



EFFECTS OF WATER DEFICIT STRESS ON ROOT AND SHOOT PHYSIOLOGICAL RESPONSES AND BIOMASS PARTITIONING IN KOCHIA (*KOCHIA SCOPARIA* L. SCHRAD)

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Abstract

Kochia (*Kochia scoparia* L. Schrad) is a tolerant plant to both saline and water deficit conditions and have recently considered as forage feed for livestock in saline and arid regions. Understanding the belowground physiological responses and biomass allocation of *Kochia*'s under water deficit conditions may help to realize more potentials of this plant. To achieve such knowledge of root and shoot, a pot experiment was carried out in a completely randomized design with three replications. Nine water deficit stress treatments imposed to plants at the vegetative and reproductive stages: control; NS-NS (no stress= 100% field capacity (FC)) during total growth period, moderate stress (MS=70% FC) during total growth period (MS-MS), severe stress (SS=30% FC) during total growth period (SS-SS), MS-NS, SS-NS, NS-MS, NS-SS, MS-SS, SS-MS. The results indicated that water deficit stress decreased relative water content, membrane stability, fresh and dry weight and increased malondialdehyde, superoxide dismutase, catalase, free amino acids, proline and soluble sugars in root and shoot of *Kochia*. Our results indicate that under water deficit stress, the amount of osmolytes and enzymatic antioxidants increased in root and shoot of *Kochia* to maintain osmotic balance and membrane stability. It is also found that water deficit stress severely decreased biomass of both roots and shoots, but allocation of photosynthates to shoots or roots was similar across all irrigation regimes. Such performance may explain *Kochia scoparia* tolerance to saline and water deficit conditions.

Key words: Drought, enzymatic antioxidants, halophyte, osmolytes, root/shoot ratio.

Introduction

Drought stress is undoubtedly a permanent constraint to agricultural yields worldwide (Slabbert and Krüger, 2014 and Abdal *et al.*, 2018; Sane *et al.*, 2016; Sane 2016). It impacts the growth, morphological, physiological and biochemical parameters and molecular mechanisms in plants (Siddiqui *et al.*, 2016; Hosseinlou *et al.*, 2014; Ardakani *et al.*, 2017). There are many studies detailing physiological and biochemical responses under drought stress. For example, it has been reported that water deficit stress reduce stomatal conductance, chlorophyll content, photosynthesis and transpiration rates, relative water content (RWC) and selective permeability in membrane and disturbed activity of different enzymes in plant tissues. There are also other reports indicating two important mechanisms of osmotic adjustment and antioxidant capacity that increase plant tolerance to water deficit stress (Siddiqui *et al.*, 2016; Jia *et al.*, 2018; Bijan Nejad *et al.*, 2017).

Water deficit enforce oxidative stress by accumulating so much reactive oxygen species (ROS). ROS induce peroxidation of lipid by malone de aldehyde (MDA) production which cause destruction of membrane structure and electrical conductivity. Biomarker of MDA and electrical conductivity are considered as indicators of water deficit tolerance (Masoumi *et al.*, 2010; Li *et al.*, 2015; Shan *et al.*, 2015; Ebrahimzadeh *et al.*, 2018).

Antioxidants benefit the body by neutralizing and removing the free radicals from the bloodstream (Jothilakshmi *et al.*, 2017). Through reducing the risk of major chronic health problems, an important role is played by antioxidants in human health (Hameurlaine *et al.*, 2018). Antioxidants such as phenolic compounds; tocopherols, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone, (TBHQ), propyl gallate (PG), lignans, flavonoids and phenolic acids), ubiquinone (coenzyme Q), carotenoids, ascorbic acids and amino acids can eliminate free radicals (Tahar *et al.*, 2019). There are two

mechanisms of ROS scavenging and disposing in plants; a) an enzymic antioxidants (superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX)), and b) non-enzymic antioxidants (carotenoid (CAR), glutathione (GSH), and ascorbic acid (AsA)). Under water deficit stress, increased activity of antioxidants can sustain dynamic equilibrium and limit membrane damage however water stress severity, duration of water deficit, genotype, and growth stage of the plant determine the antioxidant increasing level (Ghobadi *et al.*, 2013; Slabbert and Krüger, 2014; Li *et al.*, 2015; Siddiqui *et al.*, 2016; Jalalmanesh *et al.*, 2017; Valizad-Hasanlui *et al.*, 2019).

Under water deficit stress condition, osmotic adjustment is another important mechanism for protection by decreasing water potential and to conserve plant cells and subcellular structures such as membrane structure. Water deficit stress induces accumulation of compounds with low molecular mass such as soluble sugars and free amino acids termed compatible osmolyte or osmoregulation substances, in plant cells (Slabbert and Krüger, 2014; Li *et al.*, 2015; Singh and Dar, 2017; Kargarian *et al.*, 2017; Sane *et al.*, 2017). Proline is one of the most common compatible osmolytes /osmoregulations that probably reacts against water deficit stress and allows the plants to survive (Hayat *et al.*, 2012; Singh and Dar, 2017; Jalalmanesh *et al.*, 2017).

