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ARTICLE

Anatomical studies on drought-stressed wheat plants (*Triticum aestivum* L.) treated with some bacterial strains

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ABSTRACT Pot experiments were carried out during successive winter season of 2010/2011 in the greenhouse conditions at the Department of Agricultural Botany, Faculty of Agriculture, Kafrelsheikh University. The aim of this study was designed to investigate the effect of two treatments of bacterial endophytes strains *Azotobacter chrocoocum* (E1) and *Pseudomonas fluorescens*. (E2) individually whether as grains soaking and foliar application on anatomical features of two wheat plants (*Triticum aestivum* L.) cultivars (Sakha 93 and Gmiza 9) grown under three levels of irrigation water deficit stress (drought) 75, 50 and 25% field capacity (FC). Wheat stem and flag leaf anatomical parameters were take into account were vascular bundle dimensions, xylem vessel diameters as well as epidermis tissue thickness. Moreover, mesophyll tissue thickness values in flag leaf were calculated. The obtained results that, irrigation water deficit levels (75, 50 and 25% FC) treatments decreased revealed the values of the anatomical parameters such as thickness of epidermis, ground, mesophyll and phloem tissues, diameter of xylem vessel, and dimensions of vascular bundles of the tested wheat cultivars. While, treating wheat plants with *Azotobacter chrocoocum* (E1) and *Pseudomonas* sp. (E2) significantly increased the values of the tested anatomical characters. This study suggests that the effects of the tested bacterial endophytes treatments act as protective factors against irrigation water deficit and can improve water bio-productivity.
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KEY WORDS

wheat cultivars
anatomical parameters
endophytes
Azotobacter
Pseudomonas
water deficit

Wheat (*Triticum aestivum* L.) belongs to the Poaceae family and is one of the most important cereal crops and stands the top among the cereal crops in Egypt. Wheat is the world's most widely adapted crop, supplying one-third of the world population with more than half of their calories and nearly half of their protein (Rajaram 2001). Wheat grains are a staple human food and straw can be used as a fodder for live-stock. Its production in many regions of the world is below average because of adverse environmental conditions. A recent increase in Egyptian wheat production is not sufficient to meet the demands of a growing population (El-Maghraby et al. 2005). In Egypt found a big gap between wheat production and consumption in Egypt, this gap is a resultant to many problems. The most important of these problems is water deficiency, where that water stress affects growth, physiological process and yield of wheat plant (Metwaly 2012). Plants are exposed to numerous stress factors during their growth, which is of a significant effect on the growth of plants. Drought stress cause changes in normal physiological functions of all plants, including economically important cereals as well. This stress reduces biosynthetic capacity of plants and might cause some destructive damages on plants (Lichtenhaler 1996). Under severe water stress conditions caused by drought, plants stop growing completely and accumulate solutes in cells in order to maintain the cell volume and turgor against dehydration. This phenomenon is known as osmotic adjustment. Osmotic adjustment has been observed in stems, leaves, roots (Nonami 1998; Patakas et al. 2002).

Plant growth-promoting bacteria are known as the plant growth promoting rhizobacteria (PGPR) because they colonize the plant roots and promote growth to the plants. There are the two levels of complexity in relationship between plant growth promoting rhizobacteria and host plant. These levels are rhizopheric and endophytic (Hayat et al. 2010). Among different strategies to cope with drought issues seed priming (pre-sowing seed treatment) is an easy, low cost and low risk technique and this approach has recently been used to overcome the drought problem in agriculture land (Iqbal and Ashraf 2006). Although priming induced-drought tolerance has been reported in some crops, knowledge about physiological, biochemical and anatomical basis of priming induced-beneficial effects under stressful environment is still in frequent.

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Table 1. Epidermis, xylem vessels diameter, phloem thickness, vascular bundle thickness, vascular bundle width and ground tissue thickness of wheat cultivars Gmiza 9 and Sakha 93 stems as affected by different levels of irrigation water deficit and different two bacterial endophytes and their interactions.

Treatments	Sakha 93						Gmiza 9					
	Epidermis t. thick µm	X. vess. diameter µm	Phloem thickness µm	Inner V. B. dimension		Ground tissue µm	Epidermis t. thick. µm	X. vess. diameter µm	Phloem thickness µm	Inner V. B. dimension		Ground tissue µm
				V. B. thic. µm	V. B. width µm					V. B. thic. µm	V. B. width µm	
Control	5.83^{CDE}	14.17^{IJ}	16.25^{F}	61.67^{CD}	72.50^{E}	180.00^{H}	7.50^{A}	13.58^{EFG}	14.58^{CD}	49.17^{FG}	44.17^{H}	220.83^{FGH}
E 1 Gs	9.17^{A}	30.00^{A}	20.83^{BC}	66.67^{B}	103.33^{A}	306.67^{B}	7.50^{A}	18.33^{A}	19.58^{A}	60.83^{C}	59.17^{CD}	223.33^{FGH}
E 1 Sp	7.50^{ABC}	25.83^{BC}	16.67^{F}	62.50^{C}	75.00^{E}	266.67^{D}	7.50^{A}	17.08^{A}	19.17^{A}	65.83^{A}	68.33^{A}	224.17^{FGH}
E 2 Gs	7.50^{ABC}	26.67^{B}	20.83^{BC}	75.83^{A}	91.67^{B}	326.67^{A}	7.50^{A}	16.67^{ABC}	18.33^{AB}	58.33^{CD}	60.83^{BC}	220.00^{GH}
E 2 Sp	8.33^{AB}	25.00^{BCD}	24.17^{A}	69.17^{B}	86.67^{C}	266.67^{D}	7.50^{A}	16.67^{ABC}	19.17^{A}	65.00^{AB}	60.83^{BC}	220.83^{FGH}
W 1	5.00^{DE}	15.83^{HI}	12.08^{G}	48.33^{H}	54.17^{FG}	170.00^{HI}	5.83^{B}	11.25^{HI}	12.08^{EF}	54.83^{G}	43.33^{H}	196.67^{J}
W 1 + E 1 Gs	7.50^{ABC}	21.67^{FG}	16.67^{F}	58.33^{CF}	73.33^{E}	246.67^{E}	7.50^{A}	14.58^{CDE}	15.83^{CD}	55.83^{DE}	59.17^{CD}	243.33^{CD}
W 1 + E 1 Sp	8.33^{AB}	22.50^{EFG}	17.50^{EF}	55.83^{FG}	79.17^{D}	236.67^{EF}	7.50^{A}	15.83^{BCD}	18.33^{AB}	50.83^{F}	59.17^{CD}	260.00^{B}
W 1 + E2 Gs	9.17^{A}	20.83^{G}	20.83^{BC}	58.33^{EF}	86.67^{C}	293.33^{C}	5.42^{B}	16.67^{ABC}	15.42^{CD}	50.83^{F}	58.33^{CDE}	240.00^{CDE}
W 1 + E 2 Sp	7.50^{ABC}	16.67^{H}	18.33^{DEF}	57.50^{EFG}	56.67^{F}	290.00^{C}	6.67^{AB}	14.58^{CDE}	16.67^{BC}	63.33^{AB}	55.83^{EF}	260.00^{B}
W 2	6.67^{BC}	13.33^{J}	13.75^{G}	43.33^{I}	47.50^{H}	166.67^{I}	5.83^{B}	11.25^{HI}	10.83^{F}	39.17^{H}	41.67^{HI}	180.00^{K}
W 2 + E1 Gs	7.50^{ABC}	22.50^{EFG}	20.00^{BCD}	55.00^{G}	72.50^{E}	243.33^{E}	7.50^{A}	14.17^{DEF}	14.17^{DE}	57.50^{CD}	58.33^{CDE}	246.67^{C}
W 2 + E1 Sp	7.50^{ABC}	21.67^{FG}	21.67^{B}	60.83^{CDE}	83.33^{C}	243.33^{E}	6.67^{AB}	12.50^{E-H}	15.00^{CD}	61.67^{BC}	55.83^{EF}	230.00^{EFG}
W 2 + E 2 Gs	7.50^{ABC}	22.50^{EFG}	18.33^{DEF}	59.17^{DE}	86.67^{C}	236.67^{EF}	6.67^{AB}	14.17^{DEF}	16.25^{BCD}	51.67^{EF}	50.83^{G}	213.33^{HI}
W 2 + E 2 Sp	7.50^{ABC}	24.17^{CDE}	19.17^{CDE}	58.33^{EF}	74.17^{E}	240.00^{EF}	7.50^{A}	12.50^{E-H}	14.17^{DE}	55.83^{DE}	62.50^{B}	343.33^{A}
W 3	4.58^{E}	12.08^{J}	12.08^{G}	42.50^{I}	43.33^{I}	153.33^{J}	4.17^{C}	10.00^{I}	10.83^{F}	35.83^{H}	39.58^{I}	163.33^{L}
W 3 + E 1 Gs	7.50^{ABC}	24.17^{CDE}	19.17^{CDE}	60.83^{CDE}	54.17^{FG}	233.33^{EF}	7.50^{A}	13.33^{E-H}	16.67^{BC}	58.33^{CD}	51.67^{G}	226.67^{FG}
W 3 + E 1 Sp	6.25^{CD}	23.33^{DEF}	17.50^{EF}	60.83^{CDE}	55.00^{F}	226.67^{FG}	7.50^{A}	12.08^{F-I}	18.33^{AB}	60.83^{BC}	50.83^{G}	233.33^{DEF}
W 3 + Et 2 Gs	7.08^{BC}	25.00^{BCD}	20.00^{BCD}	57.50^{EFG}	55.83^{F}	233.33^{EF}	7.50^{A}	11.67^{GHI}	18.33^{AB}	58.33^{CD}	53.33^{FG}	226.67^{FG}
W 3 + E 2 Sp	6.67^{BC}	22.08^{EFG}	18.33^{DEF}	57.50^{EFG}	50.83^{G}	220.00^{G}	7.50^{A}	13.33^{E-H}	16.67^{BC}	55.83^{DE}	57.50^{DE}	206.67^{IJ}

