Pak. J. Bot., 52(3): 801-810, 2020. DOI: http://dx.doi.org/10.30848/PJB2020-3(35)

MORPHO-ANATOMICAL ADAPTATIONS OF TWO *TAGETES ERECTA* L. CULTIVARS WITH CONTRASTING RESPONSE TO DROUGHT STRESS

FAISAL ZULFIQAR[1*], ADNAN YOUNIS[1], ATIF RIAZ[1], FARAKH MANSOOR[1], MANSOOR HAMEED[2], NUDRAT AISHA AKRAM[3] AND ZAINUL ABIDEEN[4]

[1]*Institute of Horticultural Sciences, University of Agriculture Faisalabad, 38040-Pakistan;*
[2]*Department of Botany, University of Agriculture Faisalabad, 38040-Pakistan;*
[3]*Department of Botany, Government College University Faisalabad, Pakistan;*
[4]*Institute of Sustainable Halophyte Utilization, University of Karachi, Karachi 75270, Pakistan;*
**Corresponding author's email: ch.faisal.zulfiqar@gmail.com*

Abstract

Water deficit is a serious threat to the global crop production. Marigold (*Tagetes erecta* L.), an economically important annual is resistant to drought stress, however, anatomical adaptations confirming drought tolerance are still unexplored. In this context, key growth and anatomical difference of two marigold (*T. erecta* L.) cultivars, Inca (drought resistant) and Bonanza (drought sensitive), to water deficit conditions were studied. Plants of both marigold cultivars were subjected to control (T_0; 100% FC) and water deficit conditions (T_1; 60 FC and T_2; 40 FC) three weeks after transplantation. Drought stress caused a significant reduction in growth of cv. Bonanza while cv. Inca remained unaffected. However, drought stress significantly affected anatomical features in both marigold cultivars. Increasing drought stress levels enhanced the reduction in root vascular area of both cultivars. Under both levels of water stress, shoot cortex area decreased only in cv. Bonanza. Leaf thickness and cortex area decreased at 40% FC in both cultivars. The growth and biomass performance of cv. Inca was better than cv. Bonanza under water stress conditions.

Key words: Anatomy, Drought, Climate Change, Floriculture, Growth, Marigold, Physiology, Water stress.

Introduction

Marigold (*Tagetes erecta* L.) is a drought resistant plant (Riaz *et al.*, 2013) in the family *Asteraceae* which is an annual flowering herb with pinnately divided leaves (Dole & Wilkins, 2005). *Tagetes erecta* commonly known as African marigold (Yasheshwar *et al.*, 2017), is native to Mexico and has ornamental, aromatic and medicinal properties (Cicevan *et al.,* 2016). It is widely used as cut flower, and as bedding plant in landscape because of diverse flower size and availability throughout the year (Chkhikvishvili *et al.,* 2016; Younis *et al.,* 2018a). Marigold flower petals are enriched with lutein, carotenoids and various other important secondary metabolites (Pandey *et al.,* 2015). Presence of carotenoids, flavonoids, triterpene alcohol, tannins, sterol, mucilage, saponins and resin in *Tagetes* species have been reported and evaluated for their therapeutic benefits (Riaz *et al*., 2013).

Environmental stresses such as salinity and water deficit have adverse effects on plant morphology and survival (Abideen *et al*., 2019; Mahmoud *et al.,* 2018; Zulfiqar *et al*., 2020). Increasing water scarcity in arid regions is considered as a serious environmental issue restricting sustainable agriculture worldwide (Alvarez-Flores *et al.,* 2018; Ali *et al*., 2020). There is a great interest in the adoption of sustainable and resource conserving strategies in agriculture and horticulture (Zulfiqar *et al.*, 2019a, b). Similar to other abiotic stresses, plants have various strategies to survive under water stress condition. They can increase root biomass and length, reduce shoot growth, or alter leaf orientation to escape from water limitations. Reduction in leaf area, leaf shedding, and change in the anatomical features can be important parameters under water scarce conditions. Plants also triggers specific modifications in plant anatomical architecture depending upon the extent and duration of water stress. Anatomical alterations in the leaf, stem and root of a plant, can also be used as successful indicators of drought stress (Shao *et al*., 2008; Leukovic *et al*., 2009; Oliveira *et al*., 2018). For instance, reduced cell size and vascular tissues arrangements have been shown in *Olea europaea* under drought stress (Guerfel *et al*., 2009). Alteration in xylem/phloem ratio and modification of cell architecture can also be indicators (Makbul *et al*., 2011). Xylem and phloem vessel reduction under limited water regimes has been reported (Boughalleb *et al*., 2014). Thickness of phloem and xylem in the leaf vascular system of *Astragalus gombiformis* decreased under water deficit conditions (Boughalleb *et al*., 2014). Plants growing under low to moderate drought stress reduce xylem vessel diameter, and increase thickness of epidermis, phloem, and mesophyll tissues of wheat plants (El-Afry *et al*., 2012).

Water availability is an important factor affecting the growth and quality (pre and post production) of ornamental plants (Cirillo *et al*., 2017). While deficit irrigation, the practice of less water application than usual to plants to conserve water or to slow the growth in vegetables (Rouphael *et al*., 2008; Casa & Rouphael, 2014) and fruits (Egea *et al*., 2010) has been reported, this practice in ornamental plants has not been studied as intensely (Cirillo *et al*., 2017). In these aspects, it is necessary to understand their mechanisms under drought stress especially through various growth, anatomical, physiological and morphological attributes (Leukovic *et al*., 2009; Akram *et al*., 2016; Cicevan *et al*., 2016). It is necessary to understand the potential of marigold genotypes and their reaction to deficit irrigations to attain sustainable production of marigold in suboptimal conditions. Selection of genotypes with drought tolerant ability is one of the main research focus to increase the yield under limited water supply (Chaves & Davies, 2010), to conserve natural resources and to meet future demand of xeroscaping and water conservation (Cicevan *et al*., 2016). Additionally, exploration of the drought resistance mechanisms in plants can help in the future breeding of drought tolerance in economically important crops. On the other hand, study of plants with respect to water deficit conditions is becoming even more important under the changing climatic scenario (Puértolas *et al*., 2017). However, there are still no studies available describing the marigold root, shoot and leaf anatomy in relation to water deficit stress conditions. Pakistan is blessed with a diverse range of climatic conditions favoring the production of high value crops including ornamentals (Younis *et al*., 2016; Fiaz *et al*., 2018; Younis *et al*., 2018b) but also has to face drought spells due to arid and semi-arid climate with annual rainfall below than 60 cm. A complete understanding of the anatomical, physiological and biochemical mechanisms by which plant responds to normal and water deficit conditions with or without using exogenous chemicals such as plant bioregulators, is therefore essential not only to improve water use efficiency but the ornamental quality of plants especially under changing climatic scenario (Zollinger *et al*., 2006; Mansoor *et al*., 2015; Zulfiqar *et al*., 2019c). Two marigold cultivars planted widely in Pakistan were used in this study. These two cultivars differ widely in their drought tolerance ability previously confirmed and characterized by morpho-physiological traits by Younis *et al*., (2018a). The cultivar Inca is known as drought resistant while cultivar Bonanza as drought sensitive (Younis *et al*., 2018a). In view of the above discussion, we hypothesized that change in morpho-anatomical characteristics in these marigold cultivars leads to adaptation under drought stress. Therefore, the present study was performed with the key focus on two popular and commercially important marigold cultivars for characterizing their leaf, stem and root anatomical adaptations towards water stress. The knowledge about similarities and specific differences in both cultivars responses to water deficit will be useful in planning of (i) breeding strategies, (ii) irrigation.

