



Exogenously applied proline induced changes in key anatomical features and physio-biochemical attributes in water stressed oat (*Avena sativa* L.) plants

Rehmana Ghafoor¹ · Nudrat Aisha Akram¹ · Muhammad Rashid¹ · Muhammad Ashraf² · Muhammad Iqbal¹ · Zhang Lixin³

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Abstract Oat (*Avena sativa*) plants grown under 60% field capacity (water-deficit stress) were subjected to proline (40 mM) applied as a foliage spray. Water-deficit conditions suppressed plant growth, chlorophyll contents, leaf vascular bundle area, leaf phloem area and leaf midrib thickness, root diameter, root cortex thickness, stem diameter, stem vascular bundle area and stem phloem area. In contrast, water stress caused an increase in leaf proline, hydrogen peroxide, activities of peroxidase and superoxide dismutase enzymes, leaf bulliform cell area, leaf adaxial epidermis thickness, leaf sclerenchyma thickness, root metaxylem area, root epidermis and endodermis area, root stelar diameter, stem sclerenchyma thickness and stem epidermis thickness. However, exogenous application of proline significantly improved the plant growth, leaf proline contents, metaxylem area, mesophyll thickness, root diameter, root cortex thickness, root epidermis, endodermis thickness, stelar diameter, metaxylem area, stem diameter, stem vascular bundle area, stem epidermis area, stem phloem area and stem sclerenchyma thickness. Overall, foliar spray of proline was effective in improving drought stress tolerance which can be attributed to proline-induced significant modulations in physio-biochemical and anatomical features of oat plants.

Keywords Oat · Water regimes · Proline · Antioxidant enzymes · Leaf, stem and root anatomy

Introduction

Global environmental changes occurring at a fast pace are resulting in drastic stressful factors such as drought, salinity, flooding and soil acidification/alkalinity, which are a serious threat to food security (Ashikari and Feng-Ma 2015). Of these stresses, drought is the most devastating one. Water deficit conditions are known to reduce crop growth and yield by decreasing canopy absorption, efficiency of radiations, photosynthetically active radiations, chlorophyll biosynthesis, over-accumulation of reactive oxygen species, vital membrane degradation, proteins structure destabilization etc. (Earl and Davis 2003; Ashraf and Harris 2013; Kosar et al. 2015; Akram et al. 2016). Moreover, relative water content (RWC) and leaf water potential also decrease under water deficit regimes in plants, but severity of a stress depends on type of plant species (Silva et al. 2009; Arzani and Ashraf 2016). In addition, drought stress can damage the chloroplast (Mafakhari et al. 2010), as well as can reduce the turgor and plant water potentials (Rahdari and Hoseini 2012; Lum et al. 2014; Li et al. 2014).

For the survival of plants under water deficit conditions, various defense and metabolic systems are activated. For example, accumulation of osmolytes/compatible solutes, such as proline, glycine betaine, sugars, polyols, and trehalose etc. takes place, which is essential for metabolic and osmotic adjustments in plants (Shafiq et al. 2015; Akram et al. 2016). Of various compatible solutes, proline acts as a protective molecule that can unite stress generated free radicals and oxygen (Sharma et al. 2012). Proline, without

✉ Nudrat Aisha Akram
nudrataaaf@yahoo.com

¹ Department of Botany, Government College University, Faisalabad 38040, Pakistan

² University of Agriculture Faisalabad, Faisalabad, Pakistan

³ State Key Laboratory of Soil Erosion and Dryland Farming on the Loess Plateau, College of Life Sciences, Northwest A&F University, Yangling, People's Republic of China

interfering with the normal metabolic processes, allows the plants to grow or thrive under drought stress (Verbruggen and Hermans 2008).

Anatomical modifications in a plant body are capable of minimizing detrimental effects of drought stress (Hare and Cress 1997; Verbruggen and Hermans 2008; Saeed et al. 2016). Moreover, leaf is a more responsive organ than the root or stem to stress conditions (Jarret 2014). Due to continuous loss of water from leaf, the bulliform cells' area and mesophyll cells gradually become smaller (Kutluet al. 2009; Zhang et al. 2015). Furthermore, leaf vascular bundles and xylem vessels show a reduced diameter in crop plants exposed to drought stress. For example, Ennajeh et al. (2010) reported an increase in total thickness of leaf lamina, spongy parenchyma, upper palisade and trichome layer, and upper and lower epidermis in olive plant leaves. Thickness of midrib, lamina and vascular bundle area, and bulliform cell area were found to be reduced in the leaf of sewan grass (Naz et al. 2014). Water deficit conditions also cause considerable changes in roots. For example, it has been reported that root hairs increase per unit area in most plants under drought stress (Jarret 2014).

Drought tolerant and drought sensitive species differ considerably in terms of development of anatomical adaptations under drought stress. For example, drought tolerant species show increased succulence (both in root and stem), thick cuticle, enhanced deposition of wax, thick and many layered epidermis, well developed water storing tissues in the cortex and enhanced development of root endodermis (Peng et al. 2015).

Proline is most widely distributed osmoprotectant in higher plants to be involved primarily in osmotic adjustment under water stress conditions (Szoke et al. 1992; Ashraf and Foolad 2007). It is believed that proline accumulation plays an adaptive role in plants stress tolerance (Hare and Cress 1997; Ashraf and Foolad 2007). Currently, proline may cost up to US\$ 0.76/g. However, this cost may high for its application under field conditions. Therefore, more research is needed to calculate the efficiency of proline on yield attributes of crop plants particularly under field conditions. Proline accumulation during osmotic stress is mainly due to its increased synthesis or reduced degradation. It also acts as molecular chaperon that stabilizes the structure of proteins by maintaining the cell redox status. It is also involved in stress signal influencing adaptive responses in most plants (Maggio et al. 2002 (Verbruggen and Hermans 2008). Proline also works as metabolic signal that may regulate antioxidants as well as it can scavenge ROS species directly and helps the plant cell to survive under stress conditions (Hamilton and Heckathorn 2001; Kavi Kishor et al. 2005; Szabados and Savouré 2010; Liang et al. 2013; Fu et al. 2018). Ali et al. (2013) worked on maize under the foliar application of proline,

they reported that content of protein, seed sugar, oil, fiber content, moisture content, and ash were increased in both maize cultivars under normal and stress conditions. Kahlaoui et al. (2018) reported that exogenous application of proline (low concentration) can enhance the tolerance against salinity stress in both tomato cultivars.

Oat (*Avena sativa* L.) belongs to family Poaceae and is grown all over Pakistan because of being a potentially nutritive cereal. It can grow not only under moist climates, poor soil and in cool climates, but also under both rainfed and irrigated conditions throughout Pakistan. Oat has the ability to grow reasonably in acidic soil (pH up to 4.5), but 5.3–5.7 pH is best for its growth (Ovando-Martínez et al. 2013). On the basis all the afore-mentioned reports it was hypothesized that drought stress can impair a variety of physio-biochemical and anatomical features in oat and proline application can alleviate the harmful effects of drought on these attributes. Thus, the premier objective of the present study was to determine up to what extent water deficit conditions can alter some key physio-biochemical and anatomical characteristics in oat plants. Furthermore, it was also determined that how far exogenous application of proline, a vital osmo-protectant, could mitigate the injurious effects of drought on oat.

