SELECTION OF ACCURATE IRRIGATION SYSTEM BASED ON ROOT ANATOMY IN COTTON (*Gossypium hirsutum* L. cv. Stoneville 468) CULTIVATION

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Abstract. In the study it was aimed to figure out the accurate irrigation system condition for cotton cultivation. So how cotton root tissues respond to different irrigation applications was examined under different conditions created by applying different irrigation water levels and using Drip Irrigation System and Subsurface Drip Irrigation System. Finally, the effects of two irrigation systems on cotton root anatomy were compared and optimum irrigation condition was determined for both systems. In irrigation period of 2015 cotton was cultivated. Four different irrigation water levels were applied to cotton by two irrigation systems. Anatomical studies were performed by using paraffin method and light microscopy in 2016. It was anatomically determined that the optimum irrigation condition was 25% limited- irrigation under Drip Irrigation System Condition while it was 50% limited- irrigation under Subsurface Drip Irrigation System Condition. The effects of the two systems on development of the periderm tissue, which is one of the cotton root tissues, were different under drought and water stress conditions. Under both conditions, xylem vessels developed an anatomically similar response when the roots of cotton were exposed to the amount of irrigation water which was more than or less than 25% of the optimum irrigation water requirement.
Keywords: *abiotic factor, cover tissue, industrial plant, pressurized-irrigation, water resources*

Introduction

Cotton is one of the most valuable industrial plants. Cultivation of cotton is as important as its processing. In order to obtain high efficiency, the ecological needs of the plant should be met. Meeting these ecological needs directly affects the yield and fiber quality parameters of cotton. Irrigation is one of the important parameters in cultivation to optimize the ecological conditions (Özkaya and Tuylu, 2024; Tuylu and Akın, 2023). Drip Irrigation System (DIS) belongs to pressurized- irrigation methods. Subsurface Drip Irrigation System (SDIS) is known to be created as a modified DIS. Irrigation applications and the amount of irrigation water as abiotic factors are effective on plants. In some previous studies, comparing of DIS and SDIS was studied (Gençoğlan et al., 2006; Kırnak et al., 2002; Önder et al., 2005). In those studies, the comparisons were performed by evaluating morphological features such as roots lengths, plant height, etc., physiological features such as taste, active ingredient content, etc., and the other features such as water use efficiency, irrigation water usage efficiency, etc. in the plants cultivated under the conditions of DIS and SDIS. The anatomical changes can also occur in various tissues in roots as the other organs tissues. Especially xylem vessel which is responsible for the transmission of water belonging to vascular bundles is important. Traditionally, stomatal conductance and root conductivity have been considered the main controlling factors of water flow in the plant (Jones, 1983). Xylem conductivity is determined by the structure and size of the vessels (Schultz and Matthews, 1993; Tyree and Ewers, 1991). In Lovisolo and Schubert

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(1998), they used the stem heat balance (SHB) method as an adaptation to woody plants and measured the xylem sap flow. In many previous studies, it was determined that cotton roots grow very fast in the first development period (Ekinci, 2013). The situation is valid for all tissues of cotton root such as xylem vessels, cover tissue, etc.

In the study, it was aimed to figure out the answer of the question: "Why should SDIS be preferred instead of DIS in cotton cultivation?" The research was based on anatomical changes occurred in xylem vessels and periderm tissues in the cultivar (cv.) cotton roots applied different water levels under DIS and SDIS Conditions. According to the results obtained by comparing biometric measurements performed the effects of DIS and SDIS on meeting the plant water need under conditions created by applying different irrigation water levels were compared.

Materials and methods

The study was performed in the field representing Harran Plain in Harran University, Şanlıurfa, Türkiye during the spring of 2015. Cv. Stoneville 468 of Cotton (*Gossypium hirsutum* L.) was cultivated. The anatomical studies were performed in Ankara University, Ankara, Türkiye in 2016. Şanlıurfa, situated in south eastern Anatolia, Türkiye and some climate values measured in research area were presented in *Table 1*.