Halophytes cover less than 2% of the lands. These plants can be used for different commercial purposes such as vegetables, oilseed, forage, medicinal value, bioremediation, biofuel, ornamental landscaping, environmental protection such as rehabilitate the salt-affected lands and in phytoremediation of polluted soils and wild life support (Panta *et al.*, 2014; Kafi *et al.*, 2014). Hence, halophyte species should be investigated for their tolerance mechanisms and adaptations to abiotic stress to help the huge production of forage and breeding of new crops for dry and saline lands (Panta *et al.*, 2014; Rabiepoor *et al.*, 2017). Among halophytes, *Kochia* (*Kochia scoparia* L. Schrad) has an extended tolerance to salinity and water deficit stress. It can

produce large amount of forage for livestock in saline and arid regions where many other species either fail to establish or do not sustain (Jami Al Ahmadi and Kafi, 2008; Kafi *et al.*, 2010). Kafi *et al.* (2010) reported Kochia forage yield up to 11 ton ha⁻¹. Yet the above- and belowground physiological responses and biomass allocation underlying Kochia's water stress tolerance remain largely unknown. Therefore we have evaluated root and shoot physiological responses and biomass partitioning in Kochia under water deficit stress, in order to analyse responses of these traits to low water availability.

Materials and Methods

Plant Material

This study was carried out at research greenhouse of the faculty of Agriculture, Ferdowsi University, Mashhad (in 59°35' E longitude 36°17' N latitude and 1050 m elevation), Iran, in 2013. The seeds of the Sabzevar genotype were obtained from Mashkan village, Sabzevar, Iran. Five seeds were sown in each pot with 39 cm diameter, 41 cm length and 950 gr weight, filled with 29.05 kg sandy-loamy soil in 5 March 2013. Pots were kept in greenhouse at a day/night temperature regime of 24/13°C, 16-h photoperiod. At first, seeds were irrigated with regular water to ensure a good germination and to achieve good seedling survival. Then, two seedlings remained in each pot. Imposing of treatments was started when plants height were about 10 cm.

The field capacity of soil was determined based on water outflow curve. At first, 5 pots were filled with sandy-loamy soil. The pot weight with soil was 30 kg. The soil of each pot was saturated with water (hence, all micro pores were filled up by water) in such a manner that excess water was drained out from the bottom holes of pot. The pot was covered with plastic sheet to prevent evaporation and to allow downward redistribution of water. Their weight was recorded every 8 hours. When the water outflow curve was fixed, the pots were reweighted and net weight of moist soil or the amount of water held by soil at FC was obtained. Afterwards, MS (70% FC) and SS (30% FC) were calculated based on the percentage of field capacity.

Experimental Design

The pot experiment was carried out as a completely randomized design with three replicates. Nine water deficit stress treatments were imposed to the plants at the vegetative and reproductive stages, control; no stress (NS-NS=100% field capacity (FC)) during total growth period, moderate stress (MS=70% FC) during the vegetative phase (MS-NS), severe stress (SS=30% FC) during the vegetative phase (SS-NS), moderate stress during the reproductive phase (NS-MS), severe stress during the reproductive phase (NS-SS), moderate stress during the vegetative phase and severe stress during the reproductive phase (MS-SS), severe stress during the vegetative phase and moderate stress during the reproductive phase (SS-MS), moderate stress during total growth period (MS-MS) and severe stress during total growth period (SS-SS).

Each treatment consisted of 8 pots (7 for sampling during the growth season, a pot for measurements at the beginning of anthesis (forage harvest)) per replicate. Total number of pots was 216. Imposing water stress in the vegetative stage was when the plants were about 10 cm of plant height till the beginning of inflorescences formation on

the main stem. Imposing water stress in the reproductive stage was from the beginning of inflorescences formation on the main stem to beginning of anthesis (forage harvest).

Measurements

All measurements were carried out at the beginning of anthesis. Leaf samples randomly were taken from youngest fully expanded leaves. The pots were submerged in water and the roots were gently washed under water. Finally, roots were cleaned with a soft paintbrush to remove adhering soil particles. Then roots were cut by 2 cm of the root tip so that the remaining segment was mature as root samples.