Materials and methods

The current study was set-up to examine the influence of a potential osmo-protectant, proline on some key physiological attributes interlinked with anatomical features of leaf, stem and root of oat (*Avena sativa* L.) plants under water deficit conditions. A three-factor factorial (cultivars, 2; drought levels, 2; proline, 2; and replicates, 4) pot experiment was conducted in the Botanical Garden of the GC University Faisalabad (GCUF) under natural environmental conditions. Two local cultivars of oat namely F-411 and CK-1 were chosen for this experiment. The seeds of both cultivars were obtained from the Ayub Agricultural Research Institute, Faisalabad, Pakistan and at a rate of 8 seeds/pot were sown in 32 plastic pots of uniform size. Each plastic pot contained 10 kg sandy loam soil. After sprouting of the seeds, thinning was done to maintain 5 plants of uniform size per replicate. The soil water contents were maintained on daily basis. The average temperature is varied between 10–24 °C. After 15 days of seed germination, water stress treatments (control, full field capacity; water stress, 60% field capacity) started. After 32 days of water treatments, proline (40 mM along with 0.1% Tween 20) was applied as a foliage spray. This chemical was provided by Sigma Aldrich. An aliquot of 50 mL solution of proline was applied to each plant by using a hand-sprayer pump. After 15 days of foliage application, two

plants were harvested for the determination of shoot and root fresh and dry weights. At the end of experimental period, remaining plants were used for recording the data of the following physio-biochemical and anatomical attributes.

Physio-biochemical attributes

Hydrogen peroxide (H₂O₂)

A fresh leaf (0.5 g) excised with 5 mL trichloroacetic acid (0.1%) and H₂O₂ contents were determined following Velikova et al. (2000).

Malondialdehyde (MDA)

The protocol proposed by Cakmak and Horst (1991) was used to determine the malondialdehyde (MDA) contents.

Glycinebetaine (GB)

Glycinebetaine in the leaf tissues was determined following Grieve and Grattan (1983).

Ascorbic acid (AsA)

A method proposed by Mukherjee and Choudhuri (1983) was used to determine the ascorbic acid contents.

Total phenolics

A fresh leaf (0.5 g) was homogenized in 5 mL acetone (80%) and following Julkenon-Titto (1985), total phenolics were determined by using spectrophotometer (Hitachi UV-1800, Shimadzu, Kyoto, Japan).

Free proline content

The protocol proposed by Bates et al. (1973) was used to determine the proline content.

Chlorophyll contents

The chlorophyll *a* and *b* contents were determined according to Arnon (1949).

Total soluble proteins

Total soluble proteins were determined following a method proposed by Bradford (1976).

Antioxidants enzymes

The activities of catalase (CAT) and peroxidase (POD) enzymes were determined following Chance and Maehly (1955), while the activity of superoxide dismutase (SOD) enzyme according to the protocol described by Van Rossum et al. (1997).

Anatomical features

For the anatomical investigations, formalin-acid-alcohol (FAA) solution was prepared by taking 525 mL of distilled water, 750 mL of ethyl alcohol, 150 mL of formaldehyde, and 75 mL of acetic acid. An aliquot, of 30 mL of the FAA solution was taken from the stock solution and added to each bottle. Fresh leaves, stems and roots were taken (2–3 cm) from each treatment, washed well in distilled water and preserved in a (FAA) solution. The plant samples were then to be fixed in acidic alcohol solution for 24 h at room temperature. The sections of all samples were cut free hand by following Ruzin (1999). After completing the staining procedure, the sections were shifted on slides, added one drop of canada balsam (a mounting medium) on each of the sections, covered the section with a cover slip and permanent slides were preserved for taking micrographs. Finally, measurements of the micrographs were done using a light microscope (MEIJI Techno; ML2100, Japan). Then, leaf (leaf thickness, size and nature of dermal tissues, size and no of conducting tissues, size of parenchymatous tissues and size of mechanical tissues), stem (stem area, size and nature of dermal tissues, size & no of conducting tissues, size of parenchymatous tissues and size of mechanical tissues) and root (root area, size and nature of dermal tissues, size & no of conducting tissues, size of parenchymatous tissues and size of mechanical tissues) were recorded.

Statistical analysis

The collected physio-biochemical and anatomical data were subjected to analysis of variance technique (ANOVA) using a computer software (Co-Stat version 6.2, CoHort Software, 2003, Monterey, CA, USA). All mean values within each attribute were compared using the least significant difference (LSD) at 5% probability level.

Results

Growth attributes

Drought stress (60% field capacity) markedly suppressed the root and shoot lengths of both cultivars of oat. Foliar-

applied proline (40 mM) did not affect the root length, while it significantly ($P \leq 0.001$) increased shoot length of both oat cultivars under both water regimes. The response of both oat cultivars to proline was similar under control and drought stress conditions (Fig. 1; Table 1). Water-deficit conditions also considerably ($P \leq 0.001$) suppressed the root and shoot fresh weights of both oat cultivars (F-411 and CK-1). While, foliar application of proline at the concentration of 40 mM was effective in improving root and shoot fresh weight of both cultivars under water stress conditions (Fig. 1; Table 1). Water deficit conditions noticeably ($P \leq 0.001$; Table 1) suppressed the root and shoot dry weights of both oat cultivars, while foliar spray of proline was effective in improving only root dry weight of both cultivars (Fig. 1).

Physio-biochemical attributes

A significant increase in free proline contents was observed in both oat cultivars under drought stress (Fig. 1). Foliar-applied proline was also found effective in enhancing proline contents in both oat cultivars under control and water stress conditions (Table 1).

Total phenolics, MDA, glycinebetaine, and ascorbic acid contents and the activity of catalase (CAT) enzyme remained unchanged in both cultivars under water-deficit conditions as well as under foliar applied proline. The response of both oat cultivars remained unchanged under water stress and proline application (Table 1; Figs. 1, 2). Hydrogen peroxide contents increased under stress conditions, while proline application did not affect the hydrogen peroxide contents in both cultivars under control and water stress (60% of field capacity) conditions (Fig. 1).

Chlorophyll *a* and *b* contents decreased significantly in both oat cultivars subjected to water stress conditions (Fig. 2). Exogenously-applied proline (Table 1) was found to be effective in improving only chlorophyll *b* contents in oat plants under stress conditions.

Drought stress significantly increased the activity of peroxidase and superoxide dismutase in both oat cultivars. However, foliar application of proline was not effective in improving the activities of these enzymes under stress conditions (Fig. 2). The response of both cultivars was similar in terms of POD activity.

Total soluble proteins increased under water deficit conditions in both oat cultivars (Table 1). Foliar spray of proline improved the total soluble proteins. Both cultivars were similar in total soluble proteins under control and drought stress conditions (Fig. 2).

Leaf, root and stem anatomical features

Drought stress (60% field capacity) significantly ($P \leq 0.001$) increased the leaf adaxial epidermis thickness, leaf bulliform cell area and leaf sclerenchyma thickness, while it did not affect the leaf abaxial epidermis thickness, leaf blade thickness, mesophyll thickness and metaxylem area of both oat cultivars. Under water-deficit conditions, leaf vascular bundle area, midrib thickness and phloem area decreased considerably in both oat cultivars. Foliar-applied proline at the concentration of 40 mM considerably ($P \leq 0.01$) enhanced leaf mesophyll thickness and metaxylem area, while slightly suppressed leaf midrib thickness and phloem area. Other attributes (leaf abaxial and adaxial epidermis thickness, leaf blade thickness, bulliform cell area, vascular bundle area and sclerenchyma thickness) were not affected by foliar application of proline. Of both cultivars, cv. F-411 was better in leaf adaxial epidermis thickness, metaxylem area, midrib thickness, phloem area and vascular bundle area as compared to cv. CK-1 (Table 1; Figs. 3, 4).

Root cortex thickness and root diameter were found to be suppressed under water deficit conditions, while root endodermis thickness, root epidermis thickness, metaxylem area and root stele diameter increased considerably ($P \leq 0.001$) under drought stress. Foliar application of proline (40 mM) significantly improved the root cortex thickness, root diameter, root endodermis and epidermis thickness, metaxylem area and stele diameter under water deficit conditions. Cv. F-411 was better in root epidermis thickness while cv. CK-1 was better in root cortex thickness, root metaxylem area and root stele diameter (Table 1; Figs. 5, 6).