Materials and Methods

Plant materials and growth conditions: In 2017-18, a pot experiment was conducted outside under rain exclusion shelter at Floriculture Research Area of Institute of Horticultural Sciences, University of Agriculture Faisalabad, Pakistan. The climatic conditions of this area in the growing season recorded temperature/day averaged 22.1 ± 2.8°C, and air relative humidity/day averaged was 58.4 ± 5.1%. Healthy seeds of two widely grown marigold (*Tagetes erecta* L.) cultivars in the region [i.e., Bonanza (Pan American Seed 1999) and Inca F_1 (1982)] were sown on 25 August 2017. Seeds were purchased from local seed distributor in Lahore, Pakistan. Seeds were selected on the basis of uniformity in size and color. Selected seeds of both cultivars were then disinfected with sodium hypochlorite (0.58%) for 1 min followed by double rinsing using distilled water. Seeds were air dried at room temperature to avoid fungal infection. Air-dried seeds were sown in germination trays filled with peat moss. Twenty eight days after germination, healthy seedlings were transplanted and maintained one per free-draining plastic pots (24 cm x 28 cm) filled with 5 kg air dried, sieved (2.0 mm), uniform mixture of silt, sand, leaf compost and farmyard manure [1:1:1:1(v/v)]. Each pot was watered to 75-80% field capacity with the tap water having 7.8 pH, 1200 mg L^{-1} total soluble salts and 1.2 dS m^{-1} electrical conductivity until the imposition of the drought stress treatments. Three weeks after transplanting, different watering regimes viz. control (T_0; 100% FC) and water deficit conditions (T_1; 60 FC and T_2; 40 FC) were applied to the transplants. All transplants were allowed to grow for 30 days at varying water regimes. A factorial experimental arrangement of treatments was followed according to completely randomized design (CRD) with two cultivar (Inca and Bonanza) levels and three watering regimes (100, 60 and 40% field capacity). Each treatment had four replicates for material sampling and measurements.

Growth and biomass production: At the end of the study, (one month of drought treatments), each plant of treatment was uprooted carefully and rinsed with deionized water. Afterwards, the plant is separated in root and shoot. Length of root and shoot was measured using ruler in centimeter. Fresh and dry mass (oven-dried plant material at 70°C until constant weight was reached) were taken to perform growth analyses. Root and shoot mass fraction was calculated as the ratio of root and shoot fresh mass to plant mass (g g^{-1}). Specific root length was calculated as the ratio of root length to root mass (cm g^{-1}). Similarly specific shoot length was measured as the ratio of shoot length to shoot mass in cm g^{-1}. Root length ratio was calculated as the ratio of root length to plant mass (cm g^{-1}).

Anatomical analysis of leaf, stem and root: Anatomical characteristics of leaf shoot and root of both marigold cultivars 4 weeks after drought treatments were examined. For this purpose, a piece of 2 cm was excised between 8:00 to 8:30 am, from the leaf base of fully expanded leaves for leaf anatomy, from the base of the internode of the stem for shoot anatomy, and from the thickest adventitious root near the root-shoot connection for root anatomy. The root, stem and leaf samples were dipped in formalin-acid-alcohol (FAA; formalin 10%, acetic acid 5%, ethyl alcohol 50%, and distilled water 35%) solution for 48 h and subsequently shifted to acetic alcohol solution comprising 25% acetic acid and 75% ethanol for long storage. Then, free-hand sections were arranged via series of dehydration in ethanol applying double-stained technique of safranin and fast green applications were followed to prepare permanent slides of various cells and tissues of root, stem and leaf (Ruzin, 1999). The observations (measurements and micrographs) of transverse sections (root, shoot and leaf) were taken using ocular micrometer on compound microscope. The stained sections were photographed using digital camera (Nikon FDX-35) equipped light microscope (Nikon SE, Anti-Mould, Tokyo, Japan). Four characteristics were measured regarding root: meta-xylem, epidermis, cortex and vascular region; three characteristics regarding shoot: vascular bundle, cortex, meta-xylem and epidermis; five characteristics regarding leaf: leaf thickness, vascular bundle, mesophyll, the lower and upper epidermis of leaf were recorded. For these anatomical traits, data were recorded using all four plants from each replication at random.

Statistical analysis

The collected data for various attributes were analyzed using ANOVA (Steel *et al*., 1997). Means were compared using least significance difference (LSD) test following Snedecor & Cochran (1980).

Results and Discussion

Plant fresh and dry mass of both marigold cultivars decreased significantly ($p \leq 0.001$) on exposure to all drought stress levels, however, the decline was more prominent in Bonanza than Inca at 40% followed by 60% FC (Fig. 1; Table 1). Reduction of biomass is a good strategy in *Tagetes erecta* to improve water uptake under drought stress for long-term survival. Growth improvement and survival in cv. Inca under these circumstances ensure optimum biomass that can be used as potent source of ornamental, medicinal and aromatic benefits. The survival of these cultivars under drought conditions might expand its cultivation on poor degraded lands with water conservation. Reduction of biomass of *Tagetes erecta* were supported by lower shoot mass fraction levels in cv. Bonanza exposed to both T_2 and T_1 treatments than cv. Inca. The root mass fraction of both cultivars decreased significantly ($p \leq 0.01$) with the increase in drought stress levels (Fig. 1; Table 1). In contrast, shoot mass fraction of cv. Inca decreased substantially at T_2 (Fig. 1). Specific shoot length was increased in cv. Inca as compared to cv. Bonanza plants under water deficit conditions while specific root length was unchanged in each treatment (Fig. 2). Increased biomass in cv. Inca is correlated with higher shoot length (lower stem thickness) that improved the area of photosynthetic foliage under drought conditions. In addition, the stem functions as a photosynthetically active area in many cases that allowed an increase of photosynthetic active area with minimal biomass investment, transpiration and water loss at dry habitats.

