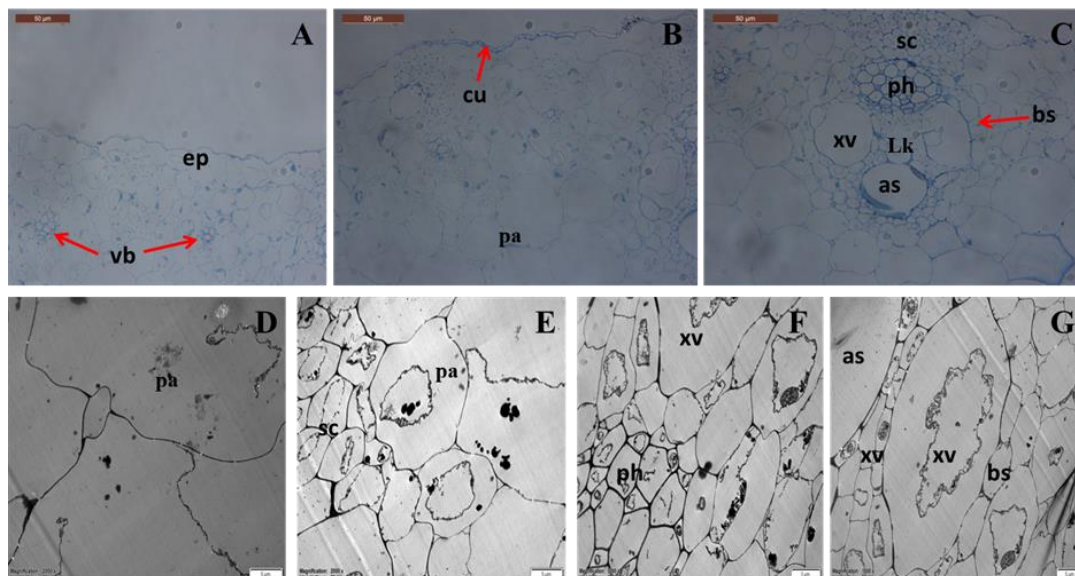


Figure 8. Histological and cytological structure of stem in *Zea mays L. indentata* under I75 condition (cross-section). A. epidermis (ep), vascular bundle (vb), bar = 50 μm , B. cuticle (cu), paranchyma (pa), bar = 50 μm , C. sclerenchyma (sc), bundle sheath (bs), phloem (ph), xylem vessel (xy), lacuna (Lc), air space (as), bar = 50 μm , D and E. paranchyma (pa), bar = 5 μm (micrograph), F. phloem (ph), xylem vessel (xy), bar = 5 μm (micrograph), G. bundle sheath (bs), xylem vessel (xy), air space (as), bar = 5 μm (micrograph)