Table 1. *Average climate values of 2015 and 1985-2016 long years (LY) in research area (Anonymous, 2015)*

Climate parameters		Maximum temperature	Minimum temperature	Mean temperature	Relative humidity	Wind speed	Hours of sunshine	Rain
Months	**Year**	**(°C)**	**(°C)**	**(°C)**	**(vv^{-1})**	**($2\ m\ s^{-1}$)**	**(h)**	**(mm)**
May	2015 LY	30.1 29.2	23.2 16.2	21.1 22.6	32.4 46.4	1.7 1.7	10.1 9.3	10.5 27.9
June	2015 LY	34.6 35.2	20.6 21.5	27.8 28.7	35.1 35.3	1.8 2.2	12.3 11.6	0.6 3.0
July	2015 LY	39.9 39.2	26.1 25.2	34.3 32.4	25.4 32.8	1.6 2.2	12.4 11.6	0.0 0.0
August	2015 LY	38.3 38.8	24.6 24.8	31.5 31.8	36.4 36.7	1.6 1.9	11.2 11.0	0.0 0.0
September	2015 LY	36.4 34.0	23.6 20.5	29.9 26.9	30.3 40.1	1.8 1.7	9.0 9.2	0.0 0.3
October	2015 LY	27.7 26.9	17.1 15.3	22.4 20.2	50.4 49.7	1.0 1.2	6.1 7.4	58.5 37.2
November	2015 LY	20.2 18.5	9.7 8.6	14.0 12.7	47.9 61.5	1.1 1.2	6.3 5.4	7.8 27.0

The study was carried out in two parts. In one part of the study, irrigation water was applied by DIS under the conditions of four irrigation issues determined. In the other part of the study, irrigation water was applied by SDIS under the same irrigation issues mentioned in the application by DIS. The part of the study applied by SDIS had been published (Tuylu and Tuylu, 2022). In that chapter published, the relationships between irrigation and the root, the stem, and the leaves anatomy of cotton and optimum irrigation condition for cotton cultivation were presented. In the present study, the relationship between irrigation and root of cotton anatomy under different irrigation issues by using DIS was examined. In addition, the two studies (The present study and Tuylu and Tuylu, 2022) carried out under the same conditions as period,

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climate parameters, etc. mentioned were evaluated together according anatomical responses of root tissues under two irrigation systems conditions and the systems were compared.

In the study area the irrigated parcels for the two parts were arranged in completely randomized experimental design with each irrigation treatments replicated three times. Each parcel was 10.5 m^2 and seeds of cotton were planted as 16 × 70 cm and 5 sequenced. Two sequences were separated as border effect in the evaluation. The soil in study area is heavy- textured and contains sand, silt and clay (*Table 2*).

Lateral depth belonging to SDIS was 1 meter. The depth was determined by considering plant root length in clay soils. The ground was compacted and covered with polyethylene material in order to prevent irrigation water from infiltrating deeply. In the study, irrigation interval was determined by considering farmer practices. In the region, furrow- irrigation method is commonly used in cotton irrigation, and the first irrigation is performed in 30-40 days after germination. These condand subsequent irrigations vary from 12 to 15 days. In the study, planting was performed on May 1, and the soil moisture content was in field capacity. The first irrigation was applied after germination (35 days). The second and subsequent irrigations were applied at intervals of 8-12 days. C_3S_1 class underground water was used as irrigation water. No washing was performed because the electrical conductivity of the water was low (*Table 3*).

Table 2. *Some of the soil physical and chemical properties of study field*

Depth (cm)	ECe (dsm^{-1})	FC (gg^{-1})	WP (gg^{-1})	BD (gcm^{-3})	Moisture (mm)	Clay (%)	Silt (%)	Sand (%)	Texture
0-30	1.04	32.50	22.10	1.15	35.88	56.56	20.00	23.44	Clay
30-60	1.07	31.40	21.20	1.40	42.84	54.56	17.00	24.44	Clay
60-90	1.08	29.60	22.08	1.16	26.17	62.56	17.00	21.44	Clay
90-120	1.55	28.70	22.07	1.20	23.87	54.56	19.00	24.44	Clay

ECe: Electrical conductivity, FC: Field capacity, WP: Wilting point, BD: Bulk density

Table 3. *Some chemical properties of irrigation water*

Electrical conductivity (µm hoscm^{-1})	Cations (meL^{-1})				Anions (meL^{-1})				pH value	Irrigation water classification
	$Ca^{++}Mg^{++}$	K^+	Na^+	Total	HCO_3^-	Cl^-	SO_4	Total		
1080	1.98	0.02	0.25	2.25	0.90	0.60	0.75	2.25	7.0	C_3S_1