E1 = *Azotobacter chrocoocum* E2 = *P. fluorescens* Gs = Grains soaking Sp = foliar application. Control = 100 % field capacity, W1 = 75% field capacity, W2 = 50 % field capacity, W3 = 25 % field capacity. Values within the same vertical areas with the same letter are not significantly different at 5% probability level by Duncan's Multiple Rage Test.

The aim of this study was designed to investigate effect of two bacterial strains *Azotobacter chrocoocum* (E1) and *Pseudomonas fluorescens* (E2) on anatomical features of two wheat cultivars Sakha 93 and Gmiza 9 grown under three levels of irrigation water deficit stress (0, 25, 50 and 75% field capacity). The wheat cultivars were treated with the two bacterial strains (10^8 cfu/ml) as grains soaking and foliar application.

Material and Methods

Source of wheat cultivars and microorganisms

Wheat grains (*Triticum aestivum* L.) cultivars Sakha 93 and Gmiza 9 were obtained from Wheat Research Dept., Agricultural research station, Sakha, Kafr El-sheikh, Egypt. Two bacterial strains {*Azotobacter chrocoocum* (E1) and *Pseudomonas fluorescens* (E2)} were obtained from Dr. Elsayed Belal, Associate professor of Agricultural microbiology, Dep. of Agric. Botany, Fac. of Agriculture, Kafrelsheikh University and these bacterial strains were isolated in previous study as endophytic bacteria from wheat plants (unpublished data). The two bacterial strains were applied on wheat as follow:

Grains treatments

Two bacterial strains were applied at the time of sowing as grains treatment. Grains were witted with 10% sugar syrup, and thoroughly mixed with an amount of bacterial suspension (10^8 cfu / ml) for 30 min. enough to obtain 10^8 cfu / per gram of grains and then dried. Grains were then sown in each pot (12 Grains / pot) during 2010/2011 season. On the other hand, grains of wheat were immersed in the manner in 10% sugar syrup and thoroughly mixed with an amount of nutrient broth medium (without bacterial growth).

Wheat plant spraying

Wheat plants (20 days from sowing) were sprayed weekly intervals with bacterial suspension (10^8 cfu / ml) from each bacterial strain to the beginning of flowering stage.

Pots, soil preparation and grains wheat planting

Each pot 30 (cm) was filled with 8 Kg of air dried clay soil. The chemical analysis was determined by conventional methods, twelve grains / pot were sown at equal distances and depth. After two weeks from sowing, the seedlings were

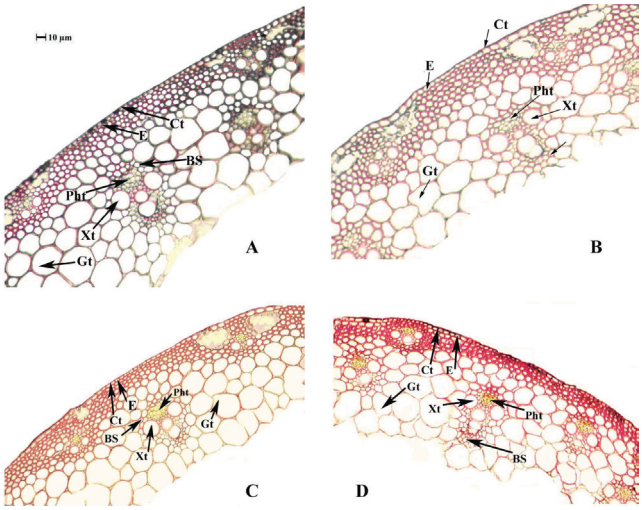

Figure 1. Transverse sections through the wheat cultivar Sakha 93 stem (stained with safranin and light green) as affected by the application of different levels of irrigation water deficit. A- Control, B- The mild irrigation water deficit level (75 % FC), C- the second irrigation water deficit level (50 % FC), D- The severe irrigation water deficit level (25 % FC). Ct: Cuticle, BS: Bundle sheath, Xt-:Xylem tissue, Pht: Phloem tissue, Gt: Ground tissue, E: Epidermis.