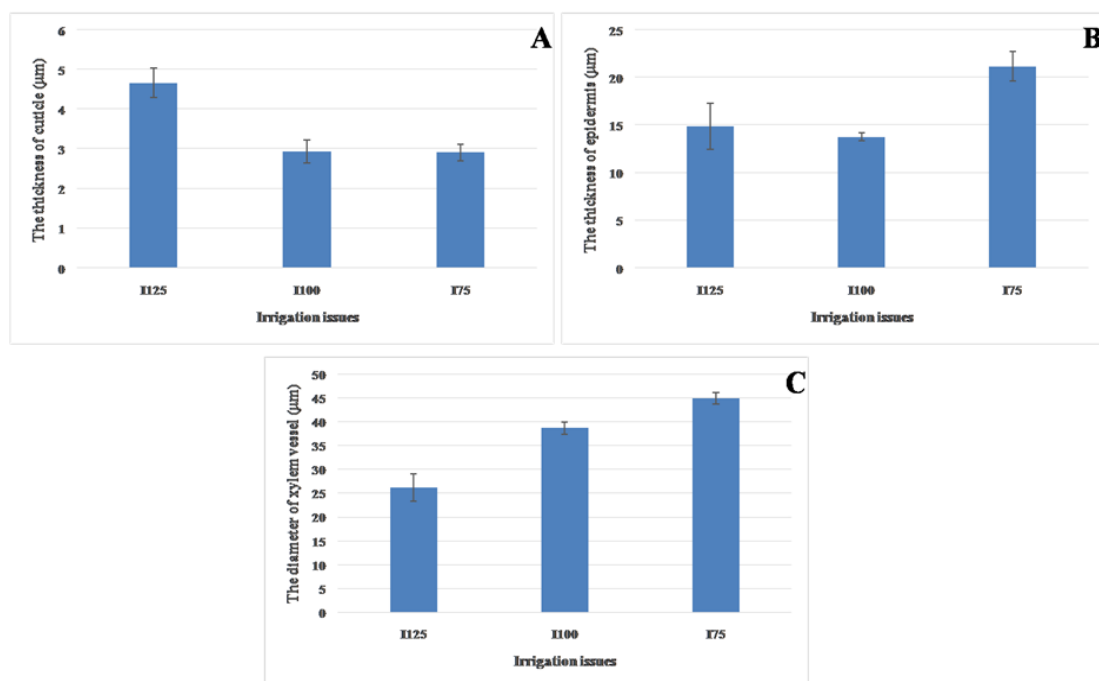


Figure 9. The relationship between the amount of irrigation water and measurements of some tissues in stems of *Zea mays L. indentata* under different irrigation conditions. A. the relationship between the amount of irrigation water and the thickness of cuticle, B. the relationship between the amount of irrigation water and the thickness of epidermis, C. the relationship between the amount of irrigation water and the diameter of xylem vessel. $p < 0.01$: highly significant, $p < 0.05$: significant, $p > 0.05$: no significant

The vascular bundles formed by xylem, phloem, lacuna and air spaces were generally surrounded by the bundle sheath. Irregularity was observed in the bundle sheath cells around small- and big-celled vascular bundles under I_{75} (Fig. 8C, 8G). The xylem and the phloem were supported by a few layered sclerenchyma tissue (Figs. 6A, 6B, 6C, 7B and 8A, 8C). The pith was paranchymatic. The cortex paranchyma cells were cytologically large and thin-walled (Figs. 6D, 6E, 7A, 7C, and 8B, 8D, 8E). Starch and lipid molecules were generally observed in paranchyma cells in the stem under I_{100} . Starch molecules were more electron-dense than lipid molecules (Fig. 6D, 6E). Starch and lipid were rarely observed under I_{125} and I_{75} . Low electron density was observed under the three conditions (Figs. 6F, 7D, 7E, and 8F, 8G).

Biometric measurements were performed in some tissues in the stems. According to the results, it was figured out that I_{125} application increased the thickness of cuticle ($p < 0.01$) while I_{75} application did not affect it clearly ($p > 0.05$). It was observed that the thickness of epidermis ($p > 0.05$) was not clearly affected under I_{125} while it increased highly significantly under I_{75} ($p < 0.01$). The diameter of xylem vessel ($p < 0.01$) increased highly significantly as long as irrigation water reduced (Fig. 9).

Leaves

The leaves cultivated under the three irrigation conditions were unifacial and amphistomatic, and stomate was in graminea type. Numerous and differently-sized bullate cells were seen in the epidermis. The bundle sheath surrounded the big vascular bundle in the midrib region and the sclerenchyma supported it on both sides. The small vascular bundles were embedded in the mesophyll (Figs. 10A, 10B, 10C, 11A, 11B, 11C, 11D, 11E, and 12A, 12B, 12C). Average number of stomate in the upper and the lower epidermis under I_{125} and I_{75} condition distinctly reduced due to stress effect. The leaf responded to over-irrigation and limited irrigation similarly with regard to the average number of stomate.

It was observed that cytologically mesophyll cells were oval or round and regular under I_{100} (Fig. 10D, 10E) while the ones under I_{125} and I_{75} were more irregular (Figs. 11A, 11G and 12A, 12C). Electron density in the vascular bundles and in bundle sheath cells around them under I_{75} was more than in the bundles under I_{100} and I_{125} (Figs. 10F, 11G, 11H, and 12D, 12E, 12F). Starch and lipid molecules were observed in the leaves under I_{125} and I_{75} (Fig. 11F). On the other hand, starch and lipid molecules were more often seen in the bundle sheath cells under I_{75} . Lipids were more electron-dense than starch (Fig. 12E). Starch and lipid molecules in the leaves under I_{100} were seldom observed.

