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Zn-priming of seed ameliorates biochemical and anatomical parameters of *Cicer arietinum* L. cultivars in drought condition

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Cicer arietinum an important legume crop is among the drought tolerant plants and cultivated for its rich nutritive value almost all over the world. The current research was led to assess the role of pre-sowing seed treatment with 0.05% $ZnSO_4$ solution on the anatomical and biochemical characteristics of four varieties of Chickpea (i.e. CM 2008, FG 0902, DO 75-09 and Pb 2008) under three different maintained drought conditions. A pot experiment was performed in GCWUF. Seed priming with distilled water and 0.05% $ZnSO_4$ solution was done. Overall, all cultivars exhibited high production of all biochemical nutrients and effective change in anatomical characters but CM 2008 and FG 0902 cultivars grown from $ZnSO_4$ rimed seeds display tolerance under drought condition and gave higher values of various biochemical and anatomical parameters compared to plants grown from seeds primed with distilled water. However, Pb 2008 cultivar gave the minimum increase in values of biochemical and anatomical parameters among all cultivars under all field capacities.
Keywords: *Cicer arietinum*, drought, stem anatomy, seed priming.

INTRODUCTION

Chickpea is an important legume plant, belongs to family Fabaceae (old name Leguminosae) (Knights *et al.,* 2007). Globally, it is sown almost on 12 million hectors of total cultivated area (FAO, 2007). In Pakistan total used area for *C. arietinum* cultivation is 1053800 ha. Total production of this crop in Pakistan is 496000 tones yield production annually (Newman *et al.,* 2012). *C. arietinum* seeds possess high nutritive value due to the presence of large amount of protein (25-28%). Morphologically this herbaceous plant is of small height rarely up to 60cm with extensive branches. Leaflets with serrated edges (9 to 15 pairs) are arranged on either side of rachis having one leaflet on the terminal end. *C. arietinum* is among the drought tolerant cereal crops. Drought is an insufficient precipitation resulting in scarcity of water over a prolonged period; generally it may occur in a season or more, triggering adverse impacts on animals, vegetation, and people (NDMA, 2008). In Arid areas which are developed by human beings drought intensify the desertification (White and Nackoney, 2003). Disturbance in metabolic processes, end of light and dark reactions and ultimately death of the plant may be result of severe water deficit conditions. Drought causes harmful impacts on several processes including photosynthesis (Pagter *et al.,* 2005; Kalefetoglu Macer and Ekmekci, 2009), water content of the cell, respiration (Silva *et al.,* 2009; Mahmood *et al.,* 2019), as well as amino acid metabolism, carbohydrates, protein, lipids or other complex organic molecules (Taiz and Zeiger, 2010; Diniz *et al.,* 2020). These physiological and biochemical changes leads to the modification in biochemical, physiological as well as anatomical structure of plants under stress conditions.

Anatomical modifications induced due to low water availability are good indicators in agriculture sector (Kanwal *et al.,* 2012). The responses of plant tissues to drought condition depend on these structural modifications that regulate the water (Hameed *et al.,* 2012). Under water scarcity, cell division and cell elongation, both are disrupted as a resulting consequent decrease in turgor loss, energy supply and enzymatic activities occurs (Hameed *et al*., 2012; Kanwal *et al*., 2012). Drought can result growth reduction by 30-70% and an increase in diameter of the stem by 70-80% in adult plants (Rahimi *et al.,* 2013). Moreover, when availability of water dropped mostly cell size reduced, number of vascular tissues increase and cell wall becomes more thickened (Guerfel *et al.,* 2009). Among all the structures which show response to drought condition vascular structure are of great importance. Mostly plants cope with the environmental stresses by changing the xylem to phloem ratio as well as modify their wall architecture (Child *et al.,* 2003).

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By retaining physiological and biochemical mechanisms as well as by maintaining turgor pressure plants can tolerate low water stress (Chandler and Bartels, 2003). Whereas, plants show better development and growth in presence of sufficient amount of minerals, macro and micro-compounds. Minerals, macro and micro-components play essential part in plant development and growth. Their deficiency can inhibits all biochemical and physiological mechanisms (Kanwal *et al*., 2013), thus disturbing the plant development (Broadley *et al.*, 2007).