Water stress conditions significantly ($P \leq 0.001$) decreased the stem diameter, stem phloem area and stem vascular bundle area, while it enhanced stem epidermis thickness and stem sclerenchyma thickness. Stem metaxylem area was not affected under stress conditions. Exogenously-applied proline considerably ($P \leq 0.001$) improved the stem diameter, stem epidermis thickness, stem metaxylem area, stem phloem area and stem vascular bundle area, while it did not affect the stem sclerenchyma thickness under stress and non-stress conditions. Cv. F-411 was better in stem phloem area as compared to the other cultivar, while cv. CK-1 was better in stem sclerenchyma thickness and vascular bundle area (Table 1; Figs. 7, 8).

Discussion

It is well known that plant growth and development is adversely affected by drought stress. The most sensitive and primary response in plants under water deficit

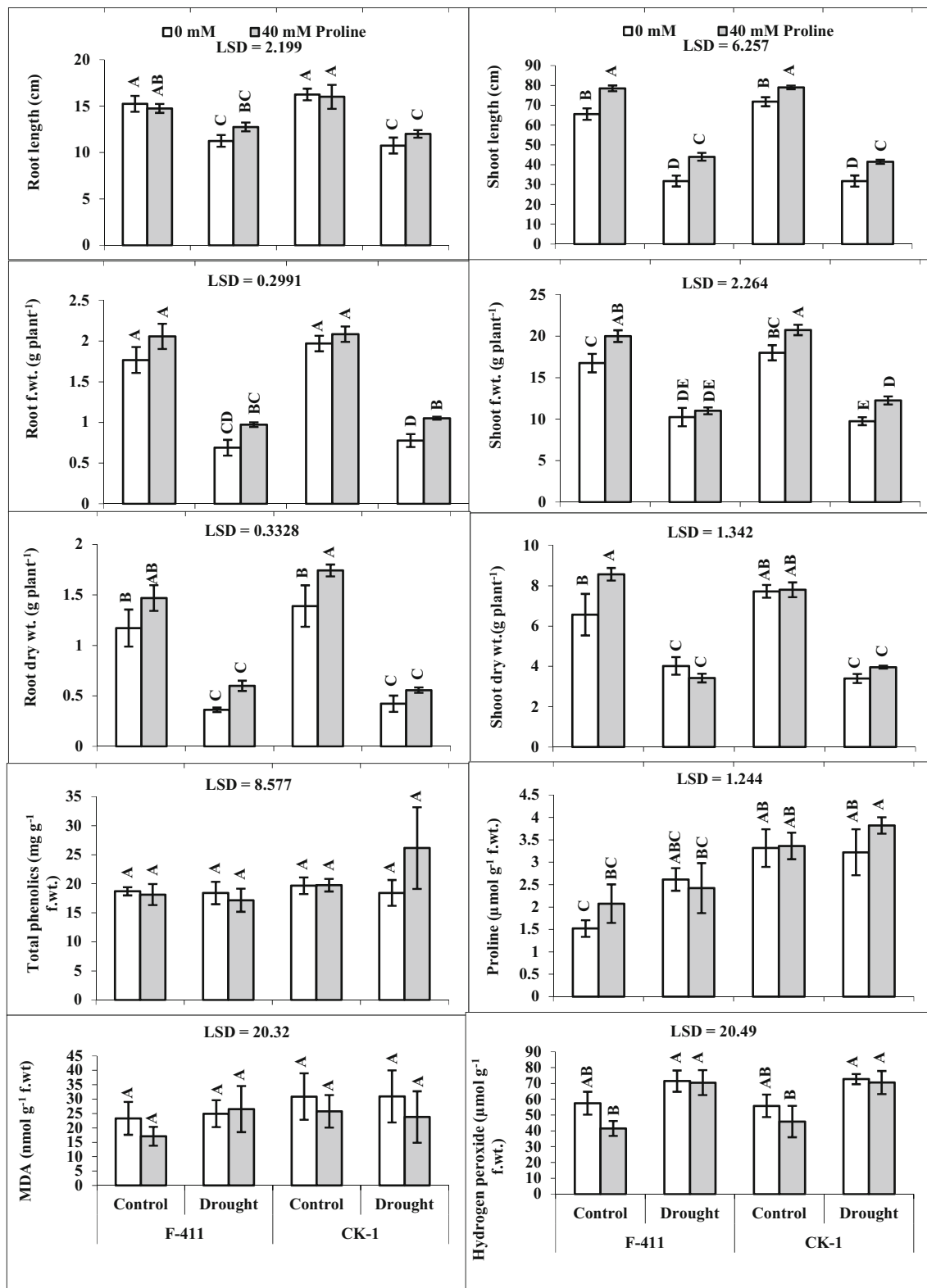


Fig. 1 Root and shoot lengths, fresh and dry weights, total phenolics, proline, malondialdehyde (MDA), and hydrogen peroxide contents of two cultivars of oat (*Avena sativa* L.) plants foliar-treated with proline

Table 1 Analyses of variance of data for different growth, physiological and anatomical characteristics of two cultivars of oat (*Avena sativa* L.) plants foliar-treated with proline grown under varying water regimes

Source of variation	df	Root length	Shoot length	Root fresh weight	Shoot fresh weight	Root dry weight	Shoot dry weight	T. phenolics	Proline
Cultivars (Cvs)	1	0.5 ns	9.031 ns	0.079 ns	3.781 ns	0.128 ns	0.045 ns	67.13 ns	5.578*
Drought (D)	1	120.1***	10621.5***	9.625***	520.0***	7.344***	125.6***	7.605 ns	0.001 ns
Proline (Pro)	1	2 ns	892.5***	0.463**	42.78***	0.522**	2.085 ns	18 ns	0.789**
Error	24	2.270	18.38	0.042	2.406	0.052	0.846	34.54	0.727
		MDA	H ₂ O ₂	Chlorophyll <i>a</i>	Chlorophyll <i>b</i>	AsA	GB	CAT	POD
Cultivars (Cvs)	1	189.2 ns	8.0 ns	0.144*	0.020 ns	0.772 ns	345.6 ns	0.132 ns	1.445 ns
Drought (D)	1	42.30 ns	3570.1***	0.114*	0.058*	3.047 ns	149.9 ns	0.026 ns	54.04**
Proline (Pro)	1	142.6 ns	420.5 ns	1.054 ns	0.061*	4.663 ns	569.5 ns	3.573 ns	0.530 ns
Error	24	193.8	197.2	0.019	0.009	1.362	136.0	0.036	6.043
		SOD	Total soluble proteins	Leaf abaxial epidermis thickness	Leaf adaxial epidermis thickness	Leaf blade thickness	Leaf bulliform cell area	Leaf mesophyll thickness	Leaf metaxylem area
Cultivars (Cvs)	1	1.483*	2.213 ns	36.125 ns	171.1***	578 ns	99235.1 ns	98 ns	652653.1***
Drought (D)	1	2.213**	235.1***	28.125 ns	128**	2664.5 ns	2309100.5***	253.1 ns	53138 ns
Proline (Pro)	1	0.076 ns	63.34*	72 ns	24.5 ns	2244.5 ns	273800 ns	6441.1**	193753.1**
Error	24	0.255	12.74	17.83	21.12	983.4	73200.1	733.9	19726.1
		Leaf midrib thickness	Leaf phloem area	Leaf vascular bundle area	Leaf sclerenchyma thickness	Root cortex thickness	Root diameter	Root endodermis thickness	Root epidermis thickness
Cultivars (Cvs)	1	177906.1***	37264.5*	334421522***	180.5 ns	3120.5*	1035.1 ns	12.5 ns	36.12*
Drought (D)	1	37264.5*	893784.5*	75393060**	11935.1***	18050***	126504.5***	406.1***	703.1***
Proline (Pro)	1	24420.5*	1889568***	22629628 ns	325.1 ns	4704.5**	103285.1***	153.1***	220.5***
Error	24	4803.6	118309.7	7118083.7	361.4	455.0	1682.1	9.187	6.5
		Root metaxylem area	Root stele diameter	Stem diameter	Stem epidermis thickness	Stem metaxylem area	Stem phloem area	Stem sclerenchyma thickness	Stem vascular bundle area
Cultivars (Cvs)	1	1386528.8***	5434.0**	12246.1 ns	1.531 ns	87780.5 ns	1029971.3**	1653.1**	33050483**
Drought (D)	1	1839841.5***	33346.5***	2388205.1***	225.7***	84872 ns	2286056.5***	5995.1***	60052580***
Proline (Pro)	1	836247.7***	7719.0**	1447551.1***	457.5***	1200475.1***	5733344.5***	32 ns	2.598***
Error	24	44895.8	600.2	39122.4	5.822	50320.4	88553.5	148.5	3790106.3