Biometric measurements were performed in some tissues in the leaves. According to the results of the measurements, under I_{125} and I_{75} conditions the thickness of cuticle highly significantly reduced ($p < 0.01$). It was figured out that the thickness of upper epidermis under I_{125} reduced ($p < 0.01$) while it increased ($p < 0.01$) under I_{75} . The thickness of lower epidermis under I_{125} was not significantly affected ($p > 0.05$) while it distinctly increased ($p < 0.01$) under I_{75} . The number of stomate in the upper and the lower epidermis under I_{125} and I_{75} conditions reduced prominently ($p < 0.01$). As long as the amount of irrigation water reduced, the width and the length of bullate cell increased clearly ($p < 0.01$). While the width of mesophyll under I_{125} distinctly increased ($p < 0.01$), it slightly increased ($p < 0.05$) under I_{75} . The diameter of xylem vessel under I_{125} narrowed ($p < 0.05$), but it highly significantly narrowed ($p < 0.01$) under I_{75} . The distance of bundles under I_{125} and I_{75} highly distinctly increased ($p < 0.01$) and was pretty

much affected by the over-irrigation condition. It was figured out that the thickness of midrib region was affected by over-irrigation ($p < 0.05$), but it highly distinctly got thinner ($p < 0.01$) under I_{75} . While the width of big vascular bundle under I_{125} increased ($p < 0.05$), it highly distinctly reduced ($p < 0.01$) under I_{75} (Figs. 13 and 14).

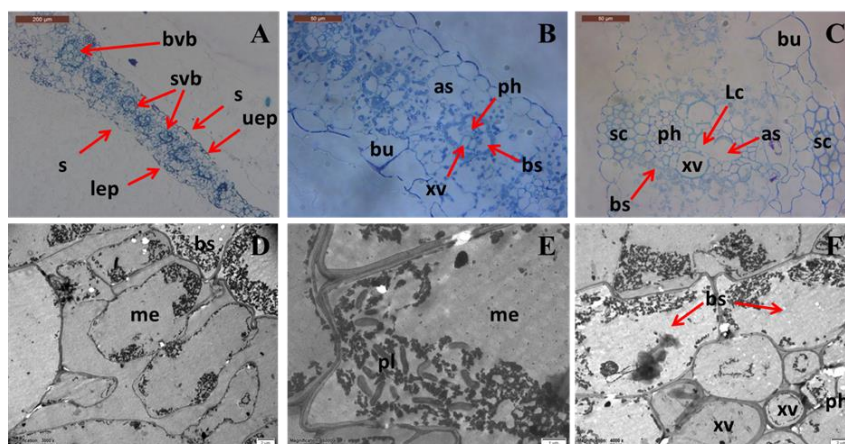


Figure 10. Histological and cytological structure of leaf in *Zea mays L. indentata* under I_{100} condition (cross-section). A. upper epidermis (uep), lower epidermis (lep), big vascular bundle (bvb), small vascular bundle (svb), stomate (s), bar = 200 μm , B. bullate cell (bu), bundle sheath (bs), xylem vessel (xy), phloem (ph), air space (as), bar = 50 μm , C. bullate cell (bu), sclerenchyma (sc), bundle sheath (bs), xylem vessel (xy), phloem (ph), lacuna (Lc), air space (as), bar = 50 μm , D. bundle sheath (bs), mesophyll (me), bar = 2 μm (micrograph), E. mesophyll (me), plastids (pl), bar = 1 μm (micrograph), F. bundle sheath (bs), xylem vessel (xy), phloem (ph), bar = 2 μm (micrograph)