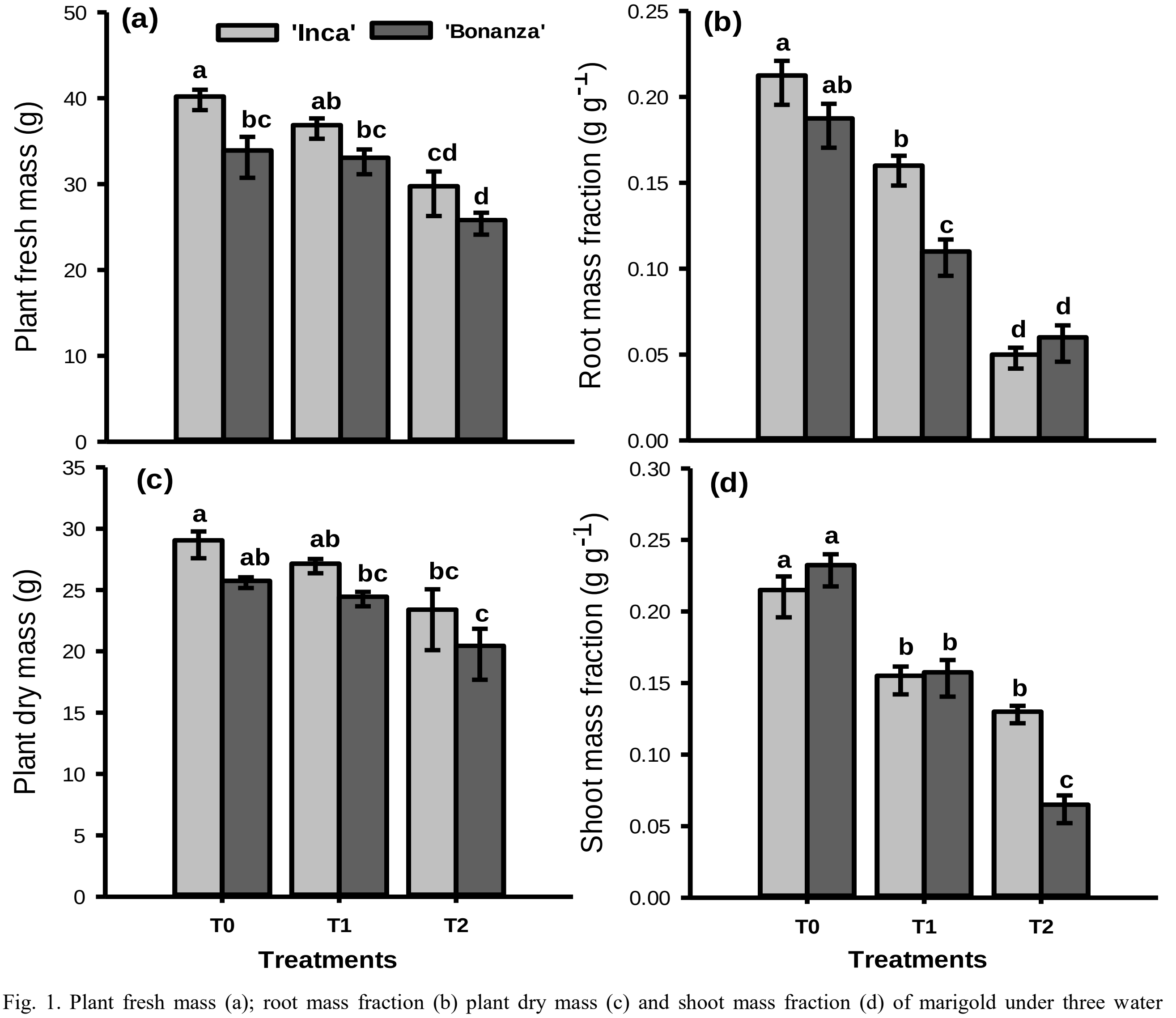

Fig. 1. Plant fresh mass (a); root mass fraction (b) plant dry mass (c) and shoot mass fraction (d) of marigold under three water regimes: T0, 100% FC; T1, 60% FC; T2, 40% FC.

Table 1. Analyses of variance (ANOVA) of the data (mean squares) for vegetative parameters of marigold cultivars subjected to water stress conditions (Mean ± S.E.).

Source of variation	df	Plant fresh mass	Plant dry mass	Root mass fraction	Shoot mass fraction	Specific shoot length	Specific root length	Root length ratio
Cultivar (C)	1	130.66667***	53.401667**	0.0030375**	2.0417e-4ns	0.0170667ns	0.0216ns	1.6667e-5ns
Treatment (T)	2	189.39125***	63.401667***	0.0429042***	0.0246167***	0.6518160***	0.2849292***	0.0166792***
C x T	2	3.8879167ns	0.1816667ns	0.0018375**	0.0048667*	0.0216167ns	0.0867125***	6.2917e-4ns
Error	18	5.6088889	3.7061111	2.0972e-4	9.6806e-4	0.0100556	0.0059361	3.8611e-4

ns= Non-significant; *, **and *** = Significant at 0.05, 0.01 and 0.001 levels, respectively

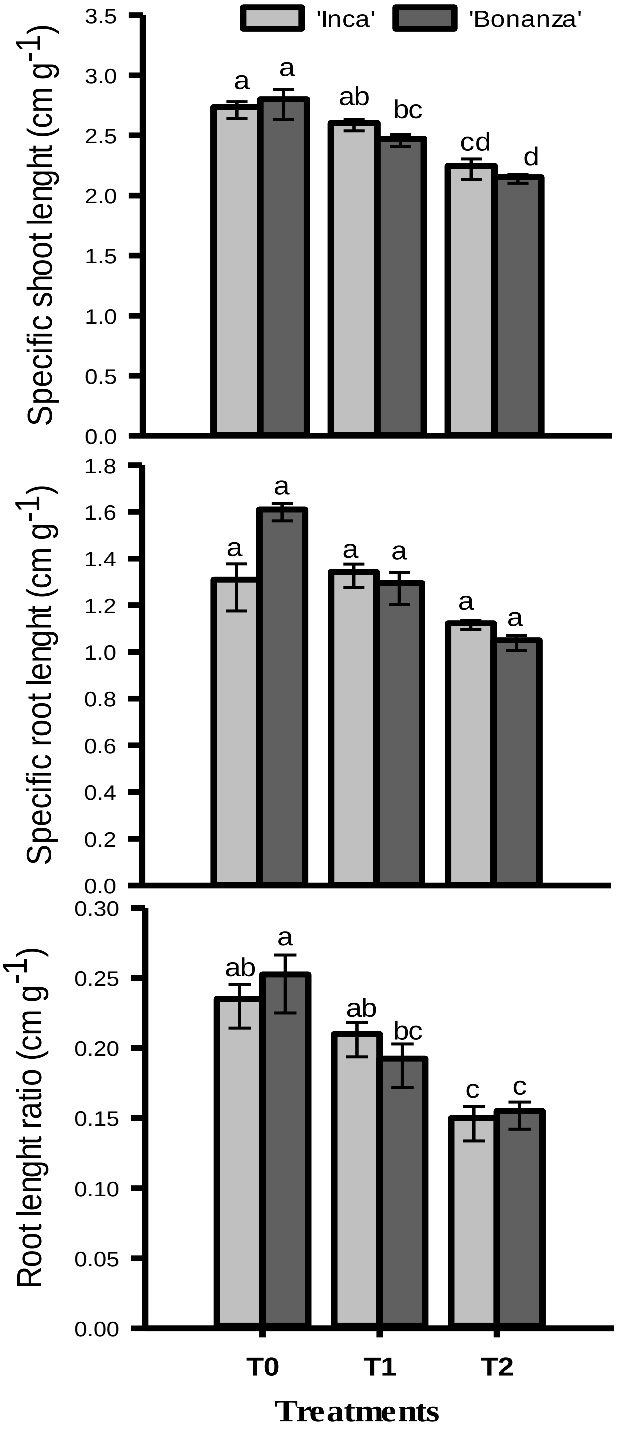

Fig. 2. Specific shoot length; specific root length; root length ratio of marigold under three water regimes: T0, 1-day interval; T1, 4-days interval; T2, 8-days interval.

Substantial reduction in root length ratio was recorded at different drought stress levels in both cultivars but root length ratio was promoted in cv. Inca than cv. Bonanza (Fig. 2). Significant increase in root length ratio of cv. Inca represents that *Tagetes erecta* modifies root length than total root biomass under drought stress conditions. Increase in root length under drought suggested as an effective strategy to improve soil water retentions and nutrient acquisition, which is associated with improved biomass in cv. Inca than cv. Bonanza (Fig. 2).