In the study, the plant water requirement was figured out by using the Class A Pan method (Demirok and Tuylu, 2017). Class A Pan Coefficient (kcp) was determined as 1 (100% (v/v)). The irrigation issues were created as I_1, I_2, I_3, and I_4 in both parts of the study. I_1 issue was determined as full- irrigation. The amounts of water in full-irrigation was decreased and I_2, I_3, and I_4 issues were obtained (25, 50, 75%, respectively). According to issues 989. 2 mm, 741. 9 mm, 494. 6 mm, and 247. 3 mm irrigation water was applied to cotton, respectively. In the study, while the irrigation issues in the part of the study DIS were named I_1, I_2, I_3, and I_4, the irrigation issues in the part of the study used SDIS were named I_{1a}, I_{2a}, I_{3a}, and I_{4a}. In two parts of the study the irrigation issues were the issues that correspond to each other, respectively. DAP fertilizer was applied as 16 kg da^{-1} (Nitrogen) and 8 kg da^{-1} Diphosphorus

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Pentoxide (P_20_5) to the soil during seed sowing. Depending on the vegetative development of the plant 20 kg da^{-1} urea fertilizer (46% N), 25 ml foliar fertilizer (NH_2), 25 ml phosphorus (P) fertilizer, and 20 kg ha^{-1} ammonium nitrate (16. 5% NH-N, 16. 5% NO-N) fertilizer were used in each parcels. The weeds were cleared by hand. No disease was encountered.

Anatomical studies were performed according to the paraffin method and light microscopy. Cross sections obtained from the roots by microtome Leica SM 2000 R were 8-10 µm thick and they were stained by toluidine blue. Leica 1000 as Light microscope was used in examination and the results were photographed by digital camera Leica EC3. The photos are completely original. Las v4.3 program was used for biometric measurements. The arithmetic mean of the values in the biometric measurements, mean standard errors, statistical evaluations (p significance levels), and graphs were obtained using Excel Computer Software (Tuylu, 2015, 2018a, b; Tuylu et al., 2017, 2018, 2019; Tuylu and Tuylu, 2019, 2022).

Results

The anatomical analyses under DIS condition

Periderm which was a cover tissue structure that continued to thicken under the degenerated epidermis tissue was observed in the root. Although it had the appearance of exodermis in some places, it generally had periderm features. The fact that the root had a wide range of small and large xylem vessels towards the pith and periderm was observed in common in all issues. Phloem was developed in accordance with the collateral structure (formed collateral). Druz crystals were found under periderm and in parenchymatic cells near the vascular bundle in I_1, I_2, I_3, and I_4. It was observed that the root was seconder structure in I_1, I_2, and I_3. It could not distinctly observed that the root was the seconder structure in I_4. In addition, 4- 5 layered periderm in I_1, I_2, and I_3 issues and 6-7, sometimes 8 layered periderm in I_4 issue were observed (*Figs. 1–4*).

Analyses of the relationship between irrigation and root anatomy under DIS condition

Periderm tissues were thinner in I_1, I_3, and I_4 than the ones in I_2 ($p < 0.01$, $p < 0.01$, $p < 0.05$; respectively). While the diameter of xylem vessels in I_1 and I_4 were insignificantly wider than the ones in I_2 ($p > 0.05$, $p > 0.05$), it was significantly narrowed in I_3 ($p < 0.05$) (*Graph 1*). According to root anatomy under DIS condition, 25% limited- irrigation issue was determined as optimum issue by evaluating biometric measurements of tissues among different irrigation issues.

The anatomical analyses under SDIS condition

According to the microscopy observations, the anatomy of cotton root cultivated under SDIS Condition in Tuylu and Tuylu (2022) and DIS Condition in the study were similar.

Tuylu and Tuylu (2022) stated that periderm thickened in I_{1a}, I_{2a}, and I_{4a} more than the one in I_{3a} ($p < 0.01$, $p < 0.01$, $p < 0.01$) in root in I_{1a}, I_{2a}, I_{3a}, and I_{4a} issues. While the diameter of xylem narrowed in I_{2a} and I_{4a} ($p > 0.05$, $p < 0.05$; respectively), it increased in I_{1a} ($p > 0.05$) (*Graph 2*). It was figured out that 50% limited- irrigation issue was the optimum irrigation issue under SDIS Condition in Tuylu and Tuylu (2022).