Relative water content (RWC): Leaves and roots fresh weight (FW) were measured after harvest. Leaves and roots were placed in deionized water for 24 hours at room temperature and they were weighed again for turgid weight (TW). Afterwards to determine their dry weight (DW), they were dried in oven at 80°C for a period of 24 hours. According to Dichio *et al.* (2009), RWC was calculated as follow:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

Membrane stability index (MSI): Leaves and roots were placed in closed tubes containing 20 ml of deionized water and incubated at room temperature for 24 h. The initial leakage (L₀) was measured by conductivity meter (JENWAY4510). Then samples boiled at 121°C for 20 min. The leakage (LD) was noted after equilibration at room temperature. The injury index (ID) and MSI were calculated (Arasimowicz-Jelonek *et al.*, 2009; Zarezadeh *et al.*, 2018) as follow:

$$ID = \frac{LD - L_0}{100 - L_0} \times 100$$

$$MSI = 1 - ID$$

For all biochemical analyses, leaf and root samples were immediately frozen into liquid nitrogen and stored in the refrigerator at -80°C temperature until analyses. Assessment of malondialdehyde (MDA) content, 2-thiobarbitoric acid (TBA) reaction was based on the method of Hodges *et al.* (1999). The activity of SOD (EC 1.15.1.1) was determined as described by Yu and Rengel (1995), CAT (EC 1.11.1.6) according to Velikova *et al.* (2000) and POD (EC 1.11.1.7) was assayed based on Srinivas *et al.* (1999). Free amino acids were determined by the method of Yemm *et al.* (1955) and assessment of free-proline concentration was performed by the method of Bates *et al.* (1973). Soluble sugars content was also measured using the phenol-sulfuric acid as described by Dubois *et al.* (1956). Total phenolic compounds were assessed by using the Folin-Ciocalteu phenol reagent method (Singleton and Rossi, 1965).

Harvested plants were separated into leaves, stems, and roots. The fresh weight of the organs were recorded then dried in an oven at 75°C for 72 hours and then dry weight and was measured.

Statistical Analysis

The data was statistically analyzed by SAS software (version 9.1) and the means were compared between treatments by LSD test ($P \leq 0.05$).

Results and Discussion

Relative Water Content (RWC)

The results of this experiment showed that both leaf and root RWC were significantly affected by deficit irrigation ($P \leq 0.01$) (Table 1). The highest leaf and root RWC was observed in control. Imposing moderate and severe water deficit stresses in vegetative stage (recovered plants) did not result a significant difference compared to control. This might be due to the leaf and root fast RWC recovery after re-watering in reproductive stage. The reduction of leaf and root RWC was increased with increasing the intensity of water deficit in reproductive stage (Table 2). The lowest leaf and root RWC was observed when severe stress imposed for the total growth period. However, there is no significant difference between MS-SS and N-SS compared with MS-MS.

It has previously been reported that water deficit stress diminishes leaf RWC in halophytes (Masoumi *et al.*, 2010; Siddiqui *et al.*, 2016). Water stress causes water loss of the plant and therefore reduction of its RWC (Sanchez-Rodriguez *et al.*, 2010). Decline in RWC is related to cell membrane properties and its adaptability to environmental changes such as drought (Farooq *et al.*, 2009; Siddiqui *et al.*, 2016). The degree of cell and tissue hydration is key indicator of relative water content, which is crucial for optimum physiological functioning and growth processes (Qayyum *et al.*, 2017). Munne-Bosch *et al.* (2003) revealed that 80% RWC value indicates a good plant water status whereas, RWC of 66-68% and RWC less than 50% reflect plant under moderately and severe water stress, respectively. Even though the reduction in leaf and root RWC was recorded in Kochia under drought stress, but still the plant was able to maintain its adequate water potential and recovered after the stress treatment which indicate some adaptive traits and tolerance mechanisms that protect the crop under stressful conditions.

Membrane stability index (MSI) and malondialdehyde (MDA)

Stress treatments resulted in significant difference in MSI ($P \leq 0.01$) (Table 1). The results show that MSI of leaf and root tissues only decreased under SS in reproductive stage (Table 2), which indicates the increasing of membrane injury and a serious loss in membrane stability in plants. Leaf MSI values in NS-SS, MS-SS and SS-SS was 21.03, 21.69 and 18.68% respectively, lower than the control. Root MSI values in NS-SS, MS-SS and SS-SS were also 17.68, 19.41 and 16.89% respectively lower than the control (Table 2). Wang and Huang (2004) reported that MSI of Kentucky blue grass exposed to drought and heat stresses was declined because of increased electrolyte leakage. Masoumi *et al.* (2010) also reported that the electrolyte leakage of Kochia leaves increased by 50% under severe stress treatment (no irrigation at reproductive stage for one month) compared with control. Biological membranes are the first target of many abiotic stresses. Water deficit can result in oxidative stress by accumulating so much reactive oxygen species (ROS) (Li *et al.*, 2015). ROS produces certain compounds, such as malondialdehyde and ethylene, which leads to reduction of cell membrane stability (Farooq *et al.*, 2009). MDA, as the final product of membranous lipid peroxidation, causes destruction of membrane structure and electrical conductivity (Ghobadi *et al.*, 2013). MSI and MDA are

physiological indicators widely used for the assessment of drought resistance (Farooq *et al.*, 2009).