Modifications in root and other plant parts are considered adaptive scheme to resists stress conditions (Shao *et al*., 2008). In the analysis of root anatomy, comparison of both cultivars at T0 (100% FC) showed greater root meta-xylem area in cv. Bonanza (Tables 2, 3). However, meta-xylem area in roots significantly decreased in both cultivars being more noticeable decrease in cv. Bonanza especially under water stress (T_2). In a previous study, Peña-Valdivia *et al*., (2005) reported a significant reduction in meta-xylem tissues of maize in response to water deficit stress. Meta-xylem area reduction was also observed in *Cenchrus ciliaris* L. under three drought stress conditions (Nawazish *et al*., 2006). Reduction in meta-xylem could be an important strategy to cope with drought as it has been suggested that reduction in meta-xylem vessels diameter lowered the embolisms risk and increase water flow passage (Vasellati *et al*., 2001). Increased meta-xylem area can be directly related to conduction of water and minerals and also important for growth of cortical parenchyma (Singh *et al*., 2013). Our results correlate with the Twumasi *et al*., (2005) who reported the reduction in xylem vessel diameter of *Zinnia elegans* under drought stressed plants. Root epidermis area generally decreased in cv. Bonanza at higher water deficit levels but in cv. Inca this attribute increased gradually under T_2 (Table 1; Fig. 3). Thick epidermis with large epidermal cells in plants are known as plant drought tolerance strategy (Boughalleb *et al*., 2014). In addition, epidermal tissues thickness offers higher resistance of plants to water loss from root surface under arid climate (Singh *et al*., 2013; Riaz *et al*., 2016).

Water deficit decreased cortical area in both marigold cultivars (Table 1; Fig. 3). Cortex area and xylem vessels both offer an opportunity to assess the mean xylem diameter which is linked to plant water conduction (Jones *et al*., 1983). Cortical parenchyma is the main water storage region in plants, therefore any problems due to environmental hazards may cause serious impact on water conservation, and disintegration of cortical region (Peña-Valdivia & Urdaneta, 2009). However, cv. Bonanza was relatively more severely affected in terms of cortical parenchyma than the cv.

Inca. The vascular area was affected severely in both cultivars under water deficit conditions (T_1) (Fig. 3; Table 1). In *Tagetes erecta,* root area is dependent on proportion of vascular region, cortical parenchyma and central pith. So changes in the length or area may affect water conservation by efficient conduction and water storage (Peña-Valdivia *et al*., 2005), while no effect was observed on xylem length and number.

Both marigold cultivars were uniform in in shoot epidermis area under drought conditions as described by treatments and cultivars interaction. Under control conditions, cv. Inca had lower epidermis area (5630 µm) as compared to cv. Bonanza (5670 µm) (Tables 2, 3). Epidermis area of shoots reduced significantly with the increasing drought stress (Fig. 4).

Table 2. Analyses of variance (ANOVA) of the data (mean squares) for the anatomical attributes of root, stem and leaf of marigold (*Tagestes erecta* L.) cultivars subjected to water stress conditions (Mean ± S.E.).

Source of variation	df	Root endodermal area	Root cortical area	Root metaxylem area	Root vascular bundle area	Leaf vascular bundle area
Cultivar (C)	1	9909183.7*	27680148***	15388030**	1.4690***	5299482**
Treatment (T)	3	720213.32ns	4985275.7*	1332285.7ns	6176175ns	2859846.9*
C x T	3	689115.1ns	729963.78ns	1286265.4ns	1.08321ns	470212.79ns
Error	24	1316401.5	1364126.4	1604311.3	54321985	637733.08
Source of variation	**df**	**Stem epidermal area**	**Stem cortical area**	**Stem metaxylem area**	**Stem vascular bundle area**	
Cultivar (C)	1	237793.04ns	21958064ns	33276278**	5.53464***	
Treatment (T)	3	877302.98ns	55064839*	508864.68ns	1.2190**	
C x T	3	202262.67ns	5038016.7ns	723459.23ns	1032290ns	
Error	24	541916.73	13972074	2974677.7	24557684	
Source of variation	**df**	**Leaf abaxial epidermal area**	**adaxial epidermal area**	**Leaf mesophyll area**	**Leaf thickness**	
Cultivar (C)	1	5218953.6ns	2372582.6**	694902.61ns	41997.816**	
Treatment (T)	3	720587.08ns	1232664*	1429531.7*	3953.9526ns	
C x T	3	296478.68ns	250651.92ns	8056.7492ns	88.851033ns	
Error	24	1920827.5	271218.37	319369.46	3260.3766	

Table 3. Anatomical characteristics of marigold (*Tagates erecta* L.) growing under different water deficit conditions.

	Bonanza Inca					
	Control (100% FC)	**60% FC**	**40% FC**	**Control (100% FC)**	**60% FC**	**40% FC**
Root anatomy						
Endodermal area (μm^2)	8049.1	7270.9	6144.3	545.0	1333.4	617.6
Cortical area (μm^2)	8016.9	7686.9	6691.3	11130.2	9262.1	7919.7
Metaxylem area (μm^2)	5641.2	4198.1	3590.4	7325.3	5626.0	4890.6
Vascular bundle area (μm^2)	43507.1	36041.4	26609.7	54110.4	50669.2	39836.4
Stem anatomy						
Epidermal area (μm^2)	5341.9	4792.8	4253.1	5769.4	5058.3	4228.0
Cortical area (μm^2)	26362.9	24276.7	19738.7	28213.8	24469.5	19873.4
Metaxylem area (μm^2)	8112.9	6989.1	3918.4	9559.5	8045.9	6646.0
Vascular bundle area (μm^2)	41142.9	36031.6	29022.6	47994.7	44481.7	38066.1
Leaf anatomy						
Abaxial epidermal area (μm^2)	5322.9	4009.4	2801.8	6561.9	5042.0	3113.0
Adaxial epidermal area (µm)	3957.5	3603.9	3075.8	4558.4	4232.9	3527.4
Mesophyll area (μm^2)	4030.4	3576.7	2900.6	4354.0	3870.7	3085.3
Leaf thickness (µm)	927.0	858.1	806.1	982.2	923.9	893.7
Vascular bundle area (μm^2)	7990.4	7676.7	6930.1	9425.4	8113.2	7618.4