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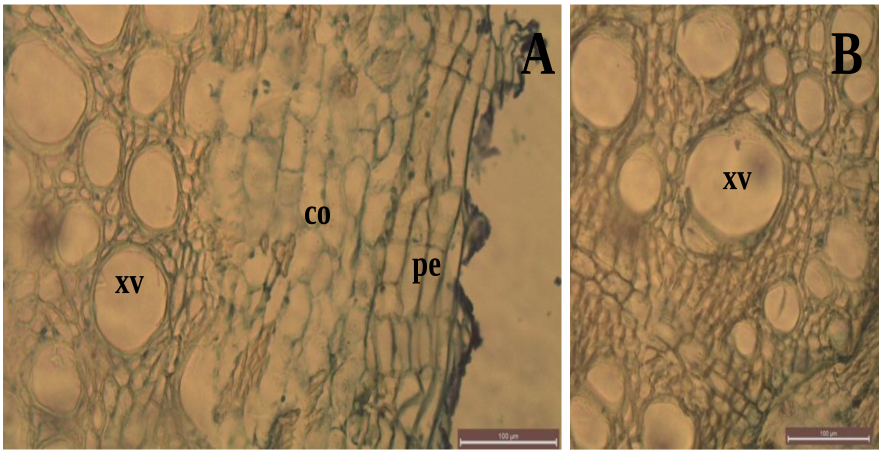

Figure 1. *Cotton root under I_1 condition (cross section). A: periderm (pe), cortex (co), bar = 100 µm, xylem vessel (xv), bar = 100 µm, B: xylem vessel (xv), bar = 100 µm*

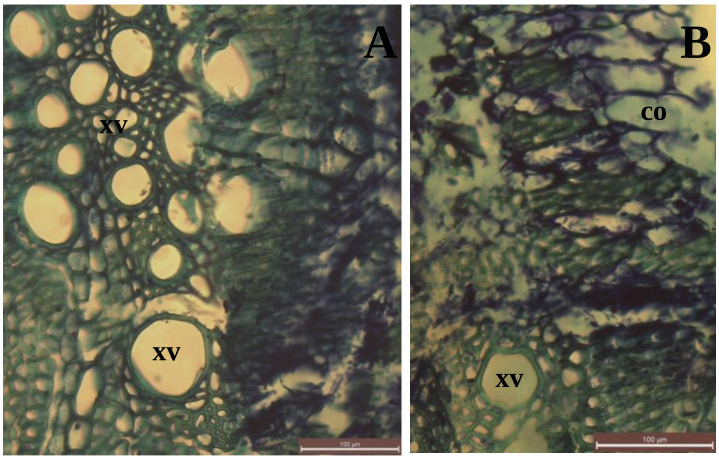

Figure 2. *Cotton root under I_2 condition (cross section). A: xylem vessel (xv), bar = 100 µm, B: cortex (co), xylem vessel (xv), bar = 100 µm*

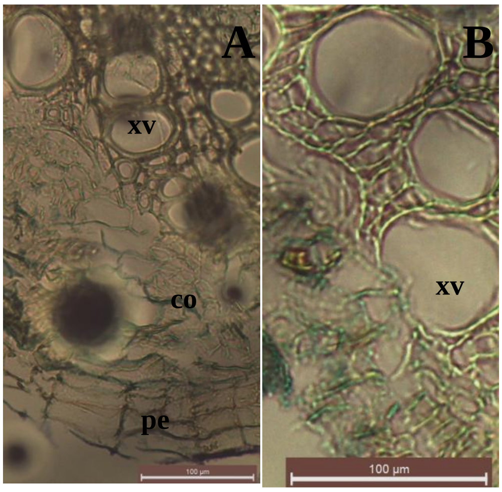

Figure 3. *Cotton root under I_3 condition (cross section). A: periderm (pe), cortex (co), xylem vessel (xv), bar = 100 µm, B: xylem vessel (xv), bar = 100 µm*