Plants derive elements carbon, oxygen and hydrogen directly from soil and atmosphere. While they obtain thirteen remaining essential elements boron, copper, calcium, chlorine, iron, manganese, magnesium, molybdenum, nickel, nitrogen, potassium, phosphorus, sulphur and zinc either from organic materials, minerals or inorganic or organic fertilizers (Uchida, 2000). There are various bio-physiological mechanisms in plants which are dependent on the availability of zinc (Zn) (Hafeez *et al*., 2013; Mahmood *et al*., 2019). Uptake of Zn inside plants takes place through xylem tissues and is transported to the shoot with the help of carrier proteins and active transport system (Broadley *et al.*, 2007). Apoplast and symplast are important pathways for the absorption of Zn in xylem tissues of root. Its translocation in large amount in phloem has also been observed (Brennan, 2005; Broadley *et al.*, 2007).

Zn deficiency is mostly induced by high percentage of soil phosphorous content which affects various crops (Grusak, 2002). A lot of methods are being used such as, by foliar spray (Johnson *et al.*, 2005), as fertilizers (Singh, 2007), seed priming (Farooq *et al.*, 2009), for artificial Zn application to increase the growth which in turn increase crop yield (Johnson *et al.*, 2005). From an economical point of view seed treatment is a best option because it needs less amount of micronutrient. Its application is easy and it is highly helpful to improve the growth of seedling (Singh *et al.*, 2003). In this method seeds are treated either by coating seeds with micronutrients or by soaking in micronutrient solution under controlled conditions (Halmer, 2003; Mahmood *et al*., 2019). Seed priming method is commercially a widely used procedure. In several crops it starts many physiological mechanisms which play vital role in sprouting process of seedling and eventually speedup the seed germination (Halmer, 2003; Mahmood *et al.*, 2019). This technique has been practiced in different countries like Australia, China, Pakistan and many others. Zinc has a significant physiological role during seed germination, stabilizing and shielding the construction of plasma membranes (Cakmak, 2008). *C. arietinum* with Zn deficiency has also been reported because it is grown in areas having water and Zn deficiency in soils. This deficiency affects the leaves and initial symptoms are yellowing of leaves, than bronzing and in severe conditions it may lead to necrosis of middle and lower leaves (Hameed *et al.*, 2012). Ameliorative effect of seed priming with Zinc solution is examined to check its ameliorative effect in plant growth and development of chickpea under water deficit conditions during this research experiment.

MATERIALS AND METHODS

Different anatomical and biochemical attributes of the study carried out by (Mahmood *et al*., 2019) were analyzed.

Total soluble proteins (mg g^{-1}fw): Bradford (1976) method was followed for measuring total soluble protein contents from fresh leaf material

Total free amino acids (mg g^{-1}fw): Hamilton and Van Slyke (1943) was adapted for measuring free amino acids from fresh plant material of chickpea

***Leaf proline content (mg g^{-1}fw)*:**Leaf free proline content was determined following the method of Bates *et al*. (1973).

Total soluble sugars (mg g^{-1}dw): Total soluble sugars were determined by following the method of Yemm and Willis (1954).

Determination of anatomical characteristics: Fresh material of stem was preserved and utilized for free-hand sectioning for anatomical studies according to Kanwal *et al*. (2012). Following anatomical characteristics of stem anatomy were studied; Size and shape of dermal, parenchymatous, mechanical and vascular tissue were observed.

Statistical Analysis: MSTAT and Microsoft Excel were used for the analysis of data. Least significant differences (LSD) test and F-test exhibited significant differences to compare means (Steel and Torrie, 1996).

RESULTS

In current study, four *C. arietinum* cultivars were evaluated to examine the influences of $ZnSO_4$ (0.05% w/v) seed priming on the basis of various anatomical and biochemical attributes. The cultivars were characterized on the basis of following attributes total soluble proteins, total free amino acids, leaf proline content, total soluble sugars and stem anatomy under normal and water deficit conditions.