ns Non-significant

*, ** and *** significant at 0.05, 0.01 and 0.001 levels, respectively

conditions is the reduction of in tissue growth due to considerable reduction in cell turgor (Akram et al. 2011; Latif et al. 2016). Under water deficit conditions or low

water potential, osmoprotectant accumulation is in dispensable to maintain a positive turgor pressure so as to protect the cellular functions (Verslues and Juenger 2011).

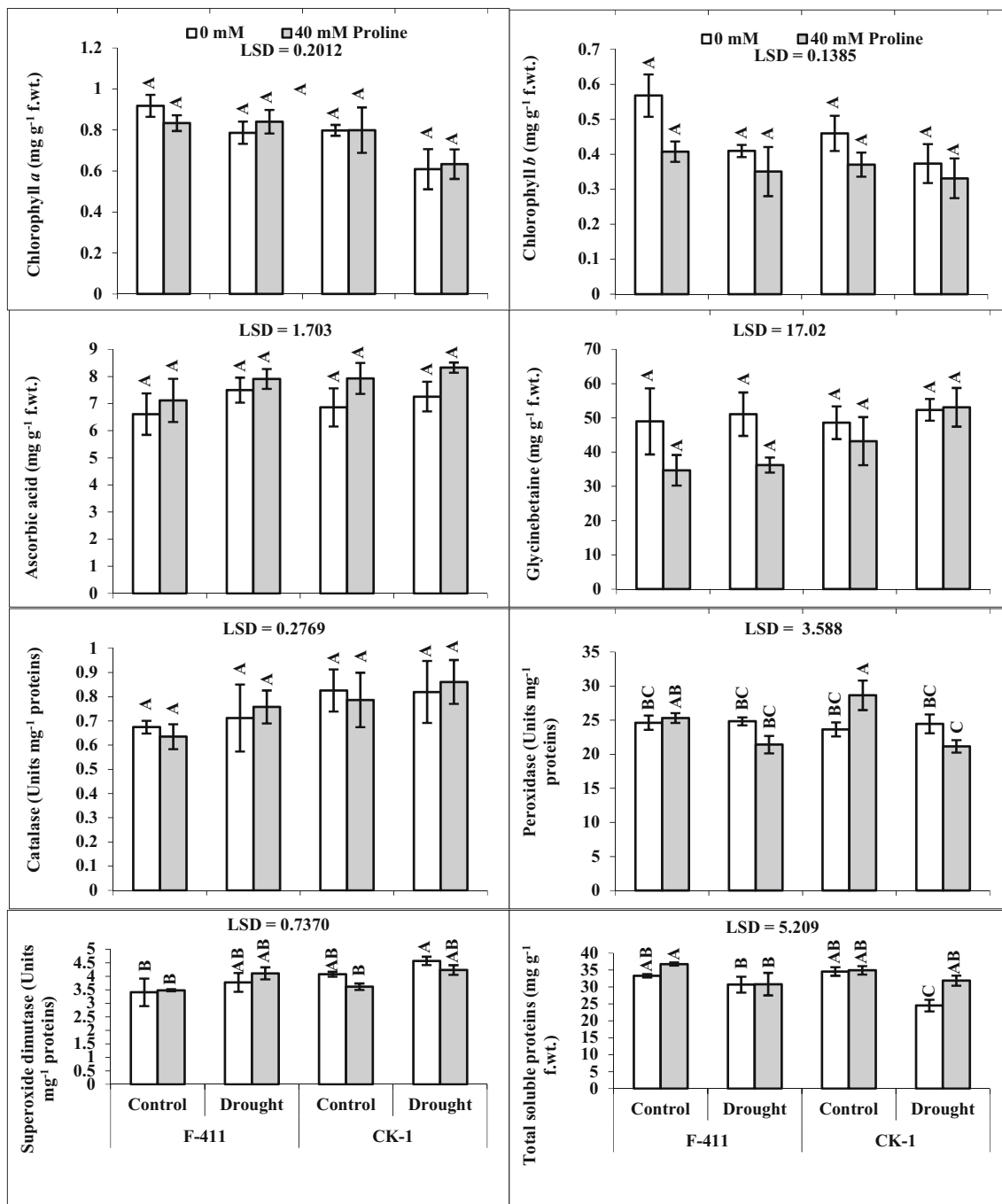


Fig. 2 Chlorophyll *a* and *b*, ascorbic acid, glycine betaine contents, activities of catalase, peroxidase, superoxide dismutase and total soluble proteins of two cultivars of oat (*Avena sativa* L.) plants

foliar-treated with proline grown under varying water regimes. Mean \pm S.E.; letters (A–B) showing least significance difference among mean values

Compatible solutes or osmoprotectants such as proline and glycinebetaine are believed to be involved in the protection of cell components during dehydration and cell osmotic adjustment (Ashraf and Foolad 2007). Free proline contents increase in plant cells under drought stress which have a clear role as an osmoticum by balancing the environmental and osmotic potential of vacuole with that of

cytosol (Ahmad and Sharma 2008; Pireivatloum et al. 2010). In the present study, foliar applied proline (40 mM) considerably improved the growth (root and shoot fresh/dry weights, lengths) of oat plants grown under water limited environment. In an earlier study on wheat, it has been observed that seed priming with 20 and 40 mM of proline improved the shoot and root fresh and dry weights as well