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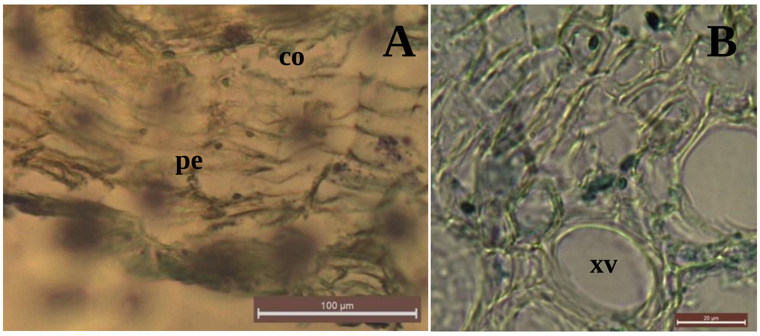
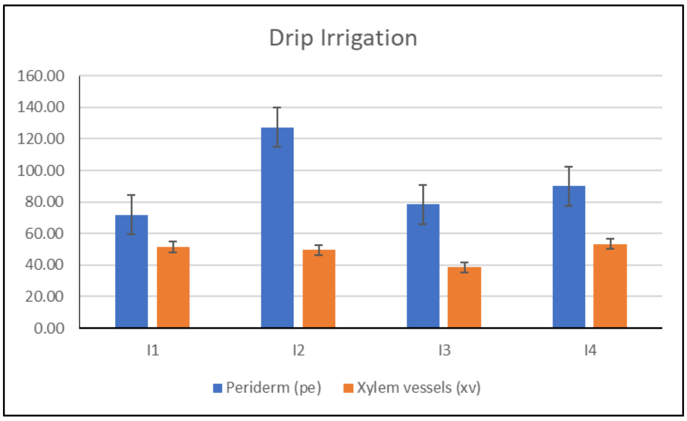
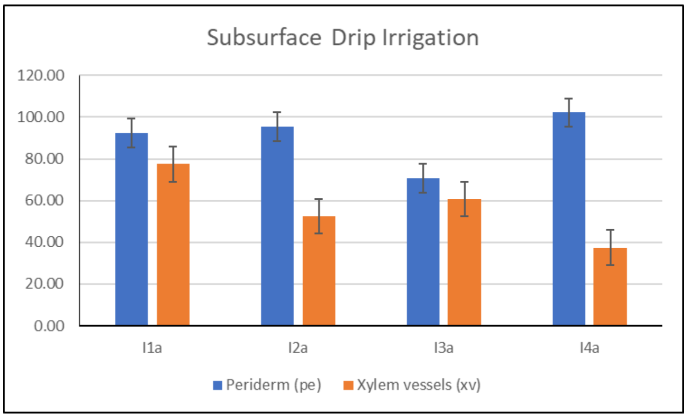


Figure 4. *Cotton root under I_4 condition (cross section). A: periderm (pe), cortex (co), bar = 100 µm, B: xylem vessel (xv), bar = 20 µm*



Graph 1. *The relationship between different irrigation water amounts and root tissues under using DIS condition ($p < 0.01$: very significant; $p < 0.05$: significant; $p > 0.05$: no significant)*



Graph 2. *The relationship between different irrigation water amounts and root tissues under using SDIS condition ($p < 0.01$: very significant; $p < 0.05$: significant; $p > 0.05$: no significant) (Tuylu and Tuylu, 2022)*

Comparing roots responses under both conditions

When the two systems were compared according to microscope observations; the fact that anatomical structures of cotton root were similar was observed in all irrigation issues

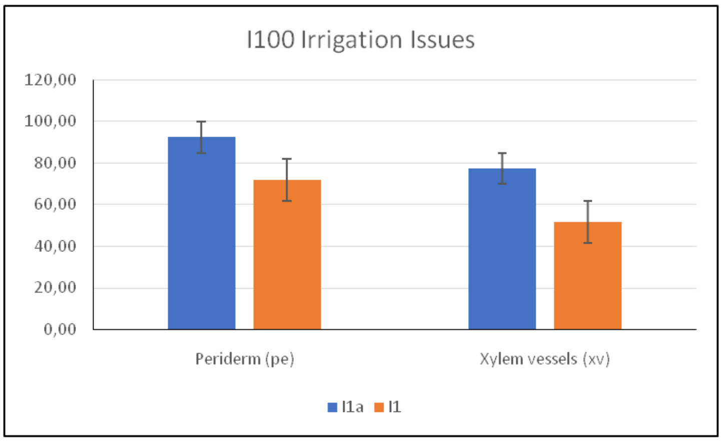
(*Figs. 1–4*; Tuylu and Tuylu, 2022). In the roots irrigated by DIS and SDIS in I_4 issue seconder structure was not distinctly observed. When the two systems were anatomically evaluated by biometric measurements among the irrigation issues in themselves, it was determined that the optimum irrigation issue was 25% limited- irrigation under DIS Condition while it was 50% limited- irrigation under SDIS Condition. It means that for both systems, the full- irrigation issue determined and applied by Class A Pan method at the beginning of the study created a water stress effect on cotton root.

