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

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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 (T0; 100% FC) and water deficit conditions (T1; 60 FC and T2; 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 (Chikhikvishvili et al., 2016; Yonis 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 gomphiformis 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 F1 (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\(_1\); 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≤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 T2 and T1 treatments than cv. Inca. The root mass fraction of both cultivars decreased significantly (p≤0.01) with the increase in drought stress levels (Fig. 1; Table 1). In contrast, shoot mass fraction of cv. Inca decreased substantially at T2 (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.

![Graphs showing plant fresh mass, root mass fraction, plant dry mass, and shoot mass fraction of marigold under three water regimes: T0, 100% FC; T1, 60% FC; T2, 40% FC.](image-url)
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 (T2). 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 T2 (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.

![Graph](image-url)
Inca. The vascular area was affected severely in both cultivars under water deficit conditions (T1) (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 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 (Tagetes erecta L.) cultivars subjected to water stress conditions (Mean ± S.E.).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Root endodermal area</th>
<th>Root cortical area</th>
<th>Root metaxylem area</th>
<th>Root vascular bundle area</th>
<th>Leaf vascular bundle area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar (C)</td>
<td>1</td>
<td>9909183.7*</td>
<td>27680148***</td>
<td>15388030**</td>
<td>1.4690***</td>
<td>5299482**</td>
</tr>
<tr>
<td>Treatment (T)</td>
<td>3</td>
<td>720213.32ns</td>
<td>4985275.7*</td>
<td>1332285.7ns</td>
<td>6176175ns</td>
<td>2859846.9*</td>
</tr>
<tr>
<td>C x T</td>
<td>3</td>
<td>689115.1ns</td>
<td>729963.78ns</td>
<td>1286265.4ns</td>
<td>1.08321ns</td>
<td>470212.79ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>1316401.5</td>
<td>1364126.4</td>
<td>1604311.3</td>
<td>54321985</td>
<td>637733.08</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Stem epidermal area</th>
<th>Stem cortical area</th>
<th>Stem metaxylem area</th>
<th>Stem vascular bundle area</th>
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<tr>
<td>Cultivar (C)</td>
<td>1</td>
<td>237793.04ns</td>
<td>21958064ns</td>
<td>33276287**</td>
<td>5.34644**</td>
</tr>
<tr>
<td>Treatment (T)</td>
<td>3</td>
<td>877302.98ns</td>
<td>55064839*</td>
<td>508864.68ns</td>
<td>1.2190**</td>
</tr>
<tr>
<td>C x T</td>
<td>3</td>
<td>202262.67ns</td>
<td>5038016.7ns</td>
<td>723459.23ns</td>
<td>1032290ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>541916.73</td>
<td>13972074</td>
<td>2974677.7</td>
<td>24557684</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>df</th>
<th>Leaf abaxial epidermal area</th>
<th>adaxial epidermal area</th>
<th>Leaf mesophyll area</th>
<th>Leaf thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cultivar (C)</td>
<td>1</td>
<td>5218953.6ns</td>
<td>2372582.6**</td>
<td>694902.61ns</td>
<td>41997.816**</td>
</tr>
<tr>
<td>Treatment (T)</td>
<td>3</td>
<td>720587.08ns</td>
<td>1232664*</td>
<td>1429531.7*</td>
<td>3953.9526ns</td>
</tr>
<tr>
<td>C x T</td>
<td>3</td>
<td>296478.68ns</td>
<td>250651.92ns</td>
<td>8056.7492ns</td>
<td>88.851033ns</td>
</tr>
<tr>
<td>Error</td>
<td>24</td>
<td>1920827.5</td>
<td>271218.37</td>
<td>319369.46</td>
<td>3260.3766</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Bonanza Inca</th>