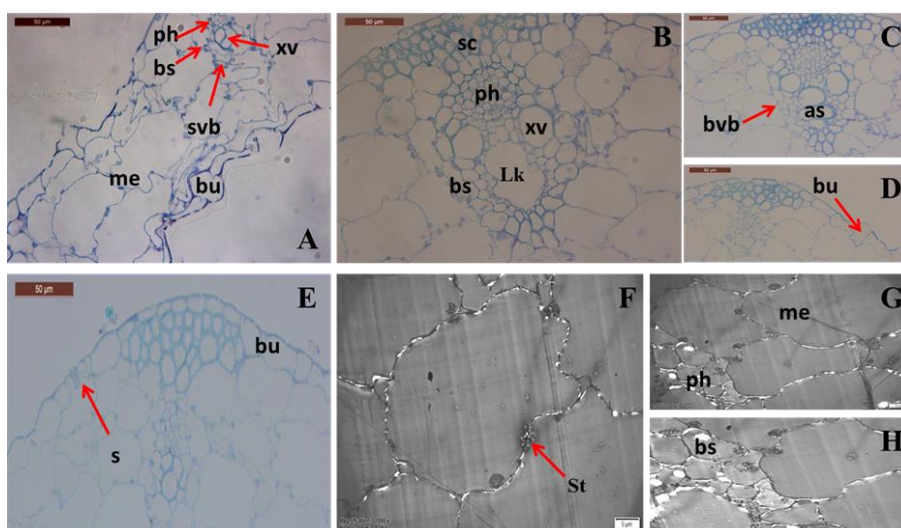


Figure 11. Histological and cytological structure of leaf in *Zea mays L. indentata* under I_{125} condition (cross-section). A. bullate cell (bu), mesophyll (me), small vascular bundle (svb), bundle sheath (bs), xylem vessel (xy), phloem (ph), bar = 50 μm , B. sclerenchyma (sc), bundle sheath (bs), xylem vessel (xy), phloem (ph), lacuna (Lk), bar = 50 μm , C. big vascular bundle (bvb), air space (as), bar = 50 μm , D. bullate cell (bu), bar = 50 μm , E. stomate (s), bullate cell (bu), bar = 50 μm , F. starch (St), bar = 5 μm (micrograph), G. mesophyll (me), phloem (ph), bar = 5 μm (micrograph), H. bundle sheath (bs), bar = 5 μm (micrograph)

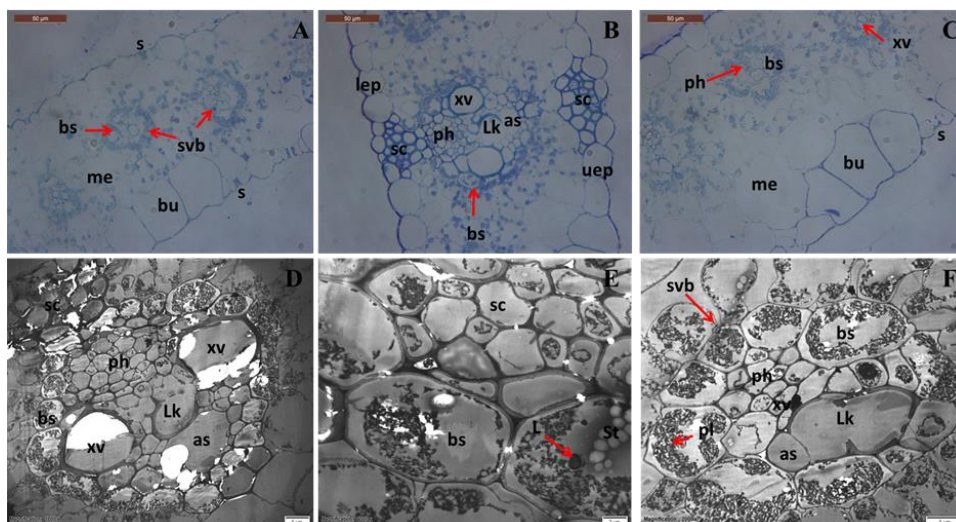


Figure 12. Histological and cytological structure of leaf in *Zea mays L. indentata* under *I75* condition (cross-section). A. stomate (*s*), bullate cell (*bu*), small vascular bundle (*svb*), bundle sheath (*bs*), bar = 50 μm , B. upper epidermis (*uep*), lower epidermis (*lep*), big vascular bundle (*bvb*), sclerenchyma (*sc*), bundle sheath (*bs*), xylem vessel (*xy*), phloem (*ph*), lacuna (*Lc*), air space (*as*), bar = 50 μm , C. stomate (*s*), bullate cell (*bu*), mesophyll (*me*), bundle sheath (*bs*), xylem vessel (*xy*), phloem (*ph*), bar = 50 μm , D. sclerenchyma (*sc*), bundle sheath (*bs*), xylem vessel (*xy*), phloem (*ph*), lacuna (*Lc*), air space (*as*), bar = 5 μm (micrograph), E. sclerenchyma (*sc*), bundle sheath (*bs*), starch (*St*), lipid (*L*), bar = 2 μm (micrograph), F. small vascular bundle (*svb*), bundle sheath (*bs*), xylem vessel (*xy*), phloem (*ph*), lacuna (*Lc*), air space (*as*), bar = 5 μm (micrograph)