Biochemical parameters: Two cultivar i.e. CM 2008 and FG 0902 raised from primed seeds with $ZnSO_4$ (0.05% w/v) showed maximum (P<0.05%) increase in values of total soluble proteins under all drought conditions (controlled, 70% FC and 35% FC) as compared to plants grown from distilled water primed seeds. DO 75-09 cultivar grown from seeds primed with $ZnSO_4$ (0.05% w/v) showed increase in the values of total soluble proteins at all drought levels than plants grown from seeds with distilled water. Overall, Cultivar CM 2008 and FG 0902 grown from seeds ($ZnSO_4$ priming) showed maximum increase in values of total soluble proteins under all drought conditions as compared to plants grown from distilled water primed seeds (Table 1).

Table 1. Mean square values of different biochemical attributes from ANOVA of four Chickpea (*Cicer arietinum* L.) cultivars raised from seeds primed with $ZnSO_4$ and distilled water under drought

Source of variation	df	Total soluble proteins	Total free amino acids	Total free proline	Soluble Sugars
Cultivars (Cvs)	3	2.3063141ns	169.70873***	12.535970***	0.1025251***
Treatment (T)	1	1.653098ns	437.68758***	29.090757***	9.7023658***
Drought (D)	2	19.489805***	1155.8078***	89.459317***	0.8248718***
Cvs x T	3	1.275594ns	1.0757ns	1.524395ns	0.0276239***
Cvs x D	6	1.074956ns	37.7566***	0.784006ns	0.0106612**
T x D	2	2.583771ns	4.0133*	3.621318*	0.0954438***
Cvs x T x D	6	0.476521ns	2.0443ns	1.623214ns	0.0062830*
Error	48	0.011247	9.7181	3.286300	0.0027076

Table 2. Mean square values of different stem anatomical attributes from ANOVA of four Chickpea (*Cicer arietinum* L.) cultivars raised from seeds primed with $ZnSO_4$ and distilled water under drought

Source of variation	df	Stem Cellular Area	Epidermis Thickness	Epidermal Cell Area	Sclarenchyma Thikness
Cultivars (Cvs)	3	0.653578***	62.438704***	21.75236***	709.1620***
Treatment (T)	1	3.242756***	45.442222***	101.53125***	34628.3470***
Drought (D)	2	3.959217***	29.024306***	113.39681***	13084.2220***
Cvs x T	3	0.012756***	0.412222**	0.05717ns	39.4954ns
Cvs x D	6	0.020822***	0.710231***	0.37180**	42.5370*
T x D	2	0.076172***	0.175972ns	0.09375ns	487.3889***
Cvs x T x D	6	0.010033***	0.539306***	0.14578ns	2.0370ns
Error	48		0.094167	0.09125	15.2500
Source of variation	**df**	**Cortical cell area**	**Vascular bundle area**	**Metaxylem**	
Cultivars (Cvs)	3	28816.204***	274.29681***	26.76167***	
Treatment (T)	1	86112.500***	509.33681***	212.18000***	
Drought (D)	2	134793.060***	756.42125***	721.03292***	
Cvs x T	3	156.944ns	0.60532***	0.48703ns	
Cvs x D	6	491.204ns	25.61180***	3.50791***	
T x D	2	1612.500**	2.85097***	0.13625ns	
Cvs x T x D	6	873.611**	1.95671***	0.73273**	
Error	48	247.222	0.05458	0.20555	

Similarly, Cultivars CM 2008 and FG 0902 also showed maximum (P<0.05%) increase in values of total free amino acids under all drought which grown from seeds primed with $ZnSO_4$ as compared to plants grown from distilled water primed seeds. (Table 3.1). Maximum (P<0.05%) increase in values of total free proline content and total soluble sugars were recorded in cultivars CM 2008 and FG 0902 grown from seeds with $ZnSO_4$ priming under controlled as well as drought conditions as compared to plants grown from seeds primed with distilled water. (Table 1).