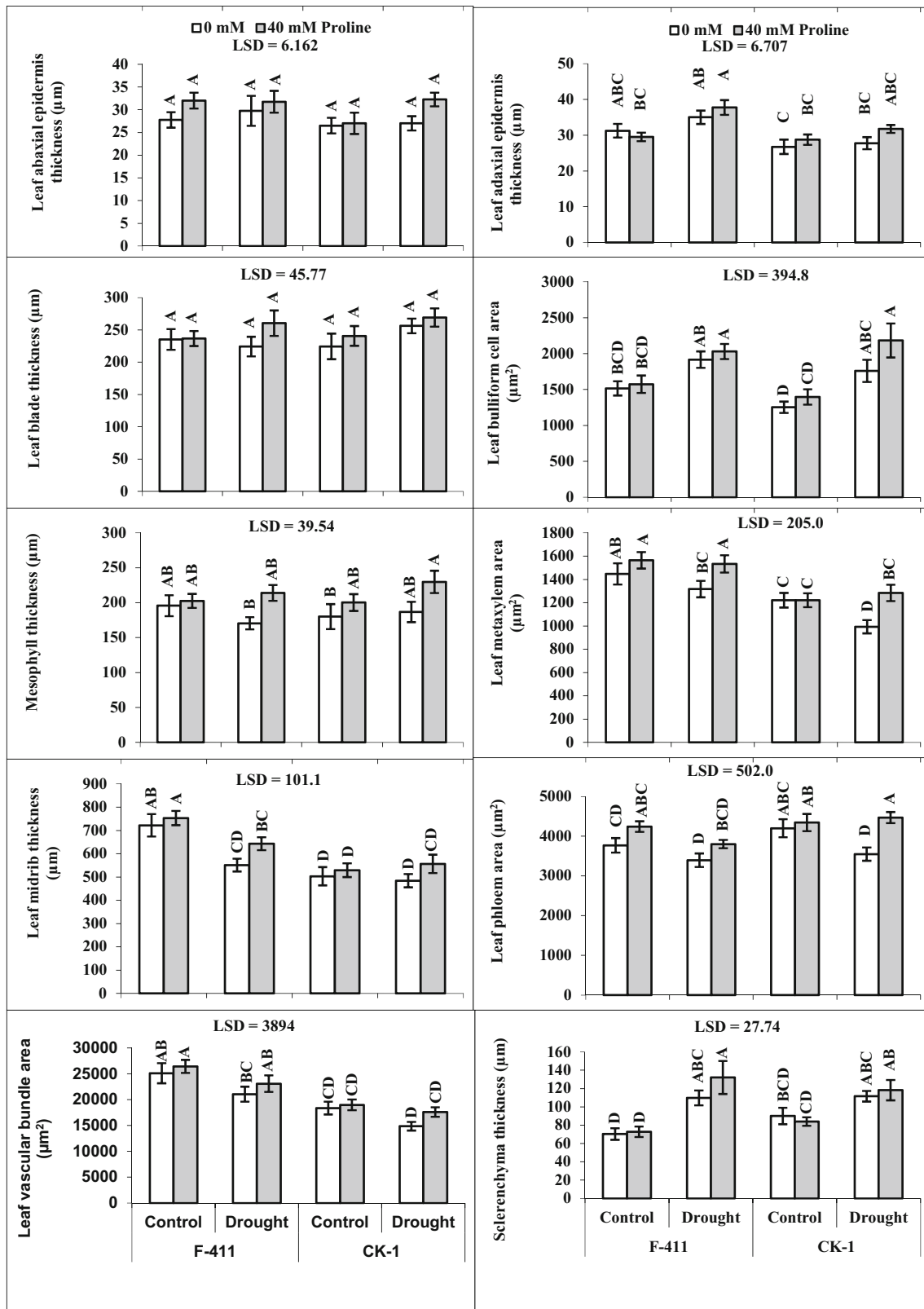


Fig. 3 Leaf abaxial epidermis, adaxial epidermis, blade thickness, bulliform cell area, midrib thickness, phloem area, vascular bundle area and sclerenchyma thickness of two cultivars of oat (*Avena sativa*

L.) plants foliar-treated with proline grown under varying water regimes. Mean \pm S.E.; letters (A–D) showing least significance difference among mean values

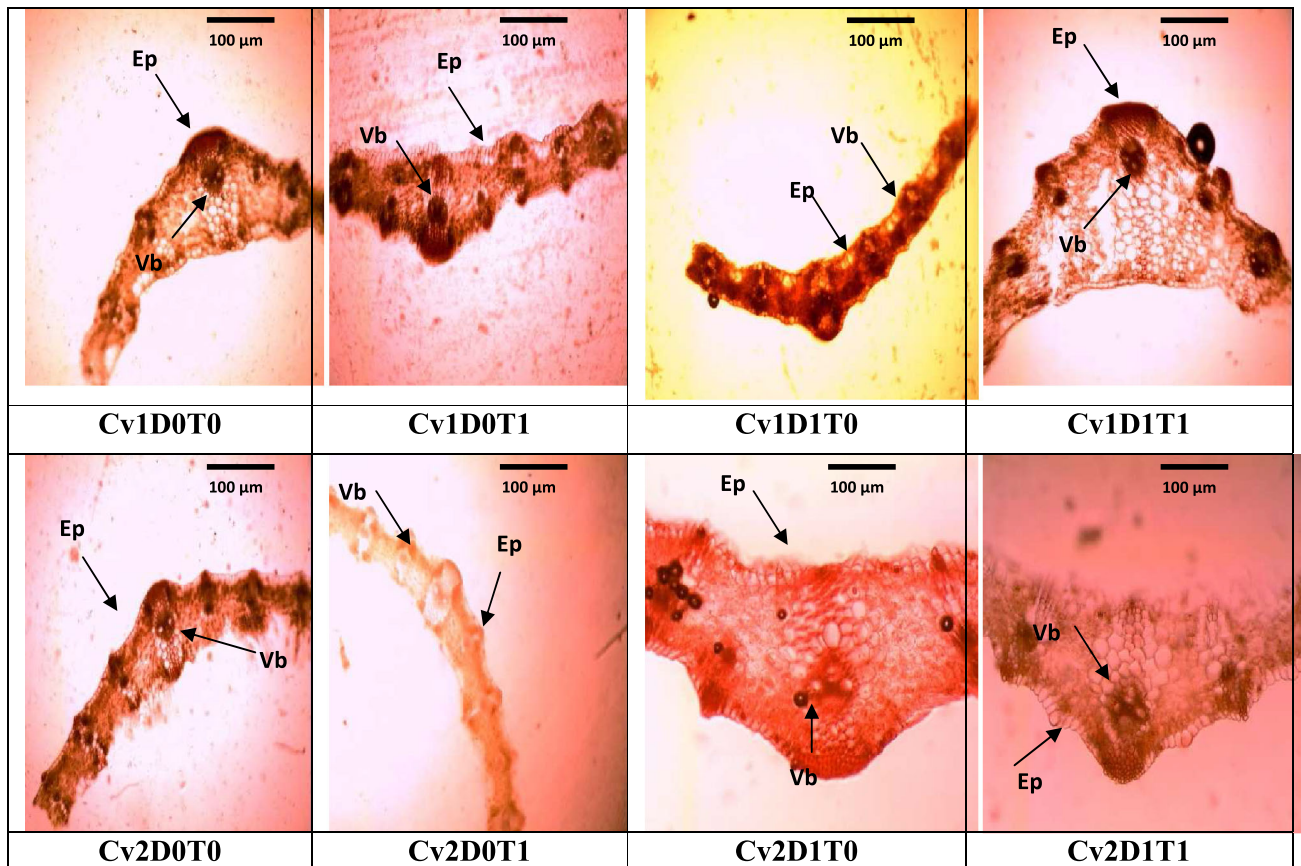


Fig. 4 Leaf abaxial epidermis, adaxial epidermis, blade thickness, bulliform cell area, midrib thickness, phloem area, vascular bundle area and sclerenchyma thickness of two cultivars of oat (*Avena sativa*

L.) plants foliar-treated with proline (40 mM) grown under varying water regimes; Cv, cultivars; D0, normal watering; D1, drought stress; T0, 0 mM proline; T1, 40 mM proline

as lengths of wheat plants and it was shown that growth improvement took place due to proline-induced changes in uptake of essential nutrients particularly N, K⁺ and Ca²⁺ (Kamran et al. 2009). It is generally believed that high synthesis of proline in plant cells/tissues provides energy for their growth as well as stress tolerance by protecting enzymes, proteins and vital membranes or organelles (Hoque et al. 2007; Kamran et al. 2009; Hayat et al. 2012). Proline as an amino acid acts as an efficient osmolyte, an antioxidative defense and signaling molecule, and a metal chelator (Hayat et al. 2012; Kahlaoui et al. 2014).