Water deficit stress significantly ($P \leq 0.01$) resulted in different MDA in leaf and root (Table 1). In this study, it was observed that only, leaf and root MDA content was significantly increased applying treatments of SS in reproductive stage (NS-SS, MS-SS and SS-SS) ($P \leq 0.01$). Moreover, the highest MDA content observed in roots under treatments of SS in reproductive stage, as their membrane structure was injured by water deficit (Table 2). In this study it is found that there was no change in root and shoot MDA content under moderate stress compared to control (Table 2). These results suggest that MDA content maintained by increasing of osmolytes and enzymatic antioxidants of root and shoot of Kochia (Tables 2 and 4). MDA content recovered quickly after rehydration in plants which experienced severe stress in vegetative stage, it seems that membrane lipid peroxidation and injury to root and leaf were not occurred (Table 2). Slabbert and Krüger (2014) reported that osmolytes and anti-oxidative enzymes may play a protective role in decreasing the damage to the cell membranes by stabilizing cellular structures or modification of cell wall proteome.

Activity of the antioxidant enzymes

The variance analysis showed that SOD activity was significantly ($P \leq 0.01$) increased by water deficit stress in Kochia (Table 1). Leaf and root SOD activity (Table 2) increased respectively in MS and SS in reproductive stage compared to control. The most increase in SOD activity was obtained in MS-SS treatment (Table 2). Leaf and root SOD values under this treatment increased 61.2 and 59.7% more than the control, respectively (Table 2). However, there were no-significant differences among NS-SS and SS-SS compared with MS-SS. Under NS-MS treatment, leaf SOD by 26.6% and root SOD by 31.9% increased compared to control. However, there were no significant difference with NS-MS in MS-MS and SS-MS. It seems that moderate and severe water deficit stress promoted the increase of SOD activity, and relieving the damage caused by water deficit. There are many studies which have reported higher SOD activity in several crops under drought stress (Masoumi *et al.*, 2010; Ghobadi *et al.*, 2013; Slabbert and Krüger, 2014; Li *et al.*, 2015; Siddiqui *et al.*, 2016). Plants under water deficit stress are affected by secondary damages caused by oxidative stress. ROS detoxification in all plants can be categorized as enzymatic and non-enzymatic antioxidants. POD, SOD and CAT are three of the key antioxidant enzymes in plant scavenging system (Ghobadi *et al.*, 2013; Li *et al.*, 2015; Siddiqui *et al.*, 2016; Mahmoodzadeh *et al.*, 2017). An increased SOD activity by water deficit stress is considered to antagonize harmful actions of superoxide radicals and this indicates that higher activities of SOD are important for water deficit resistance (Qayyum *et al.*, 2017).

Water deficit significantly ($p \leq 0.01$) increased CAT activity in leaf and root of Kochia (Table 1). CAT values of leaf and root in MS-MS increased 82% and 77.3% more than control, respectively (Table 2). However, there were no-significant differences among NS-MS and SS-MS in comparison with MS-MS. Leaf and root CAT activity increased by 206% and 202%, respectively in SS-SS treatment compared with control (Table 2). However there are no-significant differences among NS-SS and SS-SS

compared with SS-SS. The lowest CAT activity was observed in control plants. However, recovered plants (MS-NS and SS-NS) did not show any significant effect of CAT activity compared with control (Table 2). The results of this study are in agreement with other investigations (Masumi *et al.*, 2010; Shan *et al.*, 2015; Siddiqui *et al.*, 2016). Shan *et al.* (2015) reported that increased CAT activity of *Rumeria soongorica* seedling root cells prevented the harmful effects of water deficit stress. CAT effectively scavenges poisonous H_2O_2 which will cause membrane lipid peroxidation by transforming it to H_2O and O_2 (Li *et al.*, 2015; Siddiqui *et al.*, 2016; Mohammadi *et al.*, 2018). Therefore, CAT plays a key role in combating water deficit stress and maintaining substantial plant growth rate under stress (Siddiqui *et al.*, 2016).

In contrast to CAT and SOD, POD activity was not significantly changed by water deficit treatments in both leaves and roots (Tables 1 and 2). This result is in agreement with findings of Masumi *et al.*, (2010) that POD activity did not significantly differ between treatments in leaves of *Kochia* under water deficit stress.

We found that SOD and CAT activities were significantly increased in moderate water deficit stress. Therefore, both of them were able to control MDA increase. It indicated that the antioxidant enzymes were activated under water deficit stress to enhance the adaptation of the plants to such conditions. The activities of SOD and CAT increased under severe water deficit stress, but it was not sufficient to scavenge free radicals in the plants; therefore, lipid peroxidation and the damage of cell membrane was not prevented (Table 2). Taken together, we found that highest activity of the antioxidant enzymes was observed in roots, because the highest MDA content was in this tissue under water deficit stress.