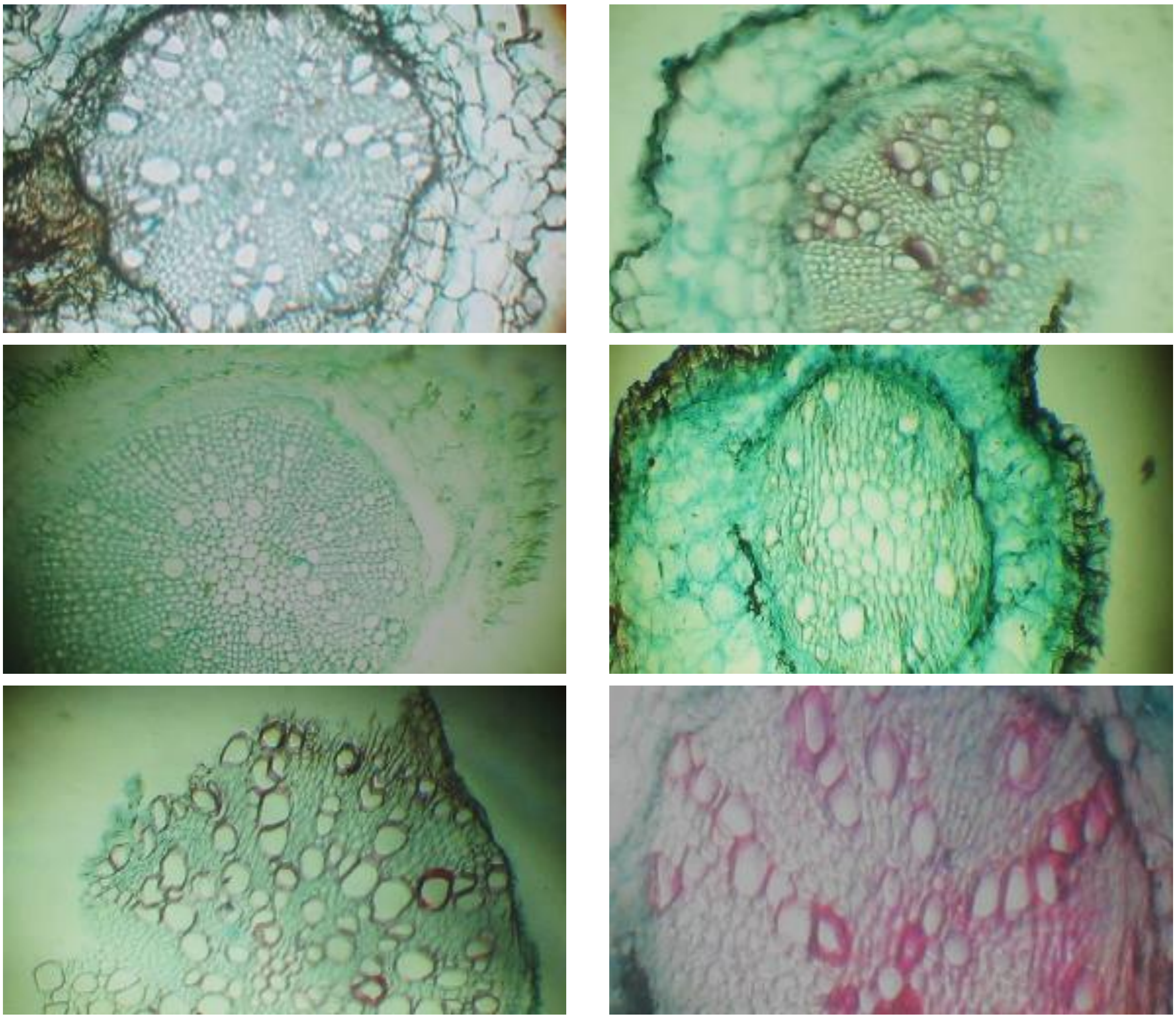

Fig. 3. Root transverse sections of *Tagates* cultivars under different watering regimes.

The performance of both marigold cultivars were unaffected regarding epidermis of shoot under T_2 treatments (Table 1). Epidermis area of *Astragalus gombiformis* (Pomel) shoot increased with increasing levels of irrigation drought intervals as reported by Boughalleb *et al*., (2014). Decrease in epidermal area and vegetative growth can be interlinked with *Tagetes erecta* water stress tolerance. Significant variation in shoot cortex area was observed under different water stress treatments. Difference between both cultivars Inca and Bonanza were significant with respect to cortex area under drought stress. Cortex area of both marigold varieties were found maximum (26507 µm^2) in control (T_0) while it was decreased with the increase in drought stress severity. Cv. Inca showed higher stem cortex area (27540 µm^2) while the cv. Bonanza remained lower in response to T_1 (Fig. 2). Shoot cortex area of cv. Inca was optimum as compared to cv. Bonanza in response to all water stress treatments. Drought stress (T_2) significantly decreased the cortex area of shoot in both cultivars as illustrated in Table 1. Environmental factors especially shortage of water significantly influence the plant anatomical characteristics (Shao *et al*., 2008), and this may have a direct impact on overall growth of plant organs (Shabala, 1996). Shoot meta-xylem area of both marigold cultivars showed different response under drought stress (Table 1). Under 100% FC, cv. Inca showed higher (9230 µm^2) meta-xylem area than cv. Bonanza (7840 µm^2). In response to drought stress, both cultivars showed least meta-xylem area under 60% FC as illustrated in Figure 2. Reduction in meta-xylem cells diameter is linked with reducing embolism risks as well as water flow (Souza *et al.*, 2009). Short distance transport of nutrients and water through stem meta-xylem vessels in annual crops like *T. erecta* may not be as important as in tall perennial and therefore, reduction in meta-xylem area may have a little impact (Vasellati *et al*., 2001). Shoot vascular bundle area was found higher in cv. Inca as compared to cv. Bonanza at normal watering (T0) (Fig. 4). Shoot vascular bundle area generally decreased with the increase in water deficit stress in both marigold cultivars (Table 1). Similar results have been reported by Kutlu *et al*., (2009) about the reduction in vascular area as a result of drought stress. Under well watered conditions, cultivar Inca, subjected to T_0 presented higher upper epidermis region area than the other cultivar Bonanza (Table 1). On exposure to drought stress, leaf upper epidermis area decreased more severely in cv. Bonanza than cv. Inca especially at T_2 (Fig. 5). These results were in agreement with an earlier study on *Nitraria retusa*, a drought resistant plant, in which the upper epidermis, palisade mesophyll thickness, stomata and trichomes density increased under drought stress (Boughalleb *et al*., 2012). Adaxial leaf surface is more exposed directly facing the sun. Thicker adaxial epidermis (along with cuticle) is important for a plant under water shortage conditions to resist environmental stress (Mulroy & Rundel, 1977). However, leaf hairiness and stomatal size, density and orientation are equally important for strict controlling of transpiration rate and stomatal conductance in plants like *T. erecta* (Xu & Zhou, 2008). Leaf lower region epidermal area was increased in cv. Inca, but decreased in cv. Bonanza under water deficit conditions (Fig. 3). Similar results were reported on olive in which comparison of olive cultivars under water stress was observed. Cv. Zalmati produced thinner leaf lower epidermis while, cv. Chemlali was not affected in terms of leaf epidermis area (Boughalleb *et al*., 2012). Leaf is the main photosynthetic organ in *T. erecta*, and protection of this organ from photo-damage is crucial for the survival of plants under unfavorable conditions. Thicker epidermis may play a vital role to minimize water loss through leaf surface (Ristic & Cass, 1991). The marigold plants grown under water-deficit conditions showed a significant reduction in the leaf mesophyll area (Fig. 5) of both marigold cultivars. Of both cultivars, cv. Inca, was comparatively better in mesophyll area at high water stress level (T_2) (Fig. 5). In an earlier study with a drought resistant plant *Nitraria retusa* an increase in upper epidermis area as well as palisade mesophyll thickness was observed under drought stress conditions (Boughalleb *et al*., 2012). Changes due to drought were also observed in leaf thickness (Table 1). Cv. Inca had considerably thicker leaf in comparison with cv. Bonanza under controlled conditions (Table 1; Fig. 5). A significant reduction was observed in leaf thickness of both genotypes of *T. erecta* under water deficit treatments. Succulence or leaf thickness is an important structural modification in plants to cope with dry habitats (Balsamo *et al*., 2006). The length of the palisade parenchyma cells determines leaf thickness, and in this regard, a thicker leaf has more ability to efficiently use water to manage drought conditions (Boeger &Wisniewski, 2002). The results are supported by an earlier study on olive cultivars, therein drought stress decreased the epidermal and mesophyll cells size with increased cell density (Bosabalidisand & Kofidis, 2002). Furthermore, Ennajeh *et al*., (2010) reported that drought resulted in 24% decrease in the leaf area of olive. In the present study, imposition of drought stress treatments significantly reduced the leaf vascular bundle area of both marigold cultivars (Table 1; Fig. 5). Comparisons of values between both marigold cultivars revealed that leaf vascular bundle area was higher in cv. Bonanza than in cv. Inca under water deficit conditions.