Chlorophyll pigments play a vital role in plants to harvest the light energy and generate reducing power of cells despite carrying out photochemical reactions (Taiz and Zeiger 2006, 2012). In this study, chlorophyll pigments were suppressed significantly in both oat cultivars under drought stress which is parallel to what has earlier been observed in different crops such as maize (Dolatabadian et al. 2009), wheat (Moaveni 2011), rice (Pattanagul 2011), and canola (Shafiq et al. 2014) etc. The exogenous application of proline enhanced the chlorophyll pigments in oat plants under drought stress that could be attributed to

proline-induced improvement in chlorophyll pigments which might have occurred due to proline-induced stabilization of chloroplast, its structure as well as activation of chlorophyll biosynthesis enzymes (Hayat et al. 2012; Ashraf and Harris 2013). While working with beans (*Vicia faba*), foliar-applied proline (8.7 µM) increased leaf chlorophyll and water contents, while it reduced membrane injury thereby improving overall plant growth under saline conditions (Gadallah 1999). In another study with maize plants, Ali et al. (2007) observed that external application of proline at the rate of 30 and 60 mM proline at different growth stages (seedling to reproductive) increased chlorophyll *a* and *b* contents in water stressed plants of two maize cultivars. They suggested that proline-induces improvement in chlorophyll biosynthesis and reduces stress-induced degradation of chloroplast and its components under water stressed environment (Ali et al. 2007).

Reactive oxygen species (ROS) accumulate in plants under water stress conditions, and plants possess a defense mechanism to defend themselves from the oxidative damage caused by ROS (Ashraf 2009; Akram et al. 2012). For example, under drought conditions, plants produce various

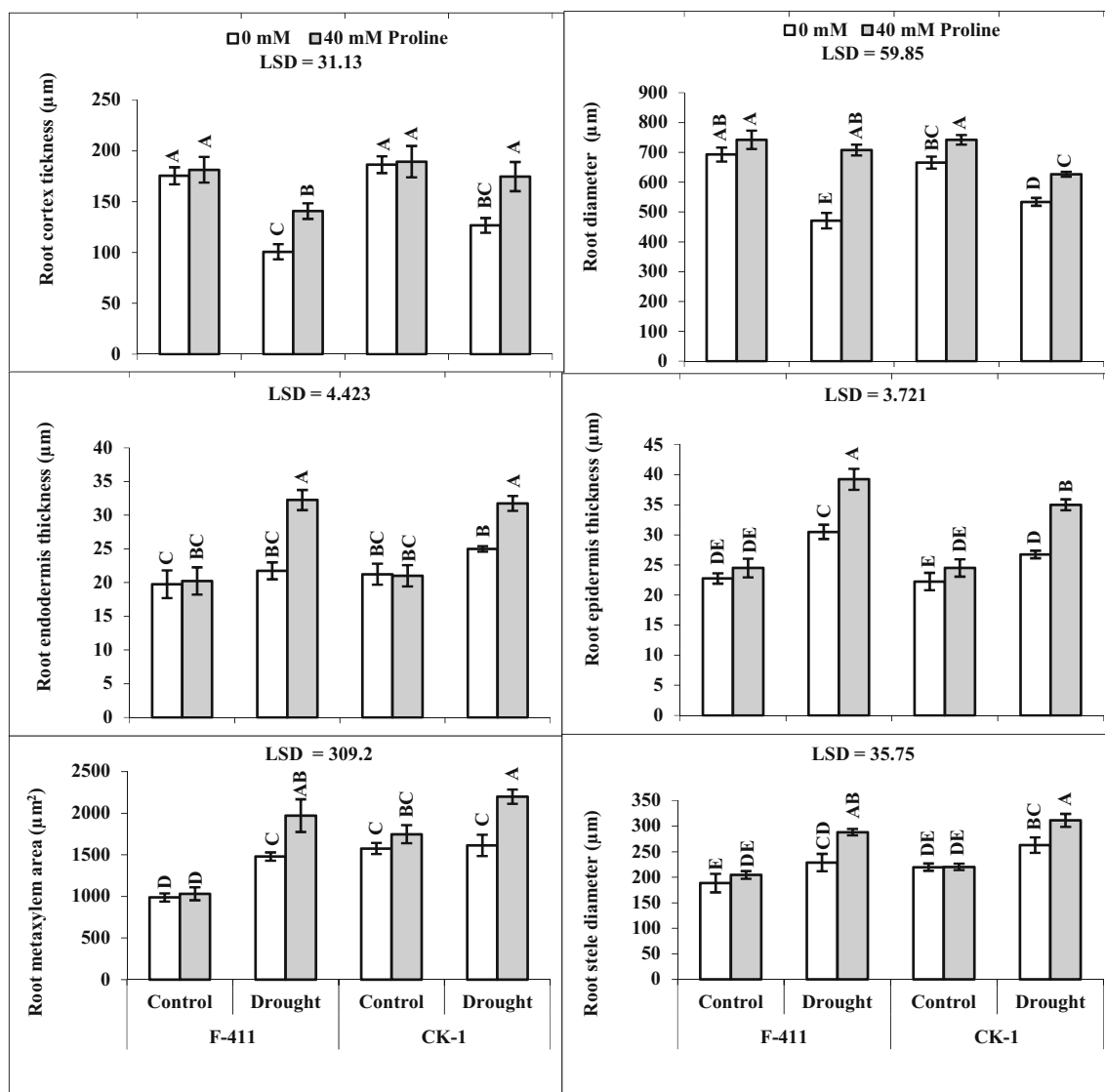


Fig. 5 Root cortex thickness, diameter, endodermis thickness, epidermis thickness, metaxylem area, and stele diameter of two cultivars of oat (*Avena sativa* L.) plants foliar-treated with proline grown under

varying water regimes. Mean \pm S.E.; letters (A–E) showing least significance difference among mean values

enzymatic and non-enzymatic antioxidants to protect themselves against the ROS-induced oxidative damage (Yamaguchi and Blumwald 2005; Noctor et al. 2014; Mukhtar et al. 2016). For example, malondialdehyde (MDA) contents usually increase in plants under stress conditions and these are considered as membrane damage indicators (Ashraf 2009; Pandey et al. 2010; Al Hassan et al. 2015). However, in the present study, exogenous application of proline remained ineffective in altering the MDA contents in both oat cultivars. ROS scavengers (catalase, peroxidase, and superoxide dismutase) are generally believed to be activated in plants under drought stress (Ashraf 2009; Khan et al. 2015; Ahmad et al. 2016, 2018a, b). Foliarly applied proline plays an important role as osmoprotectant in enhancing plant stress tolerance

(Songstad et al. 1990; Santarius 1992; Ashraf and Foolad 2007; Ahmad et al. 2008). From various studies, it was estimated that foliar-applied proline helps to promote plant growth under stress conditions (Csonka and Hanson 1991; Nounjan and Theerakulpisut 2012). Shevyakova et al. (2009) reported that exogenous application of proline suppressed the activity of SOD in common ice plant under stress. However, in the present study, it was found that the activities of SOD and POD increased significantly under deficiency of water, while exogenous application of proline was found not much effective in improving the activities of these enzymes. These findings cannot be explained in view of the general argument of Hayat et al. (2012) that exogenously applied proline can control the enhanced level of stress by elevating the activities of key enzymatic