According to the biometric measurements performed; while periderm tissue thinned under DIS Condition as it responded to water stress ($p < 0.01$) and drought stress ($p < 0.01$; $p < 0.05$), periderm thickened because of both stress factors under SDIS Condition ($p < 0.01$). In other words, when more or less than the optimum irrigation water requirement of the cotton plant was applied, the effects of the two systems on the development of periderm tissue, which is one of the cotton root tissues, was in the opposite direction. The diameter of xylem vessels under both System Conditions was statistically insignificantly affected by under over- irrigation condition ($p > 0.05$), while under 25% reduced- irrigation water condition narrowed significantly ($p < 0.05$). It means that xylem vessels under both systems conditions developed an anatomically similar response when the roots of cotton were exposed to application of irrigation water which was more than or less than 25% of the optimum irrigation water requirement.

When the two systems were compared by evaluating the biometric measurements performed according to irrigation issues; periderm tissue cultivated by application of full- irrigation under SDIS Condition was thicker and the diameter of xylem vessel was wider than the ones which were cultivated under DIS Condition ($p < 0.05$) (*Graph 3*). In addition, both systems did not affect the tissues examined in the root in the other issues anatomically when they were compared among in themselves ($p > 0.05$) (*Graphs 4, 5,* and *6*).

Discussion

In all of the cotton roots studied, anatomical features of root were generally similar to the features of Malvaceae family mentioned in Metcalfe and Chalk (1950). Under only one irrigation issue (I_4) condition, seconder structure was not distinctly observed in the roots irrigated by DIS and SDIS. It could be explained that the roots under I_4 issue were hardly exposed to drought stress.



Graph 3. *The relationship between full- irrigation and root tissues under both irrigation systems conditions ($p < 0.01$: very significant; $p < 0.05$: significant; $p > 0.05$: no significant)*

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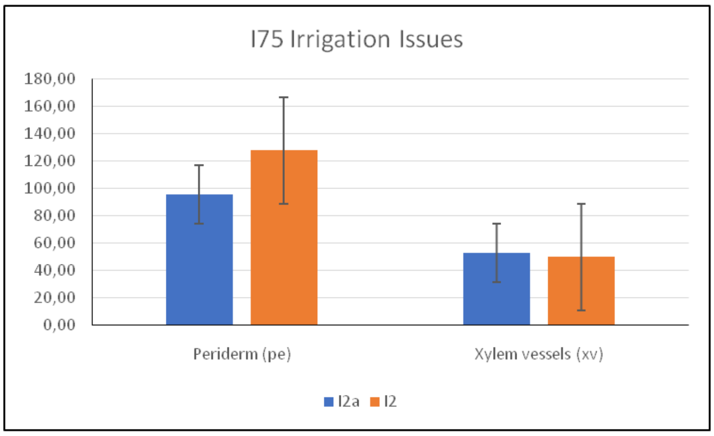
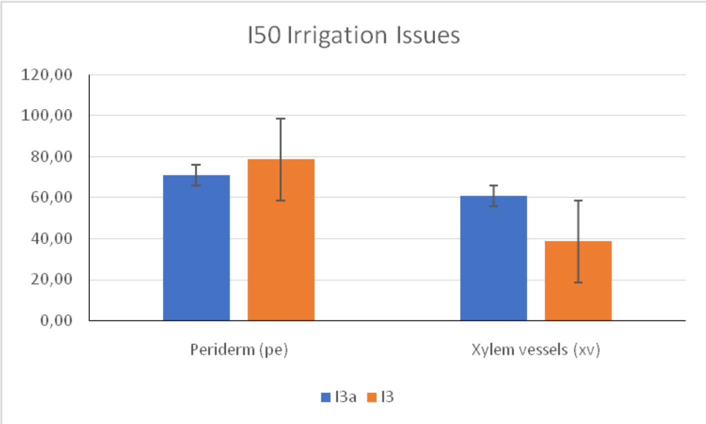
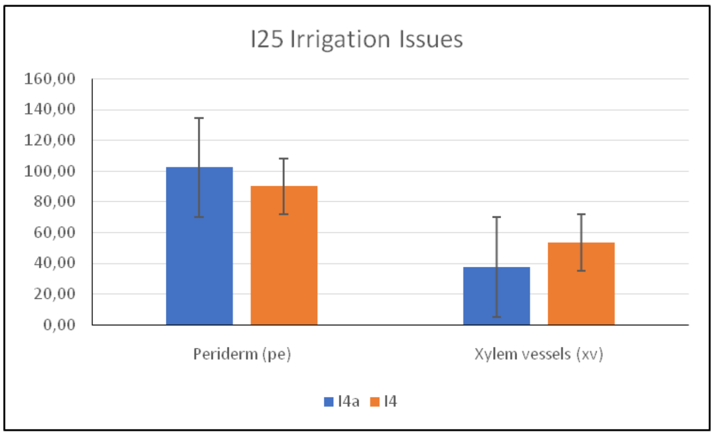