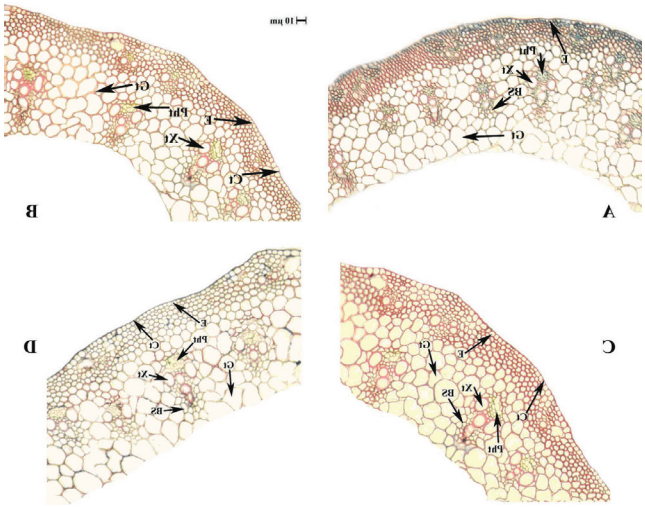

Figure 2. Transverse sections through the wheat cultivar Gmiza 9 stem (stained with safranin and light green) as affected by the application of different levels of irrigation water deficit. A- Control, B- The mild irrigation water deficit level (75 % FC), C- The second irrigation water deficit level (50 % FC), D- The sever irrigation water deficit level (25 % FC). Ct: Cuticle, BS: Bundle sheath, Xt-:Xylem tissue, Pht: Phloem tissue, Gt: Ground tissue, E: Epidermis.

thinned to leave 10 seedlings/pot. The soil used in this experiment was fertilized with nitrogen at a rate of 360 kg/h of urea fertilizer (containing 46% nitrogen). Super phosphate fertilizer (phosphorus 15%) was added at a rate of 240 kg/ha before planting. Potassium was not added because the Egyptian soil is rich in this element. These experiments were conducted at average daily temperatures ranging from 20 to 30°C.

Anatomical structure

Specimens were taken from center of the second internode of stem from apex as well as from the flag leaf from the plant tip including the midrib after 75 days from sowing. In laboratory, the different samples were cleaned with tap water, cut into suitable specimens (5mm in length). Specimens were fixed in a formalin, ethyl alcohol, and acetic acid mixture (1 : 18 : 1 v/v). Then specimens were washed and dehydrated in an alcohol series. The dehydrated specimens were infiltrated and embedded in paraffin wax (52–54°C m.p.). The embedded specimens were sectioned using a rotary microtome (Leica RM 2125) to a thickness of 8–10µm. Sections were mounted on slides and deparaffinized. Staining was accomplished with safranine and light green to enhance and improve the visibility of the specimens, cleared in xylol and mounted in canada balsam (Ruzin 1999). Ten readings from 3 slides from different specimens of the same plant were examined with electric microscope (Lieca DM LS) with digital camera (Leica DC 300) and then photographed. The anatomical manifestation was calculated using Lieca IM1000 image manager software. Lieca software was calibrated using 1 cm stage micrometer scaled at 100µm increment (Leitz Wetzler, Germany 604364) at 10x magnifications and the following data were recorded:-

Stem anatomical parameters (µm): Epidermis tissue thickness (ET) and ground tissue (GTT), length of inner vascular bundles (VBT), width of inner vascular bundles (VBW), phloem tissue thickness (PTT) and diameter of Xylem Vessels (XVD).

Leaf anatomical (µm): Thickness of mesophyll tissue (MT), upper and lower epidermis cells (UET and LET), upper and lower cuticle (UCT and LCT) and diameter of xylem vessels (XVD) and thickness of phloem tissue (PTT), of flag leaf midrib vascular bundle).

Experimental design and statistical analysis

The pots were arranged in a randomized complete block design with five replicates in every treatment and ten plants in each pot. Data of the physiological studies were tested by analysis of variance. Duncan's multiple range tests were used for comparisons among treatment mean (Duncan, 1955).

Results and Discussion

Stem anatomy

Data presented in Table (1) and Figures (1 and 2) showed that, the treatments of irrigation water deficit levels (IWD) (75, 50 and 25 % FC) in the present study decreased the most

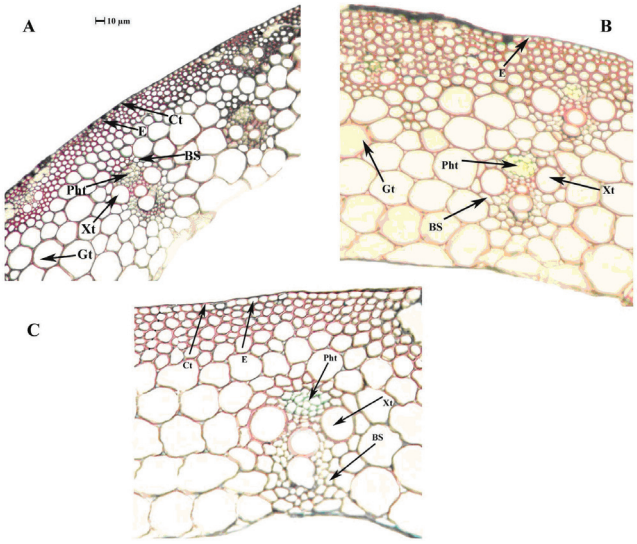

Figure 3. Transverse sections through the wheat cultivar Sakha 93 stem (stained with safranin and light green) as affected by the grains soaking application of two bacterial endophytes *A.chroccoocum* (E1) and *P. fluorescens* (E2). A- Control, B- *A chrocoocum* (E1), C- *P. fluorescens* (E2). Ct: Cuticle, BS: Bundle sheath, Xt-:Xylem tissue, Pht: Phloem tissue, Gt: Ground tissue, E: Epidermis.

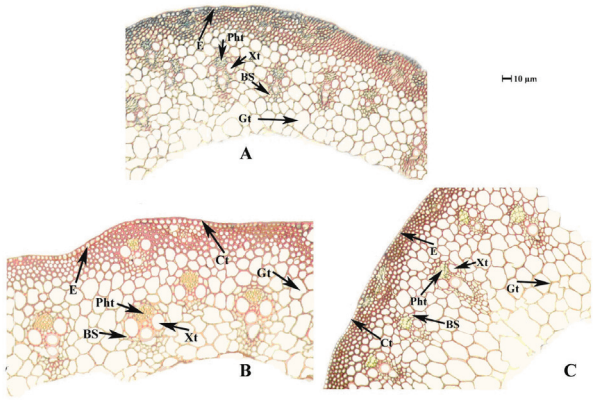

Figure 4. Transverse sections through the wheat cultivar Gmiza 9 stem (stained with safranin and light green) as affected by the grains soaking application of two bacterial endophytes *A. chrocoocum* (E1) and *P. fluorescens* (E2). A- Control, B- *A. chrocoocum* (E1), C- *P. fluorescens* (E2). Ct: Cuticle, BS: Bundle sheath, Xt-:Xylem tissue, Pht: Phloem tissue, Gt: Ground tissue, E: Epidermis.

of wheat cultivars stem anatomical features compared with control (100% FC). The significant reduction was recorded in the thickness (µm) of epidermis (ET), phloem tissue (PTT), inner vascular bundles (VBT) dimensions, and xylem vessels diameter (XVD). On the other hand, it was clear also from obtained results was found for Sakha 93 cultivar insignificantly increased in epidermis thickness (ET) by irrigation water deficit level (50% FC) and xylem vessels diameter (XVD) by irrigation water deficit level (75% FC). These obtained results were compared with control treatment (100% FC).