<th>Control (100% FC)</th>
<th>60% FC</th>
<th>40% FC</th>
<th>Control (100% FC)</th>
<th>60% FC</th>
<th>40% FC</th>
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<tbody>
<tr>
<td>Root anatomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endodermal area (µm²)</td>
<td>8049.1</td>
<td>7270.9</td>
<td>6144.3</td>
<td>545.0</td>
<td>1333.4</td>
<td>617.6</td>
</tr>
<tr>
<td>Cortical area (µm²)</td>
<td>8016.9</td>
<td>7686.9</td>
<td>6691.3</td>
<td>11130.2</td>
<td>9262.1</td>
<td>7919.7</td>
</tr>
<tr>
<td>Metaxylem area (µm²)</td>
<td>5641.2</td>
<td>4198.1</td>
<td>3590.4</td>
<td>7325.3</td>
<td>5626.0</td>
<td>4890.6</td>
</tr>
<tr>
<td>Vascular bundle area (µm²)</td>
<td>43507.1</td>
<td>36041.4</td>
<td>26609.7</td>
<td>54110.4</td>
<td>50669.2</td>
<td>39836.4</td>
</tr>
<tr>
<td>Stem anatomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epidermal area (µm²)</td>
<td>5341.9</td>
<td>4792.8</td>
<td>4253.1</td>
<td>5769.4</td>
<td>5058.3</td>
<td>4228.0</td>
</tr>
<tr>
<td>Cortical area (µm²)</td>
<td>26362.9</td>
<td>24276.7</td>
<td>19738.7</td>
<td>28213.8</td>
<td>24469.5</td>
<td>19873.4</td>
</tr>
<tr>
<td>Metaxylem area (µm²)</td>
<td>8112.9</td>
<td>6989.1</td>
<td>3918.4</td>
<td>9559.5</td>
<td>8045.9</td>
<td>6646.0</td>
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<tr>
<td>Vascular bundle area (µm²)</td>
<td>41142.9</td>
<td>36031.6</td>
<td>29022.6</td>
<td>47994.7</td>
<td>44481.7</td>
<td>38066.1</td>
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<tr>
<td>Leaf anatomy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abaxial epidermal area (µm²)</td>
<td>5322.9</td>
<td>4009.4</td>
<td>2801.8</td>
<td>6561.9</td>
<td>5042.0</td>
<td>3113.0</td>
</tr>
<tr>
<td>Adaxial epidermal area (µm)</td>
<td>3957.5</td>
<td>3603.9</td>
<td>3075.8</td>
<td>4558.4</td>
<td>4232.9</td>
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<tr>
<td>Mesophyll area (µm²)</td>
<td>4030.4</td>
<td>3576.7</td>
<td>2900.6</td>
<td>4354.0</td>
<td>3870.7</td>
<td>3085.3</td>
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<tr>
<td>Leaf thickness (µm)</td>
<td>927.0</td>
<td>858.1</td>
<td>806.1</td>
<td>982.2</td>
<td>923.9</td>
<td>893.7</td>
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<tr>
<td>Vascular bundle area (µm²)</td>
<td>7990.4</td>
<td>7676.7</td>
<td>6930.1</td>
<td>9425.4</td>
<td>8113.2</td>
<td>7618.4</td>
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</table>
The performance of both marigold cultivars were unaffected regarding epidermis of shoot under T2 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 µm²) in control (T0) while it was decreased with the increase in drought stress severity. Cv. Inca showed higher stem cortex area (27540 µm²) while the cv. Bonanza remained lower in response to T1 (Fig. 2). Shoot cortex area of cv. Inca was optimum as compared to cv. Bonanza in response to all water stress treatments. Drought stress (T2) 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 µm²) meta-xylem area than cv. Bonanza (7840 µm²). 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 T0 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 T2 (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

![Tagetes erecta L. cv. Bonanza](image1)

![Tagetes erecta L. cv. Inca F1](image2)

**Fig. 3.** Root transverse sections of *Tagetes* cultivars under different watering regimes.
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 (T2) (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 (Bosabalidis & 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.
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.

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References


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