Graph 4. *The relationship between 25% limited- irrigation and root tissues under both irrigation systems conditions (p < 0.01: very significant; p < 0.05: significant; p > 0.05: no significant)*



Graph 5*. The relationship between 50% limited- irrigation and root tissues under both irrigation systems conditions (p < 0.01: very significant; p < 0.05: significant; p > 0.05: no significant)*



Graph 6. *The relationship between 75% limited- irrigation and root tissues under both irrigation systems conditions (p < 0.01: very significant; p < 0.05: significant; p > 0.05: no significant)*

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In the study, the results obtained showed that the roots cultivated under both irrigation system conditions created by applying different levels of irrigation water underwent some anatomical changes by increasing or decreasing of their thickness or diameters and they adapted to the environment. The similar results were also figured out in previous studies (Tuylu et al., 2018; Tuylu, 2018a, b; Tuylu and Tuylu, 2019).

According to the biometric measurements belonging to cotton cultivar examined, when the irrigation issues were evaluated among in themselves, it was determined that the optimum irrigation issue was 25% limited- irrigation under DIS Condition, and it was 50% limited- irrigation under SDIS Condition. For both systems, it was figured out that the full- irrigation issue was over- irrigation issue for cotton. The situation was similar to some previous studies. For example, in Tuylu (2018b), *Zea mays* L. *indentata* was cultivated by DIS and it was histologically and cytologically examined. It was stated that the ecological condition created by applying 25% limited- irrigation could be anatomically suggested for the cultivation of maize in terms of saving water in irrigation according to the microscopy observations and the biometric measurements. In Tuylu and Tuylu (2019), *Lycopersicon esculentum* Mill. cv. Ceren was cultivated under perlite by using DIS. The full- irrigation issue was the issue allowed 20% drainage of irrigation water in that study. But 25% limited-irrigation issue was determined as optimum irrigation condition for the plant. The amount of 20% drainage caused the effect of over- irrigation on tomato cultivated.

Periderm as cover tissue thinned under DIS Condition as water stress ($p < 0.01$) and drought stress ($p < 0.01$; $p < 0.05$) responds. Under SDIS Condition, periderm thickened because of water and drought stress conditions ($p < 0.01$). In previous studies, the thickness of epidermis tissue and cuticle as cover tissue belonging to stem and leaf under water and drought stress were measured and anatomically expressed. For example, in Tuylu (2018a) it was stated that the mean thickness of epidermis in stem of cv. Ceren was more than the one in cv. Panda under hydroponic system. At the same time in that study, it was found out that upper epidermis was thicker in leaves of cv. Ceren than the one in leaves of cv. Panda. The results clearly showed the different adaptation of the two varieties to water stress in that study. In Tuylu (2018b), it was expressed that in the stems belonging to *Zea mays* L. *indentata* which were histologically and cytologically examined, the thickness of epidermis under over-irrigation was not clearly affected, but under limited- irrigation it increased highly significantly. It was also said that the thickness of cuticle and the thickness of upper epidermis belonging to cover tissue in the leaf were significantly affected under over-irrigation condition. Under limited- irrigation condition, the thickness of lower epidermis also changed significantly together with cuticle and upper epidermis. In Tuylu and Tuylu (2019), it was expressed that cuticle belonging to stem thickened under over- irrigation and limited- irrigation conditions when it was compared to the one under optimum irrigation condition. In the leaves examined in that study, it was stated that the thickness of cuticle decreased, upper and lower epidermis thickened under over- irrigation condition, and the thickness of upper epidermis under limited-irrigation condition thickened.

The diameter of xylem vessel in cotton root cultivated under full- irrigation issue by using SDIS was larger ($p < 0.05$) than the ones which were cultivated under full-irrigation issue by using DIS. 100% irrigation showed more over- irrigation effect by using SDIS than DIS. In Tuylu et al. (2018), cv. Malatya Kurucaova belonging to tomato was cultivated under two different systems conditions in the same greenhouse.