The means of reduction percentage for Sakha 93 cultivar stem anatomical characters was about 7.08% in ET (µm), 2.98% in XVD (µm), 22.24% in PTT (µm), 27.48% in VBT (µm), 33.33% in VBW (µm) and 9.26% in ground tissue thickness (GTT) (µm) under the three tested irrigation water. On the other hand, for Gmiza 9 cultivar the reduction was about 29.64% in ET (µm), 20.22% in XVD (µm), 22.86% in PTT (µm), 11.99% in VBT (µm), 5.98% in VBW (µm) and 18.49 in GTT (µm) under drought stress levels. These results are in agree with many investigators (Weryszko-Chmielewska and Kozak 2002 and Ou-Qiao et al. 2005).

Grains soaking and foliar application with two different bacterial endophytes strains *A. chrocoocum* (E1) and *P. fluorescens* (E2) individually have a positively impact on wheat cultivars stems anatomical features in the present study. The endophytic bacteria treatments increased the most of all stem anatomical characters. On the other hand, application of both endophytic bacteria didn't effect for Gmiza 9 cultivar on ET (µm). Also, application of *P. fluorescens*. (E2) didn't effect on GTT (µm). Application of *P. fluorescens* (E2) insignificantly increased GTT (µm). The obtained results were compared with control (100% FC) treatment without endophytic bacteria (Table, 1 and Figures 3 and 4).

The means increasing percentage for Sakha 93 cultivar was about 39.36% in ET (µm), 89.66% in XVD (µm), 26.92% in PTT (µm), 11.15% in VBT (µm), 22.99% in VBW (µm) and 62.04% in GTT (µm). On the other hand, for Gmiza 9 cultivar the means percentage were about 26.56 in % in XVD (µm), 30.74% in PTT (µm), 27.10% in VBT (µm), 41.02% in VBW (µm) and 1.33% in GTT (µm) by application *A. chrocoocum* (E1). Application of *Pseudomonas* sp. (E2) didn't effect on ground tissue thickness (µm).

The combination between two endophytic bacteria and all IWD levels (75, 50 and 25% FC) increased the wheat cultivars stems anatomical characters. These obtained results were compared with each of all IWD as alone without endophytic bacteria. On the other hand, for Sakha 93 cultivar foliar spraying with *P. fluorescens* (E2) insignificantly increased both of XVD (µm), VBT (µm) and VBW (µm). for Gmiza 9 cultivar spraying application of *P. fluorescens* (E2) in combination with irrigation water deficit level (75% FC) insignificantly increased ET (µm) and didn't effect on GTT (µm).

Foliar application with *A. chrocoocum* (E1) and grains soaking application with *P. fluorescens* (E2) in combination with IWD level (50% FC) insignificantly increased ET (µm), also, spraying application with *A. chrocoocum* (E1) and

Table 2. Xylem vessels (X.vess.) diameter, phloem thickness, vascular bundle (VB) thickness, vascular bundle width, mesophyll thickness and upper epidermis thickness of wheat cultivars Gmiza 9 and Sakha 93 flag leaf as affected by different levels of irrigation water deficit and different two bacterial endophytes and their interactions.

Treatments	Sakha 93					Gmiza 9				
	X. vess. diameter µm	Phloem thickness µm	V. B. dimension		Mesophyll thickness µm	X. vess. diameter µm	Phloem thickness µm	V. B. dimension		Mesophyll thickness µm
			V. B. thickness µm	V. B. width µm				V. B. thickness µm	V. B. width µm	
Control	17.67^{CD}	13.67^{FG}	74.17^{HIJ}	73.17^{GH}	100.17^{C}	24.17^{BC}	23.33^{BC}	76.67^{B-E}	73.33^{DE}	87.00^{DE}
E 1 GS	21.67^{A}	22.50^{A}	82.50^{E}	90.83^{D}	109.83^{B}	27.00^{A}	24.67^{AB}	79.33^{A}	77.67^{B}	95.33^{AB}
E 1 Sp	21.33^{A}	16.67^{CD}	71.67^{IJK}	95.83^{BC}	92.33^{D}	25.33^{AB}	24.33^{AB}	75.50^{DEF}	75.67^{BCD}	93.67^{AB}
E 2 GS	22.00^{A}	21.50^{A}	74.50^{HI}	93.33^{CD}	113.50^{AB}	26.33^{A}	26.33^{A}	79.67^{A}	78.00^{B}	95.67^{A}
E 2 Sp	20.00^{AB}	19.17^{B}	75.67^{GH}	105.83^{A}	115.33^{A}	24.33^{BC}	24.33^{AB}	75.67^{C-F}	74.33^{CDE}	93.33^{B}
W 1	16.33^{D}	11.33^{HI}	69.17^{K}	71.33^{H}	93.00^{D}	20.00^{F-I}	17.33^{EF}	71.33^{G}	70.00^{F}	84.67^{EF}
W 1 + E 1 GS	20.33^{AB}	16.67^{CD}	92.50^{CD}	104.17^{A}	109.33^{B}	22.33^{CDE}	19.33^{DE}	80.00^{A}	81.00^{A}	89.00^{CD}
W 1 + E 1 Sp	20.00^{AB}	16.67^{CD}	92.50^{CD}	105.17^{A}	92.50^{D}	22.00^{DEF}	19.67^{D}	75.67^{C-F}	76.33^{BC}	89.00^{CD}
W 1 + E2 GS	21.67^{A}	17.33^{C}	106.67^{A}	99.00^{B}	111.50^{AB}	23.00^{CD}	22.00^{C}	79.00^{AB}	80.67^{A}	89.67^{C}
W 1 + E 2 Sp	20.33^{AB}	16.50^{CD}	79.17^{EF}	82.50^{E}	109.17^{B}	21.67^{D-G}	19.33^{DE}	79.33^{A}	75.67^{BCD}	86.67^{DE}
W 2	14.33^{E}	10.00^{IJ}	74.17^{HIJ}	69.00^{H}	69.50^{I}	17.67^{JK}	16.00^{FG}	71.00^{G}	67.33^{G}	81.67^{GH}
W 2 + E1 GS	17.67^{CD}	13.83^{FG}	90.00^{D}	89.00^{D}	97.33^{C}	19.67^{G-J}	19.00^{DE}	78.00^{ABC}	75.67^{BCD}	86.67^{DE}
W 2 + E1 Sp	17.67^{CD}	14.67^{EF}	89.17^{D}	82.67^{E}	83.33^{FG}	20.67^{E-H}	18.67^{DE}	77.67^{A-D}	74.00^{CDE}	84.67^{EF}
W 2 + E 2 GS	18.67^{BC}	15.5^{DE}	78.33^{FG}	81.67^{E}	88.33^{DE}	20.00^{F-I}	17.67^{DEF}	78.50^{AB}	77.67^{B}	86.33^{E}
W 2 + E 2 Sp	18.00^{CD}	13.67^{FG}	95.83^{BC}	80.50^{EF}	88.67^{DE}	20.67^{E-H}	18.33^{DE}	74.67^{EF}	73.67^{CDE}	83.00^{FG}
W 3	14.33^{E}	9.33^{J}	68.33^{K}	70.83^{H}	63.00^{J}	16.33^{K}	14.67^{G}	70.33^{G}	65.00^{G}	72.00^{I}
W 3 + E 1 GS	16.67^{CD}	13.00^{FG}	70.83^{JK}	84.17^{E}	85.83^{EF}	18.67^{HIJ}	18.67^{DE}	74.83^{EF}	73.67^{CDE}	84.00^{F}
W 3 + E 1 Sp	17.67^{CD}	13.00^{FG}	96.67^{B}	81.00^{EF}	80.67^{G}	18.33^{IJ}	18.00^{DEF}	73.67^{F}	71.67^{EF}	80.67^{H}
W 3 + E 2 GS	18.67^{BC}	14.00^{EFG}	68.33^{K}	76.67^{FG}	81.67^{FG}	19.67^{G-J}	19.67^{D}	75.33^{DEF}	75.33^{BCD}	83.67^{FG}
W 3 + E 2 Sp	18.33^{BCD}	12.33^{GH}	82.50^{E}	84.33^{E}	75.67^{H}	20.00^{F-I}	17.67^{DEF}	73.67^{F}	70.33^{F}	80.00^{H}