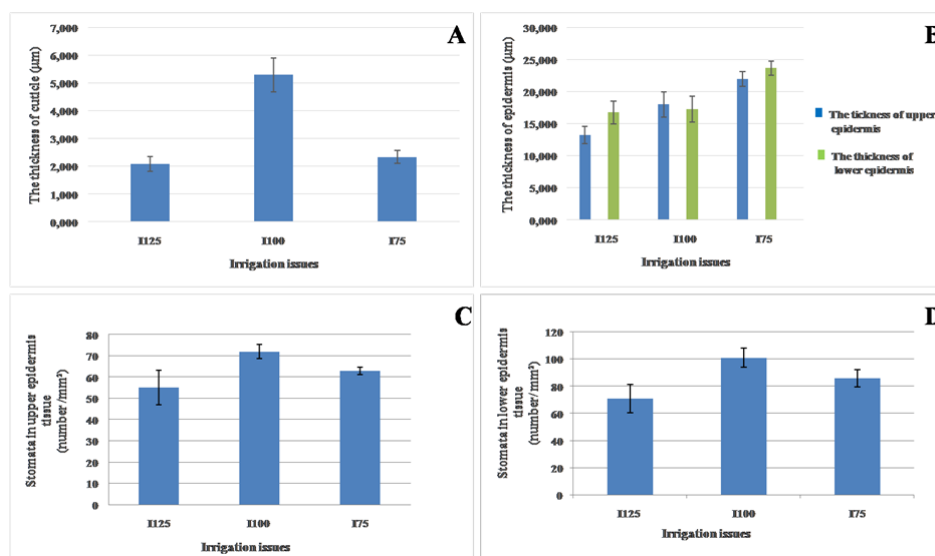


Figure 13. The relationship between the amount of irrigation water and measurements of some tissues in leaves of *Zea mays L. indentata* under different irrigation conditions. A. the relationship between the amount of irrigation water and the thickness of cuticle, B. the relationship between the amount of irrigation water and the thickness of epidermis, C. the relationship between the amount of irrigation water and the number of stomata in upper epidermis tissue, D. the relationship between the amount of irrigation water and the number of stomata in lower epidermis tissue. $p < 0.01$: highly significant, $p < 0.05$: significant, $p > 0.05$: no significant