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One of these conditions was perlite and the other one was hydroponic system. In the study mentioned, the same amount of nutrient solution was applied under both conditions and two different systems were used for cultivation. All the other features such as time, climate parameters, etc. were same under two cultivation conditions. That study with the features mentioned was similar to the present study. However, it was figured out that xylem vessels belonging to the root cultivated under hydroponic system were narrower than the ones in perlite. In there, when both systems were compared, hydroponic system showed over-irrigation effect on tomato under full- irrigation condition. In Tuylu et al. (2018) and the present study, root xylem vessels of cotton and tomato underwent reverse anatomical change against water stress.

In the present study, under both System Conditions, the diameter of xylem vessels was statistically no significantly affected under over- irrigation condition ($p > 0.05$), while under 25% reduced- irrigation water condition was significantly narrowed. The situation was similar to the results figured out in Tuylu (2018b). The diameter of xylem vessels narrowed as the amount of irrigation water was reduced in that study. In another study, the anatomical results figured out for the root showed that the diameter of xylem vessels in the root in cv. Ceren belonging to tomato narrowed under over- irrigation and limited- irrigation when they were compared to the one under optimum irrigation issue. It was expressed that the diameter of xylem vessels were anatomically affected in the same way under water and drought stress conditions (Tuylu and Tuylu, 2019).

Conclusion

According to microscope researches; the fact that the secondary structure could not be observed in the roots irrigated in I_4 issue by two systems indicated that the effect was caused by the irrigation water level, not the systems. It could be expressed that 75% limited-irrigation affected the root anatomically and prevented it from under going to the seconder structure.

According to the biometric measurements performed, when both systems were evaluated among themselves; while periderm tissue thinned under DIS Condition as water stress ($p < 0.01$) and drought stress ($p < 0.01$; $p < 0.05$) responds, periderm thickened because of both stress effects under SDIS Condition ($p < 0.01$). In this case, under SDIS, cotton roots became more robust by increasing the cover tissue against stress effects. It could be said that it is important for the vegetative and generative development of the plant.

In cotton root cultivated in full- irrigation issue by using SDIS, periderm tissue was thicker and the diameter of xylem vessel was larger ($p < 0.05$) than the ones which were cultivated in full- irrigation issue by using DIS. The situation could be expressed that under SDIS Condition, water loss can be minimized by preventing evaporation from the surface. It is easier for the root of the plant to reach the water and it is exposed to over-irrigation under full- irrigation condition. In full- irrigation issue under DIS Condition, irrigation water may be lost by evaporation from the surface and plant roots can create an anatomical response in the opposite direction to reach water when the roots were compared to the ones which were irrigated by SDIS. In other words, 100% irrigation showed more over-irrigation effect by using SDIS than DIS.

When both systems were compared to each other according to the other irrigation issues studied, there was no statistically significant difference on the anatomical features of the roots. According to the biometric measurements performed, root tissues

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belonging to cotton variety studied underwent anatomical changes in the direction of increasing or decreasing their thickness or diameter. In this way, they adapted to the environment according to the different irrigation water levels used. In this case, other advantages and disadvantages of the systems should be evaluated. When different irrigation issues were evaluated together, 25% limited- irrigation issue was figured out as the optimum condition for the roots under DIS Condition. However, the optimum condition under SDIS Condition was 50% limited- irrigation issue (Tuylu and Tuylu, 2022). Tuylu and Tuylu (2022) evaluated the cotton root, stem and leaf together anatomically and expressed the optimum condition. In that study; it had been reported that the plants cultivated in 100% (v/v), 75% (v/v), 50% (v/v), and 25% (v/v) issues created by applying different irrigation water levels were anatomically compared and the plants had optimum development in 50% limited- irrigation issue. Consequently, it was said that the condition of Kc = 0.50 was anatomically suitable for the development of the plant. In the study mentioned; root, stem, and leaf also were anatomically evaluated among themselves and the results of each one confirmed that 50% limited irrigation condition was the optimum irrigation condition to develop. In this case, the fact that not only cotton root anatomy but also the stem and the leaf anatomy cultivated under DIS Condition should be examined is suggested. In conclusion, if the stems and leaves belonging to the cotton plant are anatomically examined, the optimum irrigation issue which will be figured out can support the optimum irrigation issue under DIS in the present study. Even if such a result is obtained it can be suggested that the SDIS (Kc = 0.50) should be used in terms of plant anatomy for cotton irrigation in the Harran Plain in order to use irrigation water and water resources effectively and economically under today's conditions where water is limited and valuable.

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