E1 = *A. chrocoocum* E2 = *P. fluorescens* Gs = Grains soaking Sp = foliar application Control = 100 % field capacity. W1 = 75% field capacity, W2 = 50 % field capacity, W3 = 25 % field capacity. Values within the same vertical areas with the same letter are not significantly different at 5% probability level by Duncan's Multiple Rage Test.

Pseudomonas sp. (E2) individually in combination with IWD level (50% FC) increased insignificantly XVD. Foliar application with *A. chrocoocum* (E1) and grains soaking application with *P. fluorescens* (E2) in combination with IWD level (25% FC) insignificantly increased XVD (µm) compared with the same IWD without endophytic bacteria treatments.

Leaf anatomy

The obtained results in Tables (2 and 3) and Figures (5 and 6) showed that, generally the IWD levels (75, 50 and 25% FC) have a negatively impact on most leaf anatomical characters in the present study. The reduction values were obtained in thickness (µm) of wheat cultivars upper epidermis (UET), lower epidermis (LET), phloem tissue (PTT), mesophyll tissue (MT), vascular bundle (VBT), vascular bundle width (VBW) and xylem vessels diameter (XVD), for Gmiza 9 cultivar. The upper and lower cuticle also decreased under irrigation water deficit levels. The water stress decreased most of leaf anatomical characters (Ghanem 2008 and Hameed et al. 2002). Qureshi (2003) and Adhikary (2007) reported that, the drought tolerant and sensitive genotypes revealed differentiating parameters in leaf anatomy.

On the other hand, IWD levels treatments increased Sakha 93 cultivar upper and lower cuticle of leaf. Sensitive entry showed lower values of the characters under stress conditions (Hameed et al. 2002). Thick cuticle is the characteristic feature of xeric conditions and this may be an adaptations of xeric grasses (Ubeda 1993), as well as Ramon and Chang also reported (1982) that thick cuticle is the most reliable traits for drought resistant of four clones of tea.

Grains and foliar application with two different bacterial endophytes strains *A. chrocoocum* (E1) and *P. fluorescens* (E2) individually have a positively impact on the most of previous leaf anatomical characters and significantly increased them. The obtained results were compared with control (100% FC) without endophytes treatments. On the other hand, grains soaking application with *P. fluorescens* (E2) insignificantly increased Gmiza 9 cultivar phloem tissue thickness. Foliar application with *P. fluorescens* (E2) for Gmiza 9 cultivar insignificantly increased thickness of VBT, VBW, UE (Tables 2 and 3 and Figures 7 and 8). The increasing percentage of Gmiza 9 cultivar leaf anatomical characteristics by application of bacterial endophytes were 6.53% in XVD, 6.79% in PTT, 1.14% in VBT, 4.21% in VBW, 8.62% in MT and

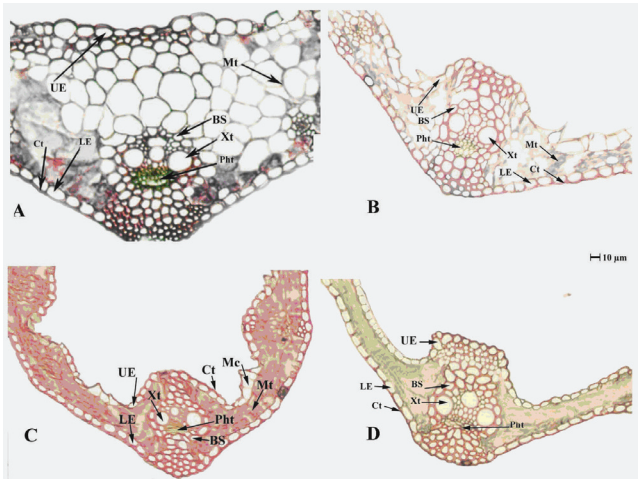

Figure 5. Transverse sections through the wheat cultivar Sakha 93 flag leaf (stained with safranin and light green) as affected by the application of different levels of irrigation water deficit stress. A- Control, B- The mild irrigation water deficit level (75 % FC), C- The second irrigation water deficit level (50 % FC), D- The sever irrigation water deficit level (25 % FC). UE- Upper epidermis, LE- Lower epidermis, CT- Cuticle, BS- Bundle sheath, Mt- Mesophyll tissue, Xt- Xylem tissue Pht- Phloem tissue, Mc- Motor cell.

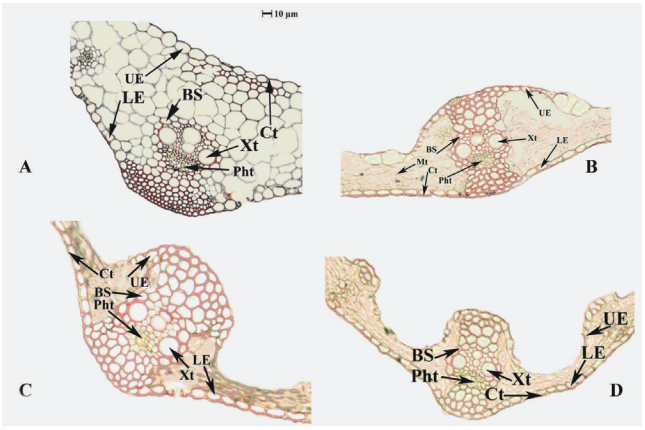

Figure 6. Transverse sections through the wheat cultivar Gmiza 9 flag leaf (stained with safranin and light green) as affected by the application of different levels of irrigation water deficit stress. A- Control, B- The mild irrigation water deficit level (75 % FC), C- The second irrigation water deficit level (50 % FC), D- The sever irrigation water deficit level (25 % FC). UE: Upper epidermis, LE: Lower epidermis, CT: Cuticle, BS: Bundle sheath, Mt; Mesophyll tissue, Xt: Xylem tissue, Pht: Phloem tissue.