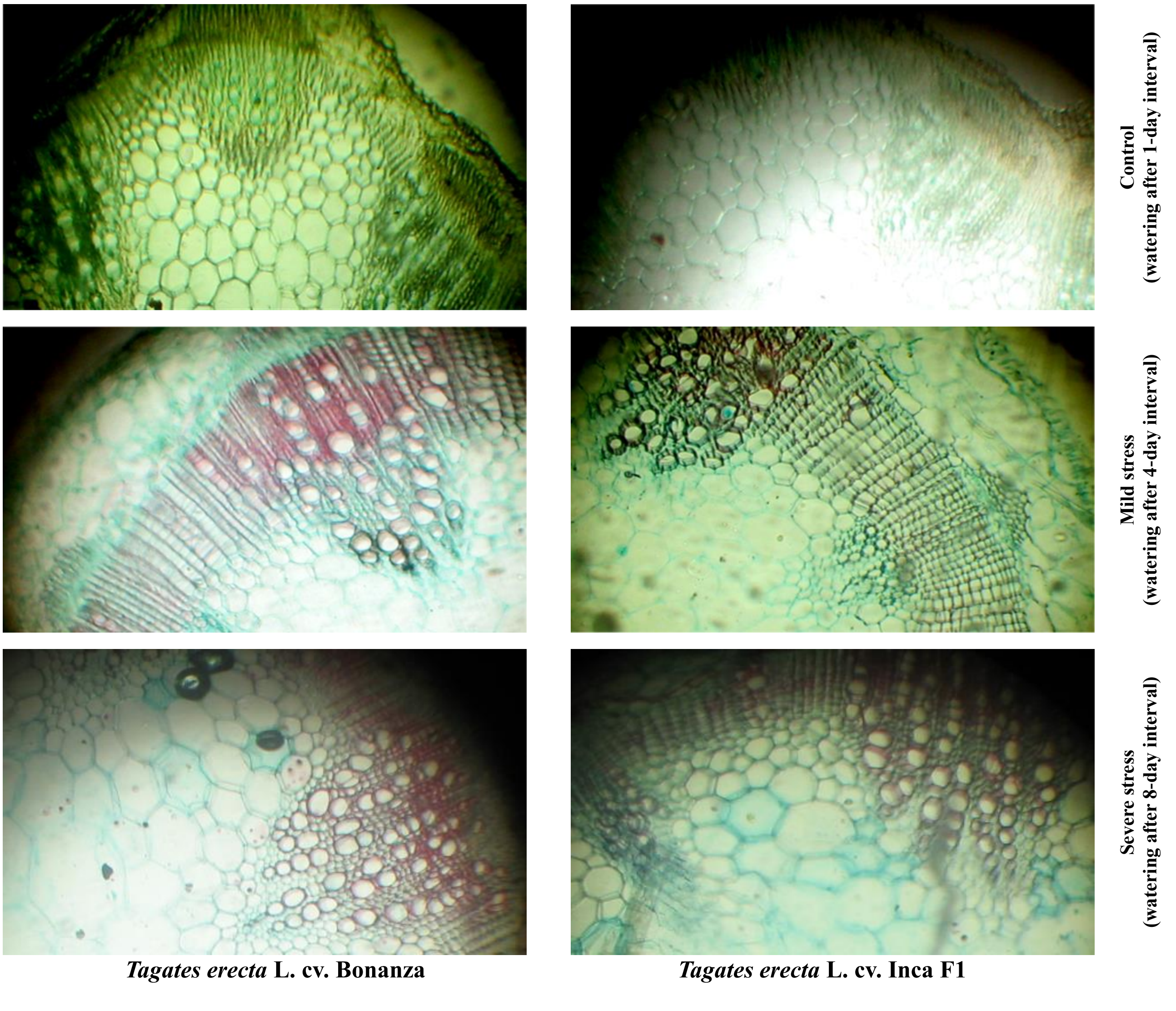

Fig. 4. Stem transverse sections of *Tagates* cultivars under different watering regimes.

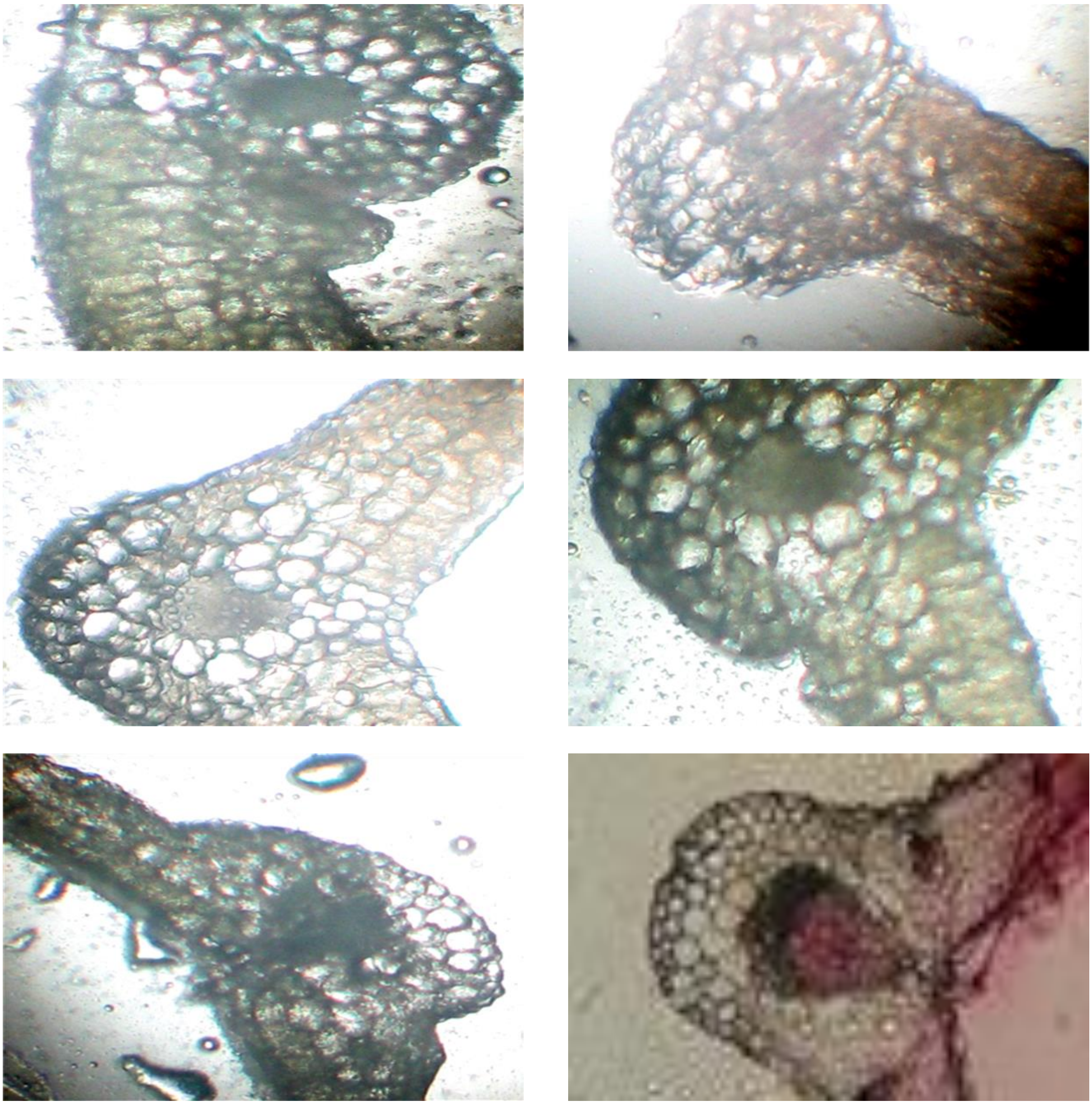

Fig. 5. Leaf transverse sections of *Tagates* cultivars under different watering regimes.