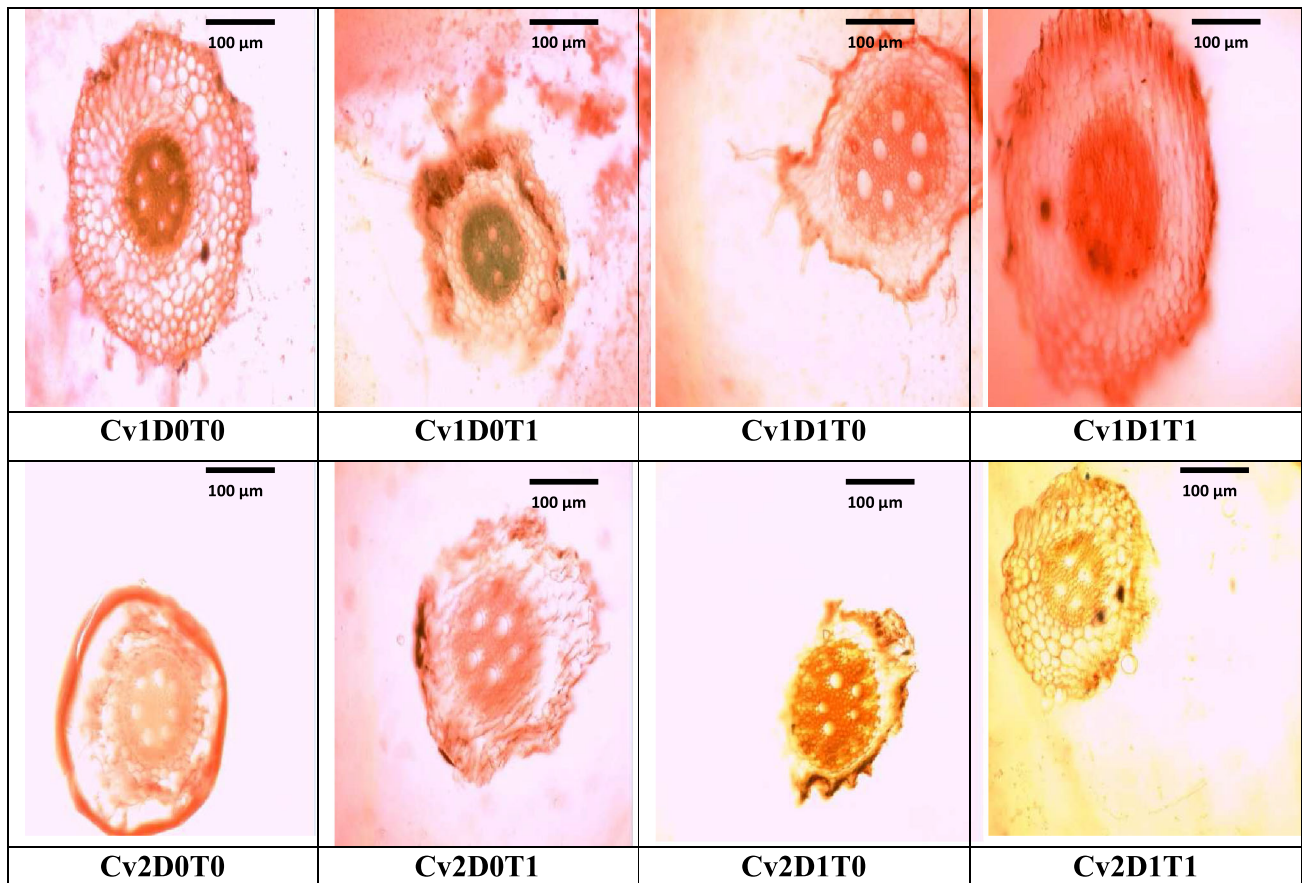


Fig. 6 Root cortex thickness, diameter, endodermis thickness, epidermis thickness, metaxylem area, and stele diameter of two cultivars of oat (*Avena sativa* L.) plants foliar-treated with proline grown under

varying water regimes; Cv, cultivars; D0, normal watering; D1, drought stress; T0, 0 mM proline; T1, 40 mM proline

antioxidants. No effect of proline on SOD and POD activity may be dose/plant species dependent, as the level (40 mM) of proline applied in the present study was not effective for these antioxidant enzymes.

Not only physio-biochemical alterations take place in plants exposed to water deficit conditions, but a variety of anatomical changes also take place which help the plants to thrive under stress conditions (Saeed et al. 2016). In the present study, water deficit conditions caused a significant increase in leaf bulliform cell area, leaf adaxial epidermis thickness, leaf sclerenchyma thickness, root metaxylem area, root epidermis and endodermis area, root stellar diameter, stem sclerenchyma thickness and stem epidermis thickness. The injurious effects of drought were reduced when sclerenchyma of leaves developed (Terletskaya and Kurmanbayeva 2017). However, a significant decrease was observed in leaf (vascular bundle area, midrib thickness and phloem area), root (cortex thickness and diameter) and stem (diameter, phloem area and vascular bundle area) anatomical features of both oat cultivars on exposure to arid environment. Recently, Akram et al. (2016) while examining the leaf anatomical features

in two radish cultivars under water stress found that water deficit stress caused a significant reduction in the leaf vascular bundle area, midrib thickness, parenchyma cell area and number of vascular bundles, while an increase was observed in leaf epidermis thickness. Some previous studies have shown that water stress can significantly reduce leaf mesophyll and midrib thickness of *Calligonum comosum* (Al-Khalifah et al. 2006), *Triticum aestivum* (Burnett et al. 2005) and *Ctenanthe setosa* (Kutlu et al. 2009). However, an increase in leaf epidermis thickness is a general observation under drought stress, but such changes entirely depend on the intensity of water shortage (Naz et al. 2014; Akram et al. 2016). In another study with *Astragalus gombiformis*, Boughalleb et al. (2014) showed a variety of anatomical adaptations under water limited environment including decreased vessel size, cortical and mesophyll parenchyma formation, while an increased wall thickness and stomatal density. These features have been suggested to be involved in maintaining water potential and energy storage under drought stress which can efficiently improve the plant survival under arid environment.