8.54% in UE under normal irrigation water (100% FC). On the other hand, for Sakha 93 cultivar the increasing percentage was 20.26% XVD, 50.06% in PTT, 2.58% in VBT, 31.82% VBW, 7.56% in MT and 16.52% in UE compared with control (100% FC) without endophytic bacteria treatments.

Grains soaking and foliar application with *A. chrocoocum* (E1) and *P. fluorescens* (E2) in combination with all IWD

Table 3. Flag leaf anatomical characters of wheat cultivars Gmiza 9 and Sakha 93 as affected by different levels of water stress and different Endophytes bacteria and their interactions.

Treatments	Sakha 93				Gmiza 9			
	Upper epidermis µm	Lower epidermis µm	Upper cuticle µm	Lower cuticle µm	Upper epidermis µm	Lower epidermis µm	Upper cuticle µm	Lower cuticle µm
Control	10.50^{D-G}	11.50^{GH}	6.07^{D}	5.17^{L}	11.67^{BCD}	10.83^{E}	6.33^{A-E}	6.93^{BC}
E 1 GS	13.00^{A}	13.67^{A}	8.50^{A}	7.33^{A}	13.00^{A}	13.50^{AB}	6.47^{ABC}	6.13^{AB}
E 1 Sp	12.17^{ABC}	12.83^{BC}	8.50^{A}	6.83^{A-D}	12.17^{ABC}	13.33^{AB}	6.37^{A-D}	6.50^{A}
E 2 GS	12.10^{ABC}	12.67^{BCD}	8.20^{AB}	6.67^{B-E}	13.17^{A}	14.17^{A}	6.67^{AB}	6.00^{ABC}
E 2 Sp	11.67^{BCD}	13.00^{AB}	8.10^{AB}	5.33^{KL}	12.33^{ABC}	13.00^{BCD}	6.83^{A}	5.97^{ABC}
W 1	9.50^{GH}	11.17^{H}	6.11^{D}	6.27^{E-H}	10.33^{E}	12.17^{D}	5.63^{FG}	5.20^{D-G}
W 1 + E 1 GS	12.00^{ABC}	12.83^{BC}	8.17^{AB}	7.10^{AB}	12.33^{ABC}	13.50^{AB}	6.07^{C-F}	6.20^{AB}
W 1 + E 1 Sp	11.33^{B-E}	12.50^{B-E}	7.83^{ABC}	7.00^{ABC}	12.00^{A-D}	13.17^{BC}	6.20^{B-F}	5.50^{B-G}
W 1 + E2 GS	11.83^{ABC}	13.00^{AB}	7.83^{ABC}	6.83^{A-D}	12.67^{AB}	14.17^{A}	6.33^{A-E}	5.67^{B-G}
W 1 + E 2 Sp	11.50^{B-E}	12.17^{C-G}	7.97^{ABC}	6.50^{C-F}	12.17^{ABC}	13.30^{AB}	6.73^{AB}	5.63^{B-G}
W 2	7.67^{I}	9.83^{I}	6.10^{D}	5.90^{HI}	8.83^{F}	10.83^{E}	5.37^{GH}	5.10^{FG}
W 2 + E1 GS	12.13^{ABC}	11.83^{E-H}	8.43^{A}	6.53^{C-F}	12.67^{AB}	13.33^{AB}	5.97^{C-F}	5.83^{A-E}
W 2 + E1 Sp	10.33^{E-H}	12.00^{D-G}	7.83^{ABC}	6.40^{D-G}	11.17^{CDE}	12.33^{CD}	6.03^{C-F}	5.33^{C-G}
W 2 + E 2 GS	10.83^{C-F}	12.33^{B-F}	8.33^{AB}	6.83^{A-D}	12.17^{ABC}	13.50^{AB}	6.17^{B-F}	5.70^{B-F}
W 2 + E 2 Sp	12.33^{AB}	11.83^{EIH}	7.67^{BC}	7.00^{ABC}	11.50^{BCD}	12.67^{BCD}	6.17^{B-F}	5.57^{B-G}
W 3	6.83^{I}	9.17^{J}	6.06^{D}	5.40^{KL}	7.67^{G}	9.67^{F}	4.97^{H}	4.97^{G}
W 3 + E 1 GS	11.67^{BCD}	11.83^{E-H}	7.67^{BC}	6.17^{FGH}	12.00^{A-D}	12.67^{CD}	5.73^{EFG}	5.87^{A-D}
W 3 + E 1 Sp	10.83^{C-F}	11.67^{FGH}	7.33^{C}	6.00^{GHI}	11.67^{BCD}	12.17^{D}	5.67^{EFG}	5.50^{B-G}
W 3 + E 2 GS	9.17^{H}	12.33^{B-F}	8.17^{AB}	5.83^{HIJ}	11.67^{BCD}	13.17^{BC}	5.87^{C-G}	5.33^{C-G}
W 3 + E 2 Sp	9.83^{FGH}	11.83^{E-H}	7.83^{ABC}	5.67^{IJK}	10.83^{DE}	11.17^{E}	5.77^{D-G}	5.13^{FG}

E1 = *A. chrocoocum* E2 = *P. fluorescens* Gs = Grains soaking Sp = foliar application Control = 100 % field capacity. W1 = 75% field capacity W2 = 50 % field capacity W3 = 25 % field capacity. Values within the same vertical areas with the same letter are not significantly different at 5% probability level by Duncan's Multiple Rage Test.

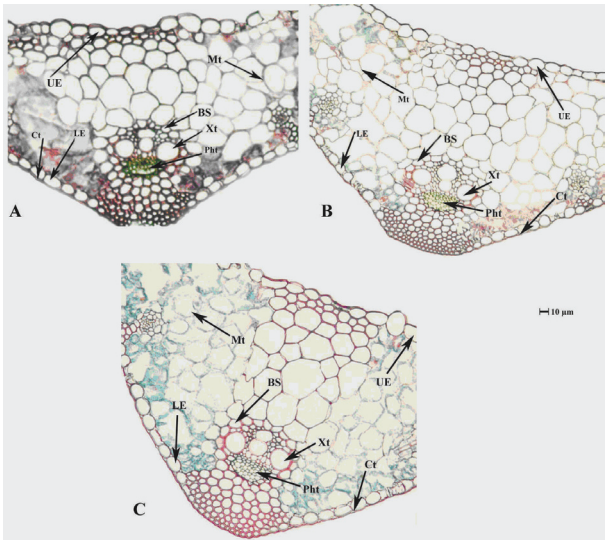

Figure 7. Transverse sections through the wheat cultivar Sakha 93 flag leaf (stained with safranin and light green) as affected by the grains soaking application of two bacterial endophytes *A. chrocoocum* (E1) and *P. fluorescens* (E2). Control, *A. chrocoocum* (E1). *P. fluorescens* (E2). UE: Upper epidermis, LE: Lower epidermis, CT: Cuticle, BS: Bundle sheath, Mt; Mesophyll tissue, Xt: Xylem tissue, Pht: Phloem tissue.