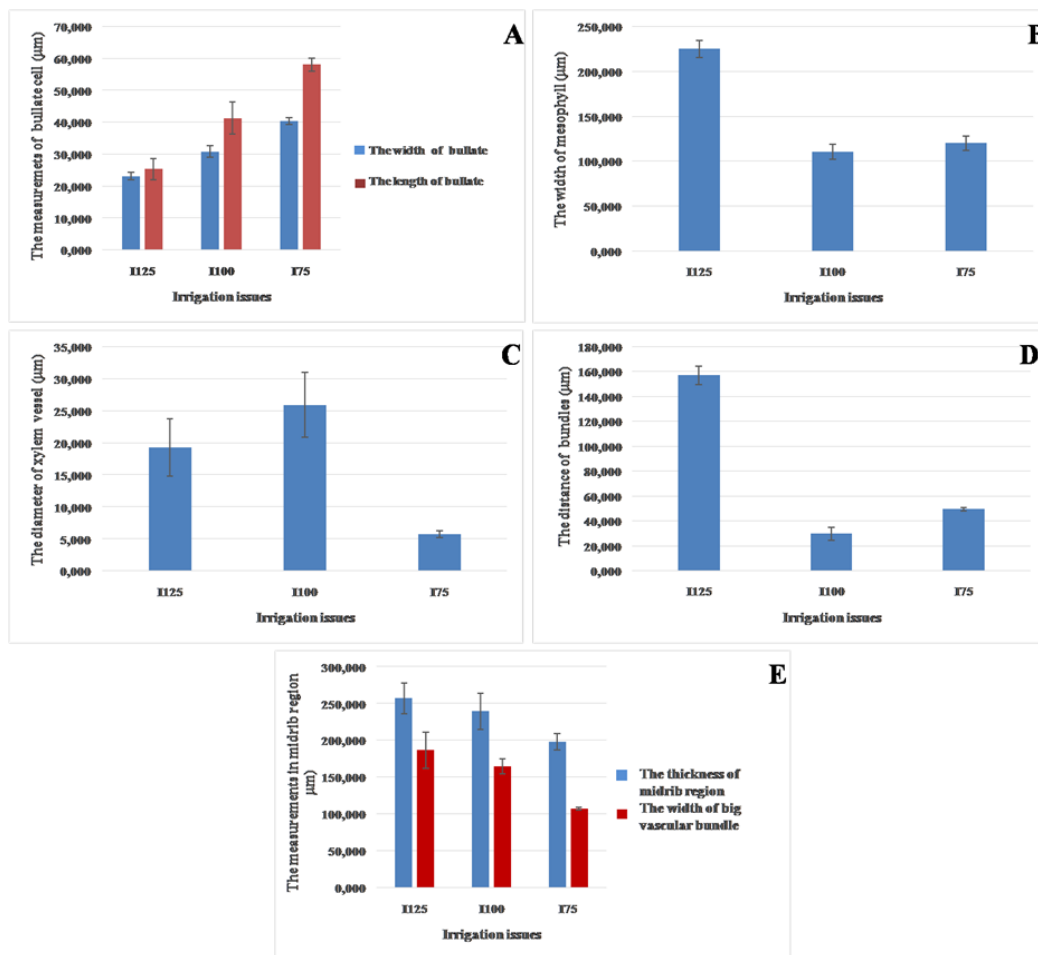


Figure 14. The relationship between the amount of irrigation water and measurements of other tissues in leaves of *Zea mays L. indentata* under different irrigation conditions. A. the relationship between the amount of irrigation water and the measurements of bullate cell, B. the relationship between the amount of irrigation water and the width of mesophyll, C. the relationship between the amount of irrigation water and the diameter of xylem vessel, D. the relationship between the amount of irrigation water and the distance of bundles, E. the relationship between the amount of irrigation water and the measurements in midrib region. $p < 0.01$: highly significant, $p < 0.05$: significant, $p > 0.05$: no significant

Discussion

Anatomical features of the root, stem and leaf of the plants cultivated under over-irrigation and limited irrigation conditions did not indicate any distinct anomaly compared to plants cultivated under optimum condition. In all of the plants studied, anatomical features of root, stem and leaf were generally similar to the features mentioned in Metcalfe (1960).

When the roots were histologically and cytologically examined, air gaps in the cortex under I₁₂₅ and I₇₅ conditions were much more than the ones under I₁₀₀. The thickness of the cortex under I₁₂₅ and I₇₅ conditions increased. The diameter of xylem vessel narrowed as the amount of irrigation water reduced. The fact that the diameter of xylem vessel under I₁₂₅ was larger than the other ones can be defined as a response to water stress. Tuylu (2018) cultivated Ceren variety of tomato under hydroponic system and stated that

xylem vessels were large and clear and phloems were generally smaller and less than xylem vessels under water stress. It was also stated that cortex and xylem vessels in the root were affected by water stress. It was observed that the thickness of the wall of paranchyma and xylem cells under I_{125} and I_{75} was relatively thicker than the ones under I_{100} . Starch grains were distinctly seen under the three conditions. While distribution of lipid in the root cultivated under I_{100} was densely observed, lipids in the roots under I_{125} and I_{75} were rarely seen. Korkmaz and Durmaz (2017) stated that plants try to survive by developing a lot of recyclable and non-recyclable reactions against all the abiotic environmental factors perceived. Electron density in paranchyma and xylem cells under I_{100} was low. Electron density in paranchyma and xylem cells in some sections under I_{125} was low, but the ones under I_{75} were more electron dense. Water deficit in the environment of life causes a negative water potential in the protoplasm. Water deficit stress tolerance was observed in almost all plant species but its amount varies from one species to another (Abdul Jaleel et al., 2007). Electron density observed in the cells under I_{75} can be ascribed to the fact that negative water potential increased in the cells under drought condition. That electron density in the cells under I_{125} was low in some sections and was high in some sections can be attributed to the fact that the plant can respond similarly to drought stress by having water stress due to over-irrigation as well.

When the stems were histologically and cytologically examined, I_{125} application increased the thickness of cuticle but it was not significantly affected under I_{75} application. The thickness of epidermis under I_{125} was not clearly affected, but under I_{75} it increased highly significantly. The diameter of xylem vessel increased highly significantly as long as irrigation water reduced. It can be explained that the plant gives a sort of storage reaction by slowing water flow in its stem due to water shortage. In the study including Malatya Kurucaova variety of tomato mentioned in Tuylu et al. (2018), it was determined that, because of water stress in the stem exposed to over-watering and cultivated under hydroponic system, xylem vessels got narrower than those cultivated in perlit condition. Starch and lipid molecules were generally observed in paranchyma cells in the stem under I_{100} condition. However, starch and lipid molecules were rarely seen in the stem under I_{125} and I_{75} conditions. As a result of this observation it can be stated that the stem of the variety examined under water and drought stress can store starch and lipid rarely. According to Sam et al. (2000), structure and functions of all plant organs are affected by water stress. It was observed that electron density in xylems was low in the three conditions. In this case, it can be stated that osmotic balance in the xylem vessels in the stems is normal in the three conditions.

When the leaves were histologically and cytologically examined, the thickness of cuticle under I_{125} and I_{75} reduced highly significantly. The thickness of upper epidermis under I_{125} reduced, but it increased under I_{75} . The thickness of lower epidermis under I_{125} was not distinctly affected, and it clearly increased under I_{75} . The number of stomate in the upper and the lower epidermis under I_{125} and I_{75} conditions was markedly reduced. The average number of stomate in the upper and the lower epidermis responded similarly to over-irrigation and limited irrigation by reducing markedly as stress effect under I_{125} and I_{75} conditions. In the study, Makbul et al. (2011) clearly stated that an average number of stomate of upper and lower epidermis in 1 mm^2 in soybean increased as a result of drought stress.