Osmolytes

Leaf, stem and root free amino acids (FAA) content were significantly ($P \leq 0.01$) influenced by water deficit stress and FAA of leaf was higher than the other tissues (Table 3). The results showed that water deficit stress induced a greater increase in FAA content under MS and SS treatments in reproductive stage than the control (Table 4). The extent of these changes was related to the intensity of the stress. For example, leaf, stem and root FAA content in plants subjected to MS-MS was 18.1%, 25.1% and 22.35% more than the control, respectively. SS-SS increased leaf, stem and root FAA content by 47.1%, 74.9% and 66.7% compared to the control, respectively. The lowest FAA content in tissues was observed in the control. Moreover, no significant difference was seen in MS-NS and SS-NS compared with control (Table 4). The results indicate that FAA content was significantly increased under water deficit stress imposed during reproductive stage. This may be the result of synthesis and/or protein degradation (Sing and Dar, 2017). According to Slabbert and Krüger (2014) stomata apertures are closed during water stress, photosynthetic rate declines while respiration rate is increased so as to provide some hydrolysate which is prerequisite for raising the osmotic potential, thus increasing cell turgor and eventually growth resumes once more after re-watering. The results of this study are in agreement with other investigations (Medeiros *et al.*, 2012; Slabbert and Krüger 2014; Sing and Dar 2017). Major roles of FAA accumulation during stress is most likely

in osmotic adjustment (initial physiological response of plant in water deficit stress) and osmoprotectants (Sing and Dar, 2017). FAA protects folded protein structures against denaturation, stabilizes cell membranes by interacting with phospholipids, contribution to osmotic adjustment and resistance of plants exposed to unfavorable environmental conditions (Shan *et al.*, 2015; Sing and Dar, 2017; Makhdoomi *et al.*, 2017).

The proline accumulation in the leaves, stems and roots was similar, increasing with low water availability, but the highest free proline content observed in roots (Table 4).

The lowest free proline content in tissues observed in the control. Moreover, no significant difference was seen in MS-NS and SS-NS compared with control (Table 4). Recovery after re-watering was fast in MS-NS and SS-NS and free proline levels reduced when the RWC increased (Table 2). We found significant differences in the effect of MS and SS on the amount of free proline accumulated. For example, leaf, stem and root free proline content in plants subjected to MS-MS was 212.1%, 204% and 172.91% more than the control, respectively. Also SS-SS increased leaf, stem and root free proline content by 448.5%, 488% and 377.1% compared to the control, respectively. Plants accumulate proline by increasing synthesis or reducing catabolism under abiotic stress (Qamar *et al.*, 2015). A greater accumulation of proline in response to water deficit stress is well documented in many plants and maintains homeostasis in leaf (Rhizopoulou *et al.*, 1990; Kumar *et al.*, 2011; Slabbert and Krüger, 2014; Siddiqui *et al.*, 2016; Sing and Dar, 2017; Kargarian *et al.*, 2017), stem (Rhizopoulou *et al.*, 1990; Sing and Dar, 2017) and root (Rhizopoulou *et al.*, 1990; Medeiros *et al.*, 2012; Shan *et al.*, 2015; Sing and Dar, 2017). Besides acting as an excellent osmolyte, proline plays three major roles during stress, i.e., as a metal chelator, an antioxidative defense molecule and a signaling molecule, which results in substantial reduction in ROS activity (Hayat *et al.*, 2012). Free proline may also act as a storage compound for both carbon and nitrogen during water deficit stress when both protein and starch synthesis are inhibited. Such a storage compound may be used for growth upon re-watering, and then the increased level of proline rapidly reduces (Slabbert and Krüger, 2014).

Leaf, stem and root of *Kochia* subjected to water deficit stress showed an increase in solute sugar content that was significant ($P \leq 0.01$) compared to control (Table 3). The results indicate that water deficit stress induced a greater increase in solute sugar content under MS and SS treatments in reproductive stage than control. The highest solute sugar content was in leaves (Table 4). For instance, leaf, stem and root solute sugar content in plants subjected to MS-MS was 20.8%, 66.4% and 52.1% more than control, respectively. SS-SS increased leaf, stem and root solute sugar content by 68.5%, 78.5% and 90.6% compared to the control, respectively (Table 4). These findings are consistent with previous studies that have demonstrated increasing soluble sugar content in different plant tissues under water deficit stress (Medeiros *et al.*, 2012; Singh and Dar, 2017). Under water deficit stress, accumulation of soluble carbohydrate content is involved in a signal transduction pathway and it is a super marker for selecting for water deficit resistance (Qayyum *et al.*, 2017). The increases in solute sugar content may occur in the beginning of the stress period either as a result of growth cease or by water deficit intensity because of

starch degradation (Medeiros *et al.*, 2012; Kazemi *et al.*, 2017).

The present study showed that the accumulation of osmolytes in response to water deficit stress has a key role in osmotic adjustment. This mechanism prevents loss of water in plants by reducing the water potential and protects the cellular membrane and the various metabolic processes. Under stress and without any stress, the highest solute sugar content and FAA was in leaves and the highest proline observed in roots.