Overall CM 2008 and FG 0902 cultivars gave higher values of all biochemical parameters (total soluble proteins, total free amino acids. total free proline content and total soluble sugars) when grown from seeds primed with $ZnSO_4$ as compared to plants grown from seeds primed with distilled water. However, Pb 2008 cultivar gave the minimum increase in values of all these biochemical parameters among four cultivars under all field capacities.

***Anatomical traits*:** Stem cellular area was reduced under all drought levels in all studied *C. arietinum* L. cultivars. However, cultivar CM 2008 and FG 0902 grown from $ZnSO_4$ primed seeds (0.05% w/v) showed higher (P<0.05%) increase in values of stem cellular area comparatively with other cultivars under two drought levels (controlled and 70% FC) as compared to plants grown from distilled water primed seeds (Fig. 1, 2: Table 2).

A decrease in epidermal cell area and epidermal thickness was also recorded under all drought levels in all *C. arietinum* L. cultivars. Whereas, cultivars CM 2008 and FG 0902 grown from $ZnSO_4$ primed seeds showed relatively greater (P<0.05%) increase in values of epidermal cell area and epidermal thickness than DO 75-09 and Pb 2008 cultivars under all drought conditions as compared to plants grown from distilled water primed seeds (Fig 3.1,3.2 : Table 3.2).

Overall, drought condition caused decrease in sclerenchyma thickness and cortical cell area in all *C. arietinum* L. cultivars. But, Cultivars CM 2008 and FG 0902 grown from $ZnSO_4$

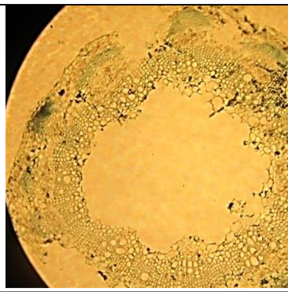
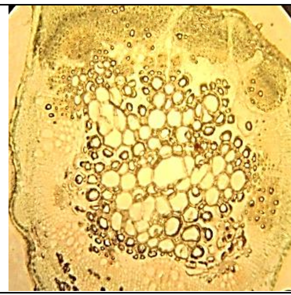
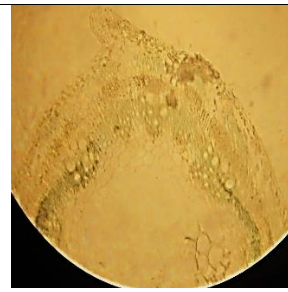
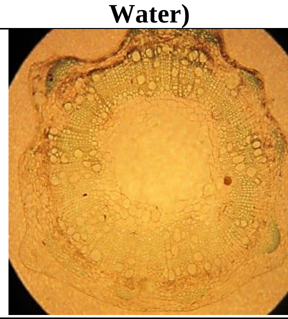
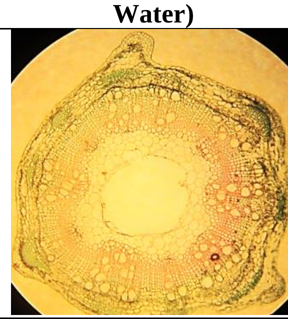
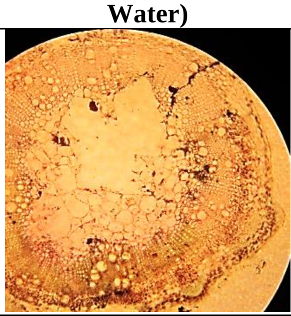
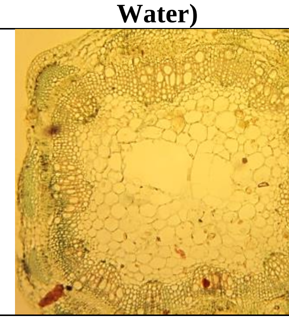
			
CM 2008 (100% FC + Priming with Distilled Water)	**FG 0902 (100% FC + Priming with Distilled Water)**	**DO 75-09 (100% FC + Priming with Distilled Water)**	**Pb 2008 (100% FC + Priming with Distilled Water)**
			