The width and length of bullate cell distinctly increased as long as the amount of irrigation water reduced. It means that the plant adapted by increasing its capacity of water storage against drought. The width of mesophyll under I_{125} condition was

distinctly increased, but it was slightly increased under I_{75} . The thickness of the midrib region was affected by over-irrigation and got thinner highly significantly under I_{75} condition. Additionally, while the width of the big vascular bundle under I_{125} condition increased, it reduced highly significantly under I_{75} condition. In this case, it can be expressed that the leaf showed the feature of curling developed as an anatomical reaction to stress under I_{125} condition. In Terzi and Kadioğlu (2006), it was stated that the response to drought stress is quite related to anatomical and morphological changes such as leaf curling. While the diameter of the xylem vessel under I_{125} condition narrowed significantly, it narrowed highly significantly under I_{75} condition. The fact that the distance of the bundles under I_{125} and I_{75} conditions increased highly significantly and was pretty much affected by over-irrigation condition was determined. Cytologically, the mesophyll cells were oval or round and regular under I_{100} , but they were irregular under I_{125} and I_{75} . Starch and lipid molecules in the leaves under I_{125} and I_{75} were observed. The vascular bundles and the bundle sheath cells around them under I_{75} were more electron-dense than the ones under I_{100} and I_{125} . It can be explained that negative water potential increased in the cells under drought condition. These results conform with Utrillas' and Alegre (1997)'s study carried out in *C. dactylon*. They stated that mesophyll cells in the leaves under drought stress were more irregular than the ones in well-watered leaves, and starch was observed in mesophyll chloroplasts under stress in *C. dactylon*.

Conclusion

In conclusion, in the study of the amount of irrigation water for optimum irrigation, over-irrigation and limited irrigation conditions applied were 704 mm, 880 mm, and 528 mm, respectively. The changes observed in the root, stem and leaf under over-irrigation and limited irrigation conditions are not anomalies but adaptation attitudes.

According to the results in the study, some features such as length, thickness and diameter in various tissues of the variety of maize examined showed changes due to adaptation to stress conditions. The thickness of cortex and the diameter of xylem vessel in the root, the thickness of cuticle and the diameter of xylem vessel in the stem, the thickness of cuticle, the thickness of upper epidermis, the number of stomate in the upper and the lower epidermis, the length and the width of bullate cell, the width of mesophyll, the diameter of xylem vessel, the distance of bundles, the thickness of midrib region, and the width of big vascular bundle in leaf significantly changed under over-irrigation condition. Under limited irrigation condition, the thickness of cortex and the diameter of xylem vessel in the root, the thickness of epidermis, the diameter of xylem vessel in the stem, the thickness of cuticle, the thickness of the upper and the lower epidermis, the number of stomate in the upper and the lower epidermis, the length and the width of bullate cell, the width of mesophyll, the diameter of xylem vessel, the distance of bundles, the thickness of midrib region, and the width of big vascular bundle in the leaf significantly changed.

As a result, it was figured out that the variety of maize examined developed basic tissues adapting to stress condition and is anatomically suitable for cultivation by over-irrigation and limited irrigation. The ecological condition created by applying 25% water shortage can be anatomically suggested for the cultivation of maize. This result is significant in terms of saving water in irrigation. In addition, morphological, physiological, etc. parameters of the plant should also be considered for good cultivation and to develop the best method in cultivation.