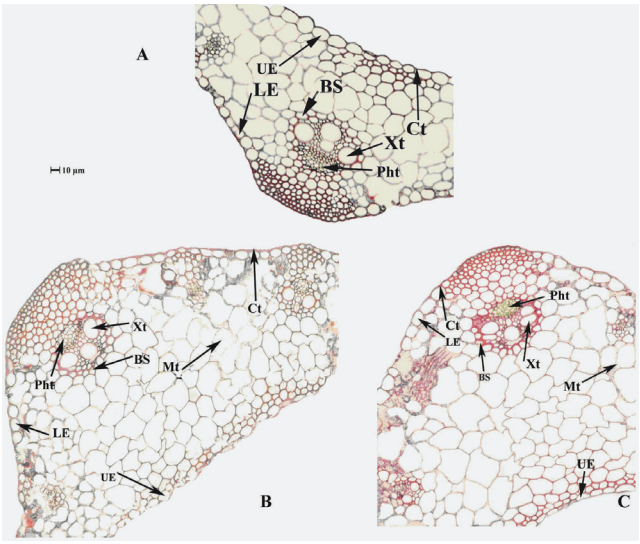

Figure 8. Transverse sections through the wheat cultivar Gmiza 9 flag leaf (stained with safranin and light green) as affected by the grains soaking application of two bacterial endophytes *A. chrocoocum* (E1) and *P. fluorescens* (E2). Control, *A. chrocoocum* (E1). *P. fluorescens* (E2). UE: Upper epidermis, LE: Lower epidermis, CT: Cuticle, BS: Bundle sheath, Mt; Mesophyll tissue, Xt: Xylem tissue, Pht: Phloem tissue.

level (75% FC) significantly increased most leaf anatomical features compared with untreated plants under the same irrigation water deficit. On the other hand, application of *A. chrocoocum* (E1) as grains soaking and *P. fluorescens* (E2) as foliar spraying insignificantly increased Gmiza 9 cultivar phloem thickness. The increasing percentage of Gmiza 9 cultivar leaf anatomical characteristics by application of bacterial endophytes were 11.25% in XVD, 15.88% in PTT, 10.05% in VBT, 12.03% in VBW, 4.62% in MT and 18.99% in UE under the same IWD. On the other hand, the increasing percentage was 26.04% XVD, 49.32% in PTT, 34.03% in VBT, 36.98% VBW, 13.58% in MT and 22.79% in UE for Sakha 93 cultivar compared with untreated plants with endophytic bacteria treatments.

Application of *A. chrocoocum* (E1) and *P. fluorescens* (E2) in combination with all IWD level (50% FC) significantly increased most leaf anatomical characters compared with untreated plants under the same IWD. On the other hand, application of *A. chrocoocum* (E1) as grains soaking and *P. fluorescens* (E2) as foliar application insignificantly increased Gmiza 9 cultivar phloem thickness. The increasing percentage of Gmiza 9 cultivar leaf anatomical characteristics by application of bacterial endophytes was 11.25% in XVD, 15.88% in PTT, 10.05% in VBT, 12.03% in VBW, 4.62% in MT and 18.99% in UE under the same IWD. On the other hand, for Sakha 93 cultivar the increasing percentage was 26.04% XVD, 49.32% in PTT, 34.03% in VBT, 36.98% VBW, 13.58% in MT and 22.79% in UE. The obtained results were compared with untreated plants with endophytic bacteria treatments.

Concerning to application of *A. chrocoocum* (E1) and *P. fluorescens* (E2) in combination with all IWD level (25% FC) significantly increased most leaf anatomical characters compared with untreated plants under the same IWD (Tables 2 and 3 and Figures 9 and 10). The increasing percentage of Gmiza 9 cultivar leaf anatomical characteristics by application of bacterial endophytes were 17.38% in XVD, 26.12% in PTT, 5.75% in VBT, 11.92% in VBW, 14.01% in MT and 50.49% in UE under the same IWD. On the other hand, for Sakha 93 cultivar the increasing percentage was 24.46% XVD, 31.68% in PTT, 16.47% in VBT, 15.12% VBW, 28.51% in MT and 51.90% in UE. The obtained results were compared with untreated plants with endophytic bacteria treatments.

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). Tissues exposed to environments with low water availability have generally shown reduction in cell size, and increase in vascular tissue and cell wall thickness (Guerfel et al. 2009).

The adverse effect of the tested irrigation water deficit on stem and leaf anatomical parameters of wheat cultivars may be attributed to the decrease in net photosynthetic rates (photoinhibition) in plants due to stomatal closure, which decreases or prevents water loss but reduces CO_2 availability for chloroplast (Lawlor and Cornic; 2002 Flexas et al. 2004; Bertamini et al. 2007). Erice et al. (2007) indicated that

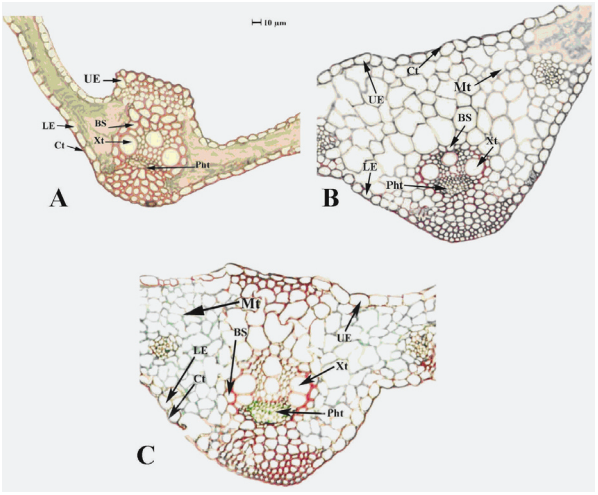

Figure 9. Transverse sections through the wheat cultivar Sakha 93 flag leaf (stained with safranin and light green) as affected by grains soaking application with *A chrocoocum* (E1) and *P. fluorescens* (E2) under the highest level of irrigation water deficit stress. A- The sever irrigation water deficit level (25 % FC). B- *A. chrocoocum* (E1). C- *P. fluorescens* (E2). UE: Upper epidermis, LE: Lower epidermis, CT: Cuticle, BS: Bundle sheath, Mt; Mesophyll tissue, Xt: Xylem tissue, Pht: Phloem tissue.