Conclusion

Marigold cultivars exhibited plasticity in growth attributes and anatomical features of root, stem and leaf under different water deficit conditions. Leaf, shoot and root morpho-anatomical adaptations to drought stress levels can explain the difference related to drought tolerance ability of two marigold cultivars. In particular, cv. Inca has better leaf, stem and roots anatomical features to withstand drought stress conditions. However, further studies with more marigold cultivars are needed to confirm these findings.

Acknowledgment

Faisal Zulfiqar gratefully acknowledge Dr. Shawn R. Wright (Department of Horticulture, University of Kentucky, Lexington-, United States) for providing technical comments and improving the language.

References

Abideen, Z., H.W. Koyro, B. Huchzermeyer, R. Ansari, F. Zulfiqar and B. Gul. 2019. Ameliorating effects of biochar on photosynthetic efficiency and antioxidant defense of *Phragmites karka* under drought stress. *Plant Biology*. doi:10.1111/plb.13054.

Ali, Z., M. Ashraf, M.Y. Ashraf, S. Anwar and K. Ahmad. 2020. Physiological response of different accessions of Sesbania sesban and Cyamopsis tetragonoloba under water deficit conditions. *Pak. J. Bot*., 52(2): DOI: http://dx.doi.org/10.30848/PJB2020-2(43)

Akram, N.A., S. Shafiq, M. Ashraf, R. Aisha and M.A. Sajid. 2016. Drought-induced anatomical changes in radish (*Raphanus sativus* L.) leaves supplied with trehalose through different modes. *Arid. Land. Res. Manag*., 30: 412-420.

Alvarez-Flores, R., A. Nguyen-Thi-Truc, S. Peredo-Parada, R. Joffre and T. Winkel. 2018. Rooting plasticity in wild and cultivated Andean Chenopodium species under soil water deficit. *Plant Soil.*, 425: 479-492.

Balsamo, R.A., C.V. Willigen, A.M. Bauer and J. Farrant. 2006. Drought tolerance of selected *Eragrostis* species correlates with leaf tensile properties. *Ann. Bot-London*., 97: 985-991.

Boeger, A.R and C. Wisniewski. 2002. Leaf structure and nutrient contents of six tree species from different successional stages at coastal plain from Parana State, Brazil. *Acta Botanica Brasilica.,* 57: 243-62.

Bosabalidis, A.M and G. Kofidis. 2002. Comparative effects of drought stress on leaf anatomy of two olive cultivars. *Plant Sci.*, 163: 375-379.

Boughalleb, F., H. Hajlaoui and M. Denden. 2012. Effect of salt stress on growth, water relations, solute composition and photosynthetic capacity of hero-halophyte *Nitraria retusa* L. *Environ. Res. J.*, 6: 1-13.

Boughalleb, F., R. Abdellaoui, N. Ben-Brahim and M. Neffati. 2014. Anatomical adaptations of *Astragalus gombiformis* Pomel. under drought stress. *Open Life. Sci.*, 9: 1215-1225.

Casa, R and Y. Rouphael. 2014. Effects of partial root-zone drying irrigation on yield, fruit quality, and water-use efficiency in processing tomato. *J. Hort. Sci. Biotechnol.*, 89: 389-396.

Chaves, M and B. Davies. 2010. Drought effects and water use efficiency: improving crop production in dry environments. *Funct. Plant. Biol.*, 37: 3-6.

Chkhikvishvili, I., T. Sanikidze, N. Gogia, M. Enukidze, M. Machavariani, N. Kipiani, Y. Vinokur and V. Rodov. 2016. Constituents of french marigold (*Tagetes patula* L.) flowers protect jurkat t-cells against oxidative stress. *Oxid. Med. Cell Longev.,* dx.doi.org/10.1155/2016/4216285.

Cicevan, R., M. Al Hassan, A.F. Sestras, J. Prohens, O. Vicente, R.E. Sestras and M. Boscaiu. 2016. Screening for drought tolerance in cultivars of the ornamental genus *Tagetes* (Asteraceae). *Peer J.*, 4: 21-33. doi.org/10.7717/peerj.2133.

Cirillo, C., V. De Micco, Y. Rouphael, A. Balzano, R. Caputo and S. De Pascale. 2017. Morpho-anatomical and physiological traits of two Bougainvillea genotypes trained to two shapes under deficit irrigation. *Trees*, 31: 173-187.

Dole, M and H. Wilkins. 2005. Floriculture: principles and species. *Prentice-Hall. Inc*. Upper Saddle River, USA.

Egea, G., P.A. Nortes, M.M. Gonza´lez-Real, Baille, A and A. Domingo. 2010. Agronomic response and water productivity of almond trees under contrasted deficit irrigation regimes. *Agri. Water. Manag.,* 97: 171-181.

El-Afry, M.M., M.M.F, El-Nady and E. B. Abdelmonteleb. 2012. Anatomical studies on drought-stressed wheat plants (*Triticum aestivum* L.) treated with some bacterial strains. *Acta. Biol. Szeged.*, 56: 165-74.

Ennajeh, M., A.M. Vadel, H. Cochard and H. Khemira. 2010. Comparative impacts of water stress on the leaf anatomy of a drought-resistant and a drought-sensitive olive cultivar. *J. Hort. Sci. Biotechnol.*, 85: 289-294.

Fiaz, M., R. Hussain, M. Amjad, S. Rashid, A. Shahid, F. Zulfiqar, M. Nafees, M.R. Shaheen, L. Ali and S. Ali. 2018. Effect of seed priming on germination, emergence and seedling growth of cocks comb (*Celosia cristata* L.) under different salinity levels. *Int. J. Biosci.*, 12: 180-193.

Guerfel, M., O. Baccouri, D. Boujnah, W. Chaibi and M. Zarrouk. 2009. Impacts of water stress on gas exchange, water relations, chlorophyll content and leaf structure in the two main Tunisian olives (*Olea europaea* L.) cultivars. *Sci. Hort. Amsterdam*., 119: 257-63.

Jones, H., A.D. Tomos, R.A. Leigh and R.G.W. Jones. 1983. Water-relation parameters of epidermal and cortical cells in the primary root of *Triticum aestivum* L. *Planta,* 158: 230-236.

Kutlu, N., R. Terzi, Ç. Tekeli, G. Şenel, P. Battal and A. Kadioğlu. 2009. Changes in anatomical structure and levels of endogenous phytohormones during leaf rolling in *Ctenanthe setosa* under drought stress. *Turk. J. Biol.*, 33: 115-122.

Leukovic, J., I. Maksimovic, L. Zoric, N. Negl, M. Percic, D. Polic and M. Putnic-delic. 2009. Histological characteristics of sugar beet leaves potentially linked to drought tolerance. *Ind. Crop. Prod*., 30: 281-86.

Mahmoud, A.A., A.S.H. Gendy, H.A.H. Said-Al Ahl, D. Grulova, T. Astatkie and T.M. Abdelrazik. 2018. Impacts of harvest time and water stress on the growth and essential oil components of horehound (*Marrubium vulgare*). *Sci. Hortic. Amsterdam*., 232: 139-144.