Total phenolic compounds

It is found that there was no significant effect of water deficit stress on total phenolic compounds of *Kochia* (Tables 3 and 4). This result concurs with the findings of Puente-Garza *et al.* (2017) that reported total phenolic compounds did not significantly differ between treatments in leaves *Agave salmiana* under water deficit stress.

Shoot and root fresh and dry weight

There were statistically significant ($P \leq 0.01$) differences among water deficit treatments for shoot and root fresh and dry weight (Table 3). These parameters displayed a reduction in response to the increasing water deficit treatments (Fig. 1 and 2). The highest and lowest shoot and root fresh and dry weight observed at control and SS-SS, respectively (Fig. 1 and 2). Under SS-SS, shoot and root fresh weight decreased by 51.2% (Fig. 1(A)) and 50 % (Fig. 2(A)), respectively, in comparison to the control. On the other hand, shoot and root dry weight of SS-SS treated plants displayed 51.1% (Fig. 1(B)) and 50.5 % (Fig. 2(B)) reduction compared to control plants, respectively. It is well known that water deficit conditions significantly reduces shoot and root fresh and dry weight of many plant species (Li *et al.*, 2010; Parida and Jha, 2013; Siddiqui *et al.*, 2016; Jia *et al.*, 2018). Kafi *et al.* (2014) reported that *Kochia* produced 90% biomass when was irrigated by 25% water less than optimum water application. Root as the initial part to be affected by soil water deficit may have influenced stomatal responses by chemical signals such as abscisic acid (Shan *et al.*, 2015). Therefore, CO₂ assimilation by leaves would be decreased mainly by stomatal closure, membrane damage and disturbed activity of various enzymes, particularly those of CO₂ fixation and adenosine triphosphate synthesis. Increased metabolite flux through the photo-respiratory pathway increases the oxidative load on the tissues as both processes generate reactive oxygen species. Injury caused by ROS to biological macromolecules under water stress is among the major deterrents to growth (Farooq *et al.*, 2009). A large number of ATP molecules are exhausted in the synthesis of osmolytes and enzymatic antioxidants. Thus, their biosynthesis which is energy demand (Singh and Dar, 2017)

decreased fresh and dry weight of root and shoot (Fig. 1 and 2). On the other hand, Leaf and stem are main outlet of water via transpiration (Li *et al.*, 2008). Thus, plants would invest less to these two parts to decrease the water loss under water deficit so fresh and dry weight shoot would be reduced significantly. When the shoot growth decreases, the tap root would be inhibited and its diameter reduces, so root biomass would be low (Li *et al.*, 2010; Shan *et al.*, 2015; Saberi *et al.*, 2018). In general, our study found that water deficit stress severely decreased biomass of both roots and shoots.

Allocation of photosynthates to shoots or roots was similar between different irrigation regimes (Fig. 3). Similar ratios indicate that plants have established a balance between transpiring area and absorbing mass. Following the functional equilibrium theory, plants under water deficit stress should increase root biomass allocation. Whereas shoot growth is well known to be early and strongly reduced by water deficit (Parent *et al.*, 2009; Tisné *et al.*, 2010; Cai *et al.*, 2017; Massah *et al.*, 2017). However, in all cases, root growth seems to be less affected than shoot growth, which could lead to an increased root/shoot ratio. This is observed in many cases (Lei *et al.*, 2006; Padilla *et al.*, 2009; Erice *et al.*, 2010), but not in all the cases. Some studies report a constancy of the root/shoot ratio under water deficit conditions (Osorio *et al.*, 1998; Heilmeyer *et al.*, 2001; Nasiri *et al.*, 2019). This absence of consensus on the root/shoot ratio could be explained by the equal importance of shoot and root growth maintenance for *Kochia* to maintain water and mineral uptake by conserving root growth, and to maintain photosynthesis and biomass production at the shoot level.

Conclusions

Our results showed that, water deficit stress decreased leaf and root RWC, shoot and root fresh and dry weight and increased leaf and root MDA, SOD and CAT, leaf, stem and root FAA, free proline and soluble sugars. Results showed no significant effect of water deficit stress on POD and total phenolic compounds. Under both water deficit stress and without any stress, the highest solute sugar content and FAA was obtained in leaves and the highest proline, enzymatic antioxidants (SOD, CAT and POD) and total phenolic compounds were observed in roots. It appears from this study that under water deficit stress, root and shoot of *Kochia* undergo key physiological changes via increasing the amount of osmolytes and enzymatic antioxidants that help *Kochia* to maintain osmotic balance and membrane stability respectively. We also found that water deficit stress decreased biomass of both shoot and root severely, but allocation of photosynthates to shoots or roots was similar between different irrigation regimes. However, all change may be the important reason why *Kochia scoparia* can withstand arid and semi-arid regions.