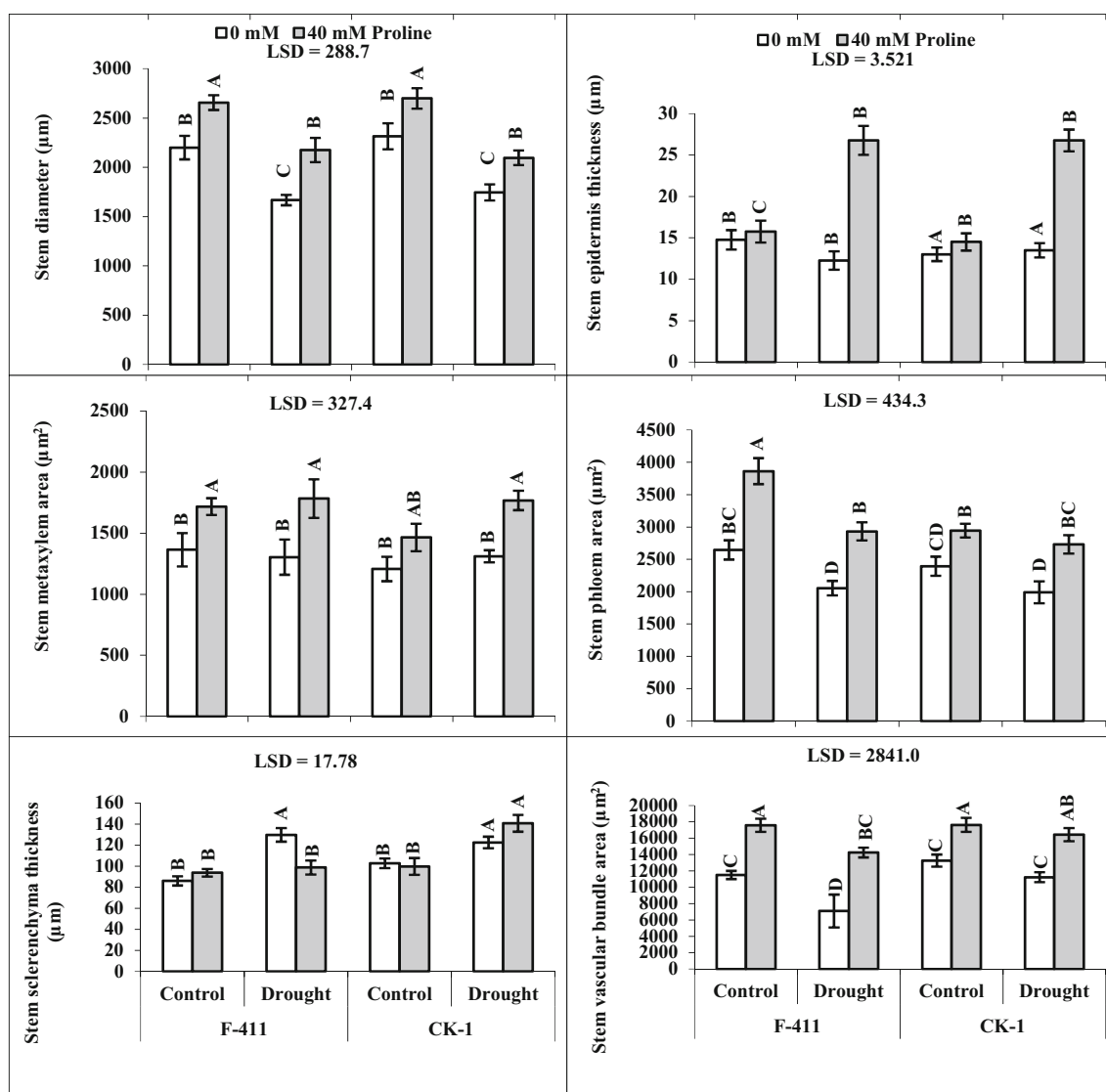


Fig. 7 Stem diameter, epidermis thickness, metaxylem, phloem area, stem sclerenchyma thickness and vascular bundle area of two cultivars of oat (*Avena sativa* L.) plants foliar-treated with proline

grown under varying water regimes. Mean \pm S.E.; letters (A–D) showing least significance difference among mean values

Of anatomical features of various plant organs, root architecture is most important because root can act as a sensor to sense water shortage (Olmos et al. 2007; Naz et al. 2014). In the present study, exogenous application of proline significantly improved various leaf (metaxylem area, mesophyll thickness), root (diameter, cortex thickness, epidermis, endodermis thickness, stelar diameter, metaxylem area), and stem (diameter, vascular bundle area, epidermis area, phloem area and sclerenchyma thickness) anatomical features of both oat cultivars grown under water-deficit conditions. These results clearly indicate that exogenous application of proline induced a variety of anatomical changes in oat plants. So, proline-induced growth improvement under drought stress in oat plants can be attributed to proline-induced increase in vascular bundle

area, epidermis/sclerenchyma thickness, and phloem area. However, we cannot compare these results as there are no reports available in the literature on proline-induced changes in plant anatomical features.

Conclusion

In conclusion, water-deficit conditions induced a variety of morphological, anatomical and physiological changes in both oat cultivars (CK-1 and F-411). A significant decrease was observed in chlorophyll *a* and *b* contents, while an increase was observed in leaf proline, hydrogen peroxide, activities of peroxidase and superoxide dismutase enzymes under water scarce conditions. However, exogenous

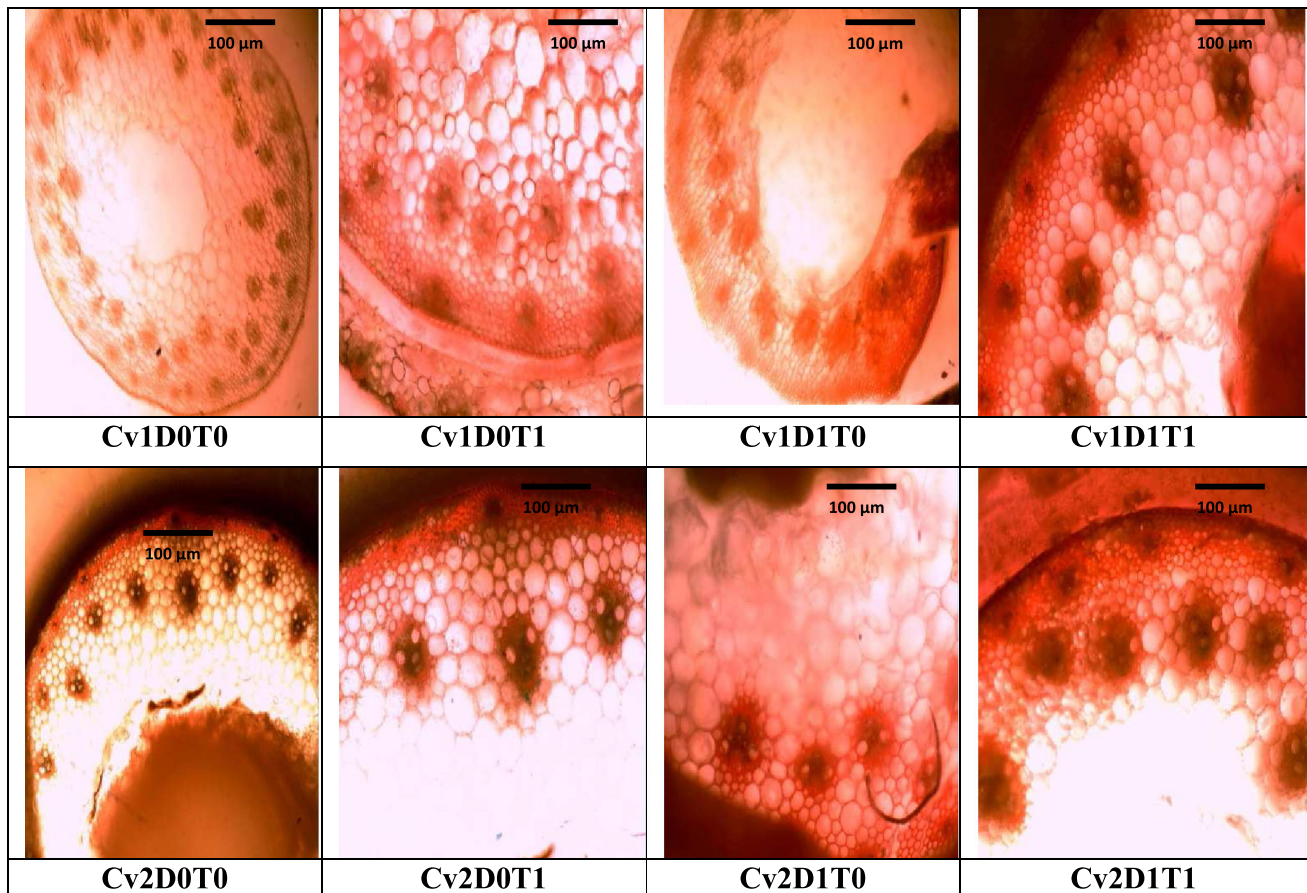


Fig. 8 Stem diameter, epidermis thickness, metaxylem, phloem area, stem sclerenchyma thickness and vascular bundle area of two cultivars of oat (*Avena sativa* L.) plants foliar treated with proline

application of proline significantly improved the plant growth (root and shoot fresh/dry weights, lengths), leaf proline contents, metaxylem area, mesophyll thickness, root diameter, root cortex thickness, root epidermis, endodermis thickness, stelar diameter, metaxylem area, stem diameter, stem vascular bundle area, stem epidermis area, stem phloem area and stem sclerenchyma thickness. However, application of proline induced reduction in leaf phloem area and leaf midrib thickness. Overall, foliar spray of proline was effective in improving drought stress tolerance which can be attributed to proline-induced significant modulation in physio-biochemical and anatomical features of oat plants.

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