CM 2008 (70% FC + Priming with Distilled Water)	**FG 0902 (70% FC + Priming with Distilled Water)**	**DO 75-09 (70% FC + Priming with Distilled Water)**	**Pb 2008 (70% FC + Priming with Distilled Water)**
			
CM 2008 (35% FC + Priming with Distilled Water)	**FG 0902 (35% FC + Priming with Distilled Water)**	**DO 75-09 (35% FC + Priming with Distilled Water)**	**Pb 2008 (35% FC + Priming with Distilled Water)**

Figure 1. Stem anatomy of four Chickpea (*Cicer arietinum* L.) cultivars raised from seeds primed with distilled water under controlled and drought stress conditions

primed seeds showed more (P<0.05%) increase in values of sclerenchyma thickness under controlled and drought conditions as compared to plants grown from distilled water primed seeds than the other two cultivars. Overall, Pb 2008 gave minimum increase in cortical cell area under 35% FC (Fig. 1, 2 :Table 2).

Drought condition directly affected the metaxylem and vascular bundle area of *C. arietinum* L. cultivars. Whereas, $ZnSO_4$ seed priming treatment facilitated the plants to increase their metaxylem and vascular bundle area under all water deficit treatment levels. Cultivars CM 2008 and FG 0902 grown from $ZnSO_4$ primed seeds showed larger (P<0.05%) increase in values of vascular bundle and metaxylem area than DO 75-09 and Pb 2008 cultivars under water deficit conditions as compared to plants grown from distilled water primed seeds. Overall, minimum increase in metaxylem and vascular bundle was recorded in Pb 2008 under 35% FC (Fig. 1, 2: Table 2).

DISCUSSION

Pre-sowing seed treatment with zinc can help the plants to overcome the adversative properties of drought condition Plants get Zinc in the form of cation (Zn^{+2}) from the soil. It play important role to improve the activity of various biochemical procedures and anatomical structures in plants