Makbul, S., N. Suhan Güler, N. Durmuş and S. Güven. 2011. Changes in anatomical and physiological parameters of soybean under drought stress. *Turk. J. Bot*., 35: 369-77.

Mansoor, U., M. Naseer, M. Hameed, A. Riaz, M. Ashraf, A. Younis and F. Ahmad. 2015. Root morpho-anatomical adaptations for drought tolerance in *Cenchrus ciliaris* L. ecotypes from the Cholistan desert. *Phyton-Ann. Rei. Bot.,* 55: 159-179.

Nawazish, S., M. Hameed and S. Naurin. 2006. Leaf anatomical adaptations of *Cenchrus ciliaris* L. from the salt range, Pakistan against drought stress. *Pak. J. Bot*., 38: 1723-1730.

Oliveira, I., A. Meyer, S. Afonso and B. Gonçalves. 2018. Compared leaf anatomy and water relations of commercial and traditional *Prunus dulcis* (Mill.) cultivars under rain-fed conditions. *Sci. Hort. Amsterdam*., 229: 226-232.

Pandey, V., A. Patel and D.D. Patra. 2015. Amelioration of mineral nutrition, productivity, antioxidant activity and aroma profile in marigold (*Tagetes minuta* L.) with organic and chemical fertilization. *Ind. Crop. Prod*., 76: 378-385.

Peña-Valdivia, C., A. Sánchez-Urdaneta, C. Trejo, R.J. Aguirre and S.E. Cárdenas. 2005. Root anatomy of drought sensitive and tolerant maize (*Zea mays* L.) seedlings under different water potentials. *Cereal. Res. Commun*., 33: 705-712.

Peña-Valdivia, C.B and A. B. Sánchez-Urdaneta. 2009. Effects of substrate water potential in root growth of *Agave salmiana* Otto ex Salm-Dyck seedlings. *Biol. Res.*, 42: 239-248.

Puértolas, J., E.K. Larsen, W.J. Davies and I.C. Dodd. 2017. Applying 'drought' to potted plants by maintaining suboptimal soil moisture improves plant water relations. *J. Exp. Bot.*, 68: 2413-2424.

Riaz, A., A. Younis, A.R. Taj, A. Karim, U. Tariq, S. Munir and S. Riaz. 2013. Effect of drought stress on growth and flowering of marigold (*Tagetes erecta* L.). *Pak. J. Bot.*, 45: 123-131.

Riaz, A., U. Tariq, M. Qasim, M.R. Shaheen, A. Iqbal and A. Younis. 2016. Effect of water stress on growth and dry matter partitioning of *Conocarpus erectus*. *Acta. Hort.,* 1112: 163-172.

Ristic, Z and D.D. Cass. 1991. Leaf anatomy of *Zea mays* L. in response to water shortage and high temperature: a comparison of drought-resistant and drought-sensitive lines. *Bot. Gaz.*, 152: 173-185.

Rouphael, Y., M. Cardarelli, G. Colla and E. Rea. 2008. Yield, mineral composition, water relations and water use efficiency of grafted mini-watermelon plants under deficit irrigation. *Hort. Sci.*, 43: 730-736.

Ruzin, S.E., 1999. Plant micro technique and microscopy. New York: *Oxford University Press*, USA.

Shabala, S.N. 1996. Leaf temperature kinetics measure plant adaptation to extreme high temperatures. *Aust. J. Plant Physiol.*, 23: 445-452.

Shao, H.B., L.Y. Chu, C.A. Jaleel and C.X. Zhao. 2008. Water-deficit stress-induced anatomical changes in higher plants. *C. R. Biol*., 331: 215-225.

Singh, A., M.D. Shamim and K.N. Singh. 2013. Genotypic variation in root anatomy, starch accumulation, and protein induction in upland rice (*Oryza sativa*) varieties under water stress. *Agri. Res.*, 2: 24-30.

Snedecor, G.W and W.G. Cochran. 1980. *Statistical methods*. 7th edition, Iowa State University Press, Ames, Iowa.

Souza, T.D., E.D. Castro, F. J. Pereira, S.N. Parentoni and P.C. Magalhães. 2009. Morpho-anatomical characterization of root in recurrent selection cycles for flood tolerance of maize (*Zea mays* L.). *Plant. Soil. Environ*., 55: 504-510.

Steel, R.G.D., J.H. Torrie and D.A. Dickey. 1997. Principles and procedures of statistics. A biometrical approach. *McGraw Hill Co*. New York., 178-182.

Twumasi, P., W. Van Ieperen, E.J. Woltering, A.M.C. Emons, J.H.N. Schel, U. van Meeteren and D. van Marwijk. 2005. Effects of water stress during growth of xylem anatomy, xylem functioning and vase life in three *Zinnia elegans* cultivars. *Acta Hort.*, 669: 303-312.

Vasellati, V., M. Oesterheld, D. Medan and J. Loreti. 2001. Effects of flooding and drought on the anatomy of *Paspalum dilatatum*. *Ann. Bot. London*., 88: 355-360.

Xu, Z and G. Zhou. 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. *J. Exp. Bot.*, 59: 3317-3325.

Yasheshwar., S. Umar, S, M. P. Sharma, W. Khan, S. Ahmad. 2017. Variation in ornamental traits, physiological responses of *Tagetes erecta* L. and *T. patula* L. in relation to antioxidant and metabolic profile under deficit irrigation strategies. *Sci Hortic*., 214: 200-208.

Younis, A., A. Riaz, F. Zulfiqar, A. Akram, N.A. Khan, U. Tariq, M. Nadeem and M. Ahsan. 2016. Quality lady palm (*Rhapis excelsa* L.) production using various growing media. *Int. J. Adv. Agric. Sci.*, 1: 1-9.

Younis, A., A. Riaz, M. Qasim, F. Mansoor, F. Zulfiqar, U. Tariq, M. Ahsan, M.K. Naseem and Z.M. Bhatti. 2018a. Screening of marigold (*Tagetes erecta* L.) cultivars for drought stress based on vegetative and physiological characteristics. *Int. J. Food Allied Sci.*, 3: 56-63.

Younis, A., M.S. Akhtar, A. Riaz, F. Zulfiqar, M. Qasim, A. Farooq, U. Tariq, M. Ahsan and Z.M. Bhatti. 2018b. Improved cut flower and corm production by exogenous moringa leaf extract application on gladiolus cultivars. *Acta. Sci. Pol. Hort*., 17: 25-38.

Zollinger, N., R. Kjelgren, T. Cerny-Koenig, K. Kopp and R. Koenig. 2006. Drought responses of six ornamental herbaceous perennials. *Sci. Hort. Amsterdam.*, 109: 267-274.

Zulfiqar, F., A. Casadesús, H. Brockman, and S. Munné-Bosch. 2019a. An overview of plant-based natural biostimulants for sustainable horticulture with a particular focus on moringa leaf extracts. *Plant Sci*., 110194.

Zulfiqar, F., A. Younis, Z. Abideen, A. Francini, A. Ferrante. 2019c. Bioregulators Can Improve Biomass Production, Photosynthetic Efficiency, and Ornamental Quality of *Gazania rigens* L. *Agronomy*., 9(11): 773.

Zulfiqar, F., M. Navarro, M. Ashraf, N.A Akram and S. Munné-Bosch. 2019b. Nanofertilizer use for sustainable agriculture: Advantages and limitations. *Plant Sci*., 110270.

Zulfiqar, F., N.A. Akram and M. Ashraf. 2020. Osmoprotection in plants under abiotic stresses: new insights into a classical phenomenon. *Planta*., 251(1): 3.

(Received for publication 28 November 2019)