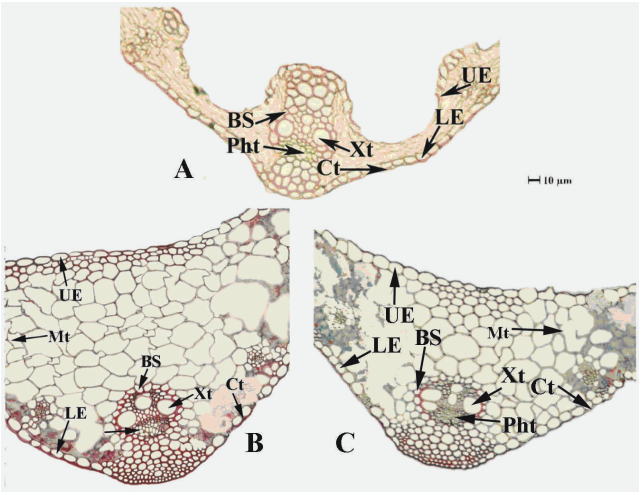

Figure 10. Transverse sections through the wheat cultivar Gmiza 9 flag leaf (stained with safranin and light green) as affected by grains soaking application with *A. chrocoocum* (E1) and *P. fluorescens* (E2) under the highest level of irrigation water deficit stress. A- The sever irrigation water deficit level (25 % FC). B- *A. chrocoocum* (E1). C- *P. fluorescens* (E2). UE: Upper epidermis, LE: Lower epidermis, CT: Cuticle, BS: Bundle sheath, Mt; Mesophyll tissue, Xt: Xylem tissue, Pht: Phloem tissue.

total dry matter of alfalfa plants significantly reduced to the well-watered ones, but the reduction was less under elevated CO_2. Moreover, this effect may be attributed to the results of massive and irreversible expansion of small daughter cells produced by meristematic divisions and growth inhibition is therefore related to the inhibition of cell expansions as well as reduced rates of new cell production may make additional contribution to the inhibition of growth (Hsiao 1973). Water stress causes losses in tissue water content, which reduce turgor pressure in the cell, thereby inhibiting enlargement and division of cells causing a reduction in plant growth (Shao, 2007). Moreover, under the water stress conditions, IAA and GA_3 decrease while ABA increases in different plants (De Souza et al. 2006; Shao 2007; Maria, et al. 2008; Kutlu et al. 2009). Cell division and cell enlargement were inhibited leading to a reduction of growth. Moreover, reduction in anatomical structure of wheat stem grown under the tested irrigation water deficit related to essential mineral uptake (El-Afry, et al. 2012). Generally, drought reduces both nutrient uptake by the roots and transport from the roots to the shoots, because of restricted transpiration rates and impaired active transport and membrane permeability (Viets 1972; Alam 1999). The decline in soil moisture also results in a decrease in the diffusion rate of nutrients in the soil to the absorbing root surface (Alam 1999).

Highly developed vascular system in the flag leaf was noted by Skoromnyi (1980) in drought tolerant wheat genotypes which were higher values for yield components especially grain weight per plant, 1000grain weight and number of grains /spike. Venora and Calcagno (1991) noted the higher number of vascular bundles with notable thick cuticle and prominent vascular bundles in high yielding drought resistant durum wheat variety as compared to high yielding variety in normal condition, which was not well developed vascular bundles and with imperceptible cuticle. A better understanding of the morpho-anatomical and physio-biochemical characteristics of changes in drought resistance could be used to select or create new varieties of crops to obtain a better productivity under water stress conditions (Nam et al. 2001; Martinez et al. 2007)

Plant growth-promoting bacteria include both free living and symbiotic bacteria, typically found in the soil, that facilitate the growth and development of plants (Glick et al., 1999). This can occur directly to promote plant growth either by providing the plant with a compound that is synthesized by the bacterium or by facilitating the uptake of nutrients from the soil. Thus, plant growth-promoting bacteria can directly facilitate the proliferation of plants by fixing atmospheric nitrogen; producing siderophores which can mineral solubilize and provide it to plants; synthesizing phytohormones, such as auxin, cytokinin and gibberellin, which can enhance various stages of plant growth; solubilizing minerals such as phosphorus; and synthesizing enzymes that can modulate plant growth and development (Glick 2007).

Obtained results indicated that, treating wheat cultivars with *A. chrocooccum* (E1) and *P. fluorescens* (E2) significantly increased the most of all anatomical characters. Microbial inoculants that can promote plant growth and pro-

ductivity is internationally accepted as an alternative source of N-fertilizer. It is environmental friendly and can be used to ensure a sustainable wheat production. In this bio-fertilizer technology new systems are being developed to increase the biological N_2 fixation (BNF) with cereals and other non-legumes by establishing N_2-fixing bacteria within the roots (Cocking 2000). Nitrogen fixation and plant growth promotion by plant growth promoting bacteria are important criteria for an effective bio-fertilizer. Inoculation of associative and free living N_2-fixing bacteria have been shown to produce beneficial effects on plant growth, thus they are termed plant growth promoting rhizobacteria (PGPR) (Kloepper et al. 1980; Bashan and Holguin 1998). Significant increases in crop yields following application of PGPR have been documented under diverse field conditions (Bashan 1998). They have been widely reported to fix atmospheric nitrogen with grasses and cereals (Dobereiner 1997) and enhance nutrient uptake (Lin et al. 1983; Murty and Ladha 1988; Bashan and Holguin 1998). Azotobacter sp besides fixing nitrogen it is also secrete certain growth hormones such as IAA, GA_3 and Cytokinins (Coppola 1971) which promote vegetative growth and root development.

Auxin is recognized as a key factor, which is directly beneficial of plants. The role of microorganisms as plant growth stimulators is widespread in nature, especially in relation to a group of plant hormones that are implicated in the regulation of diverse biological processes including cell division, elongation, differentiation, root elongation and tropistic responses (Spaepen et al. 2007). Moreover, Cytokinin stimulates the synthesis of chlorophyll pigments (Jelić and Bogdanović 1989). In addition, increasing in wheat cultivars treated with endophytic strains was due to the enhancement of essential elements uptake especially nitrogen element (N_2). Nitrogen is necessary for chlorophyll synthesis and as part of chlorophyll molecules, is the focal point of photosynthesis. Also, N_2 is an essential component of amino acids, which building blocks of protein. Generally, the literature review indicates that there are possibly some positive effects of endophytic bacteria treatment on growth and reproduction of plants. It is interesting to indicate that, the reduction in internal growth parameters of wheat stem and leaf explain and contributed to the morphological and physiological and yield component parameters under the same irrigation water deficit levels (El-Afry et al. 2012; El-Nady et al. 2012). They investigated the effects of the same endophytic bacteria strains on physiological, growth and yield component characters of wheat plants grown under different drought stress levels.

Conclusion

Treating wheat plants with two bacterial endophytes strains *Azotobacter chrocoocum* (E1) and *Pseudomonas fluorescens* (E2) increased most of and anatomical features under normal or irrigation water deficit stress and overcome the adverse effects of different IWD stress (drought).

So, it could be recommended that, using the *Azotobacter chrocoocum* (E1) and *Pseudomonas fluorescens* (E2) especially treating with grains soaking before sowing whether under normal or IWD stress to increase the wheat yield under drought stress (IWD).

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