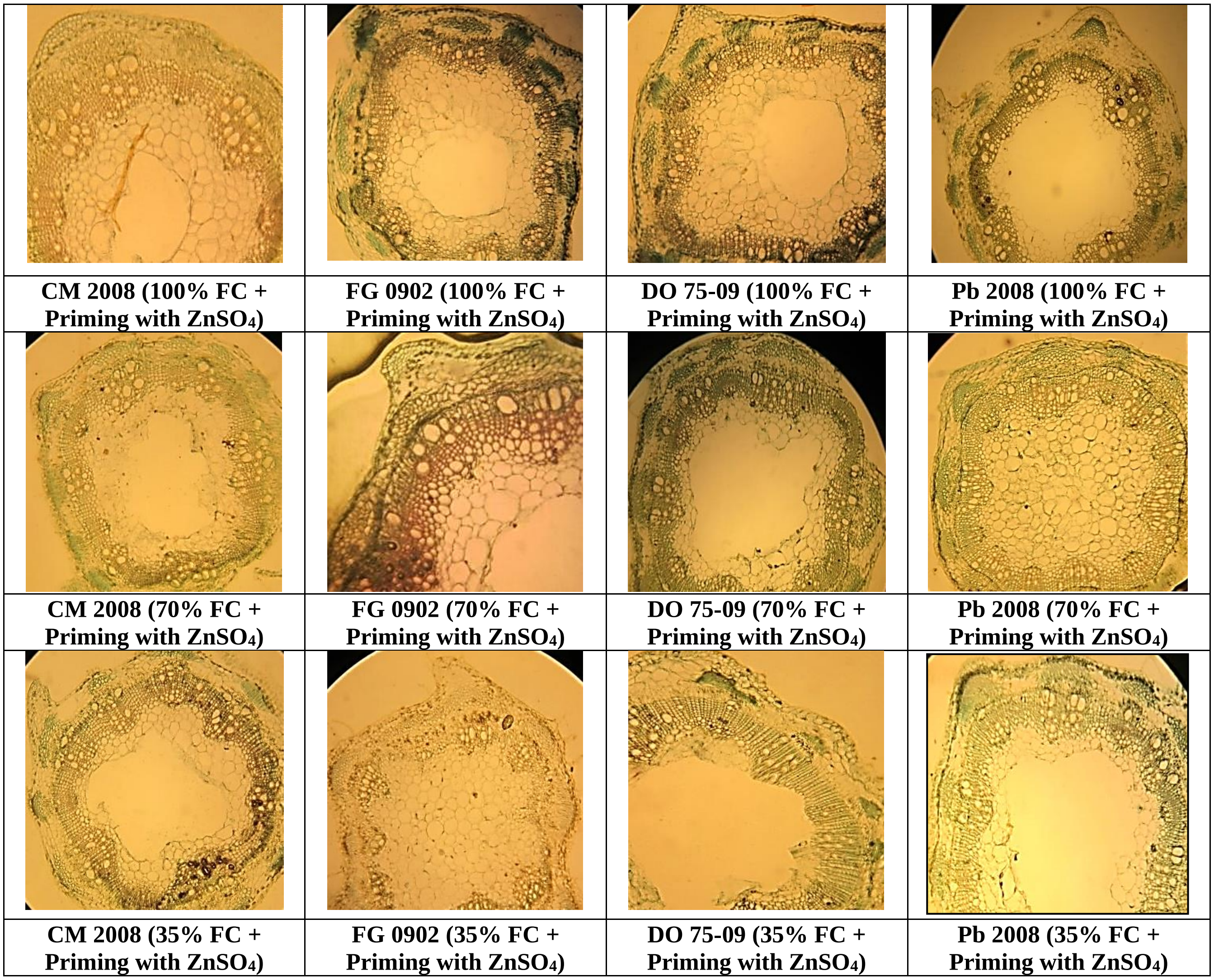


Figure 2. Stem anatomy of four Chickpea (*Cicer arietinum* L.) cultivars raised from seeds primed with $ZnSO_4$ under controlled and drought stress conditions

because it is vital structural and functional part of various enzymes. It is one of the vital nutrient for plant development because it is a co-factor of many enzymes such as RNA polymerase, carbonic anhydrase,alcohol dehydrogenase, Cu-Zn superoxide dis mutase etc and play an important in the functioning of these enzymes which control the various biochemical processes in plants (Reddy, 2006; Mahmood *et al*., 2019).

Different plant species accumulate osmoprotectants like free amino acids and free proline content in their tissues in response to different environmental stresses. The accumulated proline helps the plant tissues to equalize the osmotic potential of cytosol and vacuole in correspondence to the atmosphere under stress conditions. Boughalleb and Mhamdi (2011) studied that with the substantial increase in drought, proline content in olive cultivars was increased significantly. They observed that its increase protected the plant tissues from serious consequences of dehydration uder low availability of water.

The results of this research revealed that CM 2008 and FG 0902 *C. arietinum* cultivars grown from $ZnSO_4$ primed seed gave higher values of total leaf proline content in camparison to plants grown from seeds treated with distilled water under all drought levels. This increase in proline accumulation prevents the plants from decrease in osmotic potential of cytosol. Farooqi *et al.* (2010) observed similar results in *Cajanus cajan* and *Cucumis sativus*. He also observed that carbohydrates level directly determined the accumulation of proline content. Verbruggen and Hermans (2008) revealed that the cells maintained their balance by using accumulated proline in leaves. During maintenance of cells water potential of vacuole is also balanced with its external surroundings

(Yokota *et al*., 2006). Accumulated proline content supplies energy to the plant tissues and played a vital role in better survival of plants under harsh conditions (Kumar *et al*., 2011). In the current study, increase in drought levels reduced the osmoprotectants of all *C. arietinum* cultivars while pre sowing seed treatment with $ZnSO_4$ results in enhanced values of all these biochemical parameters under all drought treatments. An increased level of total soluble sugars in plants grown from $ZnSO_4$ primed seeds cosuld be the result of depletion of starch in leaves. In another study Patakas and Noitsakis (2001) noted that in leaves of grapevine the level of starch depletion was increased in response to low water availability thus resulting an increased level of total soluble sugars. In many metabolic processes of plants soluble sugars play complex role. They are involved in the production of energy. Soluble sugars are the substrate of many biosynthetic as well as signalling mechanisms. Gibson (2005) claimed that sugar flux might be playing a role of signalling molecule for regulations of metabolic processes which produce energy.