Table 1: Analysis of variance (mean square) of the effect of the drought on relative water content (RWC), membrane stability index (MSI), malondialdehyde (MDA), super oxide dismutase (SOD), catalase (CAT) and peroxidase (POD) activity in leaf and root of *Kochia**

Source of variance	df	Leaf RWC	Root RWC	Leaf MSI	Root MSI	Leaf MDA	Root MDA	Leaf SOD	Root SOD	Leaf CAT	Root CAT	Leaf POD	Root POD
Drought	8	117.77 **	118.72 **	221.31 **	166.97 **	114.60 **	117.78 **	2825.47 **	2911.43 **	121753.12 **	203854.74 **	64.17 ^{ns}	68.55 ^{ns}
Error	18	0.76	1.37	1.49	1.16	0.66	0.65	1.41	0.81	1.30	1.08	1.5	1.84
Total	26												

*ns and ** are no-Significant and Significant at $p \leq 0.05$, respectively.

Table 2: Mean comparison of the effects of drought stress on relative water content (RWC), membrane stability index (MSI), malondialdehyde (MDA), super oxidase (SOD), catalase (CAT) and peroxidase (POD) activity in leaf and root of Kochia*

Traits	Parts of plant	Drought stress treatments								
		control (no stress, NS-NS)	moderate stress during vegetative stage (MS-NS)	serious stress during vegetative stage (SS-NS)	moderate stress during reproductive stage (NS-MS)	serious stress during reproductive stage (NS-SS)	moderate stress during vegetative + serious stress during reproductive (MS-SS)	serious stress during vegetative + moderate stress during reproductive (SS-MS)	moderate stress during total growth period (MS-MS)	serious stress during total growth period (SS-SS)
RWC (%)	Leaf	87 a	85.70 a	84.40 a	81.20 b	71.06 c	70.56 c	78.16 b	79.03 b	70.33 c
	Root	83.22 a	81.12 a	81.03 a	78.87 b	69.64 c	69.38 c	77.97 b	76.70 b	69.04 c
MSI (%)	Leaf	86.86 a	86.10 a	86.67 a	82.51 a	68.95 b	68.02 b	83.25 a	86.35 a	70.63 b
	Root	88.27 a	87.06 a	87.86 a	85.90 a	72.66 b	71.13 b	86.06 a	87.63 a	73.36 b
MDA ($\mu\text{mol.g}^{-1}\text{fw}$)	Leaf	37.05 b	38.76 b	38.43 b	38.78 b	52.28 a	50.51 a	38.70 b	37.16 b	51.14 a
	Root	40.14 b	42.06 b	41.89 b	43.28 b	54.84 a	53.61 a	43.93 b	41.72 b	55.04 a
SOD ($\text{unit.g}^{-1}\text{fw}$)	Leaf	148.97 c	145.68 c	143.23 c	188.55 b	238.17 a	240.12 a	182.15 b	181.21 b	237.78a
	Root	172.39 c	170.34 c	176.37 c	227.03 b	270.21 a	274.75 a	221.26 b	225.44 b	272.41a
CAT ($\mu\text{mol.g}^{-1}\cdot\text{min}^{-1}\text{fw}$)	Leaf	203.52 c	198.78 c	195.46 c	361.73 b	624.22 a	639.89 a	367.52 b	370.25 b	622.32a
	Root	236.17 c	232.19 c	221.72 c	418.21 b	711.16 a	708.34 a	421.23 b	418.63 b	712.41
POD ($\text{unit.g}^{-1}\text{fw}$)	Leaf	18.11 a	18.98 a	18.42 a	19.79 a	21.37 a	20.11 a	20.46 a	19.26 a	20.44 a
	Root	22 a	22.87 a	23.31 a	23.81 a	22.29 a	23.25 a	23.38 a	21.51 a	22.32 a

*Mean comparisons was done by LSD test at $p \leq 0.05$.**Table 3:** Analysis of variance (mean square) of the effect of the drought on free amino acid, free proline, soluble sugars, total phenols, fresh and dry weight in shoot and root of Kochia*

Source of variance	df	Free amino acids			Free Proline			Soluble Sugars			Total Phenols			Shoot Fresh Weight	Shoot Dry Weight	Root Fresh Weight	Root Dry Weight
		Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root				
Drought	8	1340.53**	1299.12**	1300.84**	1.60**	1.52**	1.77**	523.09**	228.65**	299.67**	1.77 _{ns}	1.70 _{ns}	1.71 _{ns}	1385.43**	137.66**	93.62 **	3.59 **
Error	18	20.15	11.44	19.63	0.02	0.02	0.01	0.56	0.27	0.21	0.01	0.01	0.02	38.45	3.65	2.51	0.10
Total	26																

*ns and ** are no-Significant and Significant at $p \leq 0.01$, respectively.**Table 4:** Mean comparison of the effects of drought stress on free amino acid, free proline, soluble sugar and total phenols in leaf, stem and root of Kochia*