REFERENCES

- [1] Abdul Jaleel, C., Manivannan, P., Kishorekumar, A., Sankar, B., Gopi, R., Somasundaram, R., Panneerselvam, R. (2007): Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in *Catharanthus roseus* exposed to water deficit. – *Colloids and Surfaces B: Biointerfaces* 59: 150-157.
- [2] Anonymous (2017): Mardin Climate Values (Mardin İklim değerleri). – Turkish State Meteorological Service (Meteoroloji Genel Müdürlüğü), Ankara, Turkey (personally received).
- [3] Anonymous (2018): Report about Maize (Mısır Raporu). – http://www.zmo.org.tr/genel/bizden_detay.php?kod=26263 (accessed 01.01.2018).
- [4] Demirok, A. (2017): Searching Applications of Surface and Subsurface Drip Irrigation for Corn (*Zea mays* L. *indendata*) Plant in Harran Plain (Harran Ovası'nda Mısır (*Zea mays* L. *indendata*) Bitkisi için Toprak Üstü ve Toprak Altı Damla Sulama Uygulamalarının Araştırılması). – Harran University Graduate School of Natural and Applied Sciences (Harran Üniversitesi Fen Bilimleri Enstitüsü), Master Thesis (Yüksek Lisans Tezi), Şanlıurfa, Turkey.
- [5] Demirok, A., Tuylu, G. İ. (2017): Evaluation of planning and actual irrigation time scheduling for the maize (*Zea mays* L.) plant in Harran Plain (Harran Ovası'nda Mısır Bitkisi (*Zea mays* L.) için Planlanan ve Gerçekleşen Sulama Zamanı Programının Değerlendirilmesi). – *Harran Tarım ve Gıda Bilimleri Dergisi* 21(1): 84-90.
- [6] Korkmaz, H., Durmaz, A. (2017): Responses of plants to abiotic stress factors (Bitkilerin Abiyotik Stres Faktörlerine Verdiği Cevaplar). – *GUFBED* 7(2): 192-207.
- [7] Luft, J. H. (1961): Improvements in epoxy resin embedding methods. – *J. Biophys. Biochem. Cytol.* 9: 409.
- [8] Makbul, S., Saruhan Güler, N., Durmuş, N., Güven, S. (2011). Changes in anatomical and physiological parameters of soybean under drought stress. – *Turk J Bot.* 35: 369-377. DOI: 10.3906/bot-1002-7.
- [9] Metcalfe, C. R. (1960): *Anatomy of Monocotyledons*. – Clarendon Press, Oxford, pp. 532-536.
- [10] Orhangazi, R. (2017): Investigation of Surface and Subsurface Drip Irrigation Applications for Pepper (*Capsicum annuum* L.) Plant in Harran Plain (Harran Ovası'nda Biber (*Capsicum annuum* L.) Bitkisi için Toprak Üstü ve Toprak Altı Damla Sulama Uygulamalarının Araştırılması). – Harran University Graduate School of Natural and Applied Sciences (Harran Üniversitesi Fen Bilimleri Enstitüsü), Master Thesis (Yüksek Lisans Tezi), Şanlıurfa, Turkey.
- [11] Öktem, A. G., Öktem, A., Özel, M. R. (2017): Methods of Drying and Storing Maize (Mısır Tanesini Kurutma ve Depolama Yöntemleri). – 12.Tarla Bitkileri Kongresi Kongre Kitabı, 12-15 Eylül, Kahramanmaraş, pp. 70-73.
- [12] Sam, O., Jerez, E., Dell'Amico, J., Ruiz Sanchez, M. C. (2000): Water stress induced changes in anatomy of tomato leaf epidermis. – *Biologia Plantarum* 43(2): 275-277.
- [13] Stempak, J. G., Ward, R. T. (1964): An improved staining method for electron microscopy. – *J. Cell Biol.* 22: 697.
- [14] Terzi, R., Kadioğlu, A. (2006): Drought stress tolerance and the antioxidant enzyme system in *Ctenanthe setosa*. – *Acta Biologica Cracoviensia Series Botanica* 48(2): 89-96.
- [15] Tuylu, M. (2015): Leaf and Stem Anatomy, Histology, Cytology of Several *Marrubium* L. (*Lamiaceae*) Taxons (Bazı *Marrubium* L. (*Lamiaceae*) Taksonlarının Yaprak ve Gövde Anatomisi, Histolojisi, Sitolojisi). – University of Süleyman Demirel Institute of Science (Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü), Phd. Thesis (Doktora tezi), Isparta, Turkey.
- [16] Tuylu, M. (2018): Examination of anatomical features of tomato (*Lycopersicon esculentum* Mill.) varieties cultivated under hydroponic system. – *Applied Ecology and Environmental Research* 16(3): 3381-3391.

- [17] Tuylu, M., Tuylu, G. İ., Söylemez, S., Büyükkartal, H. N. (2018): Comparing Some Anatomical Features of Tomato (*Lycopersicon esculentum* Mill. cv. Kurucaova) Cultivated under Perlit and Hydroponic Culture (Perlit ve Su Kültürü Ortamlarında Yetiştirilen Domates (*Lycopersicon esculentum* Mill. cv. Kurucaova) Bitkisinin Bazı Anatomik Özelliklerinin Karşılaştırılması) – Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi. DOI: 10.19113/sdufbed.93255.
- [18] Utrillas, M. J., Alegre, L. (1997): Impact of water stress on leaf anatomy and ultrastructure in *Cynodon dactylon* (L.) Pers. under natural conditions. – International Journal of Plant Sciences 158(3): 313-324. <https://about.jstor.org/terms/> (accessed 06.04.2016).