Hoekstra and Buitinik (2001) revealed that osmoprotectants (soluble sugars and proline) might be responsible for resistance mechanism against low water potential and dehydration. Thus, osmoprotectants were typically involved in maintenance of the cell membranes and turgor pressure which eventually helped the plant to increase their root and shoot length. During low water availability sugars started two types of defence mechanisms to protect the plant tissues. First mechanism starts against dehydration, to stabilize the interaction (hydrophilic) between membranes and proteins thus preventing the proteins from denaturation. Second mechanism starts in dehydrated cells by the contribution of a factor responsible for vitrification (production of a biological gas) (Bernacchia and Furini, 2004).

The present research showed that drought negatively affects the all anatomical attributes. Epidermal thickness, diameter of xylem, phloem and paranchyma cells decreased in plants raised from seeds primed with distilled water. However, structural modifications were observed in chickpea plants grown from $ZnSO_4$ primed seeds. Many researches revealed that plants show anatomical modifications in their various organs while facing water deficit conditions. For example stem anatomy is a good indicator to show resistance against drought. Structural modifications in stem are indicators for adaptations due to drought (Kanwal *et al*., 2012; Hameed et al., 2012; Ola *et al*., 2012). Overall, in this research experiment, the CM 2008 and FG 0902 *C. arietinum* cultivars exhibited better tolerance to drought in terms of anatomical modifications.

The present study showed that all cultivars facing drought conditions have thin stem epidermis. But $ZnSO_4$ seed priming treatment helped the plant to develop thick epidermis of stem. As thick epidermis can reduce the water loss from stem (Hameed *et al*., 2012). Water stress reduced the size of sclerenchyma cells but treatment of $ZnSO_4$ seed priming increased the sclerification of stem cells in all *C. arietinum* cultivars. As sclerification gives rigidity to the stem cells and prevent the water loss. The results were similar to previous studies of Shao *et al*. (2008). Adesoji *et al*. (2013) reported the significant increase in the size of cell, number of cells and cell division due to Zn enhanced the growth of *Zea mays* and other legume crops. On the other hand Zn application increased the water conductance in stem of *Hordeum vulgare* plant. Same results were observed in *Brassica juncea* when treated with Zn priming. Zn increased the area of vascular tissues of the stem. Drought caused decrease in diameter of pith of all cultivars of *C. arietinum* grown from distilled water primed seeds but Zn treatment helped the plant cells to increase diameter of parenchymatous area of pith cells. Zn deficiency retarded the primary and secondary growth of the stem cells.

***Conclusion*:** All above discussion inferred that different levels of drought condition applied adversely affect all the examined attributes in present research i.e. biochemical attributes and anatomical characteristics in both sets of plants. Overall, all cultivars exhibited high production of all biochemical nutrients and effective changes in anatomical characters but CM 2008 and FG 0902 cultivars grown from seeds primed with ZnSO4 showed tolerance in drought condition under all field capacities and gave higher values of all biochemical and anatomical parameters compared to plants grown from seeds primed with distilled water. However, Pb 2008 cultivar gave the minimum increase in values of biochemical and anatomical parameters among all cultivars under all field capacities.

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***Authors' contributions*:** Authors one Plan the research and arranged the funding to complete this research plan. The other authors contributed in execution of the plant, data keeping analysis and write up the article with author one.

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***Ethical statement*:** This article does not contain any studies with human participants or animal performed by any of the authors.

***Availability of data and material*: N/A**

Code Availability: Not applicable

***Consent to participate*:** All authors are participating in this research study

***Consent for publication*:** All authors are participating in this research study.

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