Traits	Part of plant	Drought stress treatments								
		control (no stress, NS-NS)	moderate stress during vegetative stage (MS-NS)	serious stress during vegetative stage (SS-NS)	moderate stress during reproductive stage (NS-MS)	serious stress during reproductive stage (NS-SS)	moderate stress during vegetative + serious stress during reproductive (MS-SS)	serious stress during vegetative + moderate stress during reproductive (SS-MS)	moderate stress during total growth period (MS-MS)	serious stress during total growth period (SS-SS)
Free amino acids (mg.gfw)	Leaf	97.66 c	98.66 c	100 c	116 b	142.66 a	140.66 a	117.66 b	115.33 b	143.67 a
	Stem	73 c	79 c	79.33 c	87 b	122.33 a	120.33 a	97.33 b	91.33 b	127.66 a
	Root	82 c	88 c	88.33 c	96 b	131.33 a	129.33 a	106.33 b	100.33 b	136.66 a
Free Proline (mg.gfw)	Leaf	0.33 c	0.35 c	0.42 c	0.63 b	1.61 a	1.56 a	0.68 b	0.70 b	1.48 a
	Stem	0.25 c	0.29 c	0.35 c	0.55 b	1.33 a	1.29 a	0.57 b	0.51 b	1.22 a
	Root	0.48 c	0.53 c	0.61 c	0.76 b	1.93 a	1.87 a	0.78 b	0.83 b	1.81 a
Soluble Sugars (mg.gfw)	Leaf	43.33 c	43.67 c	44 c	51.66 b	72.81 a	71.67 a	52.67 b	52.33 b	73 a
	Stem	18.63c	19.43 c	19.38 c	28.66 b	39.31 a	39.84 a	29.13 b	31 b	40 a
	Root	25 c	26.21 c	25.33 c	37.69 b	48.56 a	49.83 a	36.83 b	38.02 b	47.66 a
Total Phenols (mg galic.g ⁻¹ fw)	Leaf	9.81 a	10.03 a	10 a	10.38 a	10.91 a	9.64 a	10.55 a	10.78 a	9.93 a
	Stem	6.07 a	5.50 a	5.48 a	5.83 a	6.37 a	5.16 a	6.05 a	6.29 a	5.97 a
	Root	22.04 a	21.41 a	21.55 a	21.80 a	22.31 a	21.06 a	21.49 a	22.21 a	21.83 a

*Mean comparisons was done by LSD test at $p \leq 0.05$.

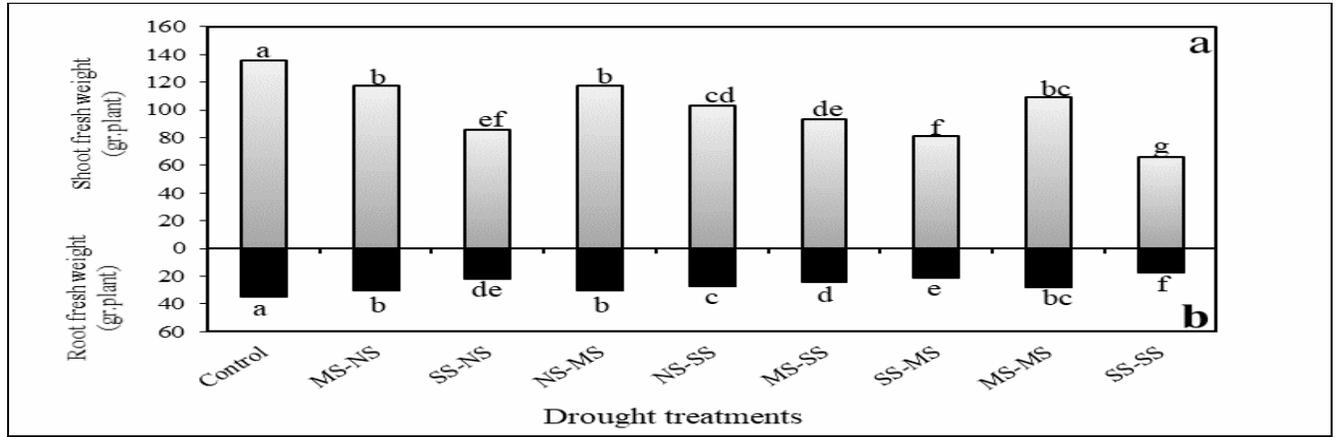


Fig. 1: Shoot (a) and root fresh weight (b) of Kochia under drought stress. No Stress (NS=100% field capacity), Moderate stress (MS=70% field capacity), Severe stress (SS=30% of field capacity). The first abbreviation of the bottom is related to the drought stress during the vegetative stage and the second abbreviation after the interval line represents drought stress during the reproductive stage. Means followed by similar letter(s) are not significantly different at $p \leq 0.05$ by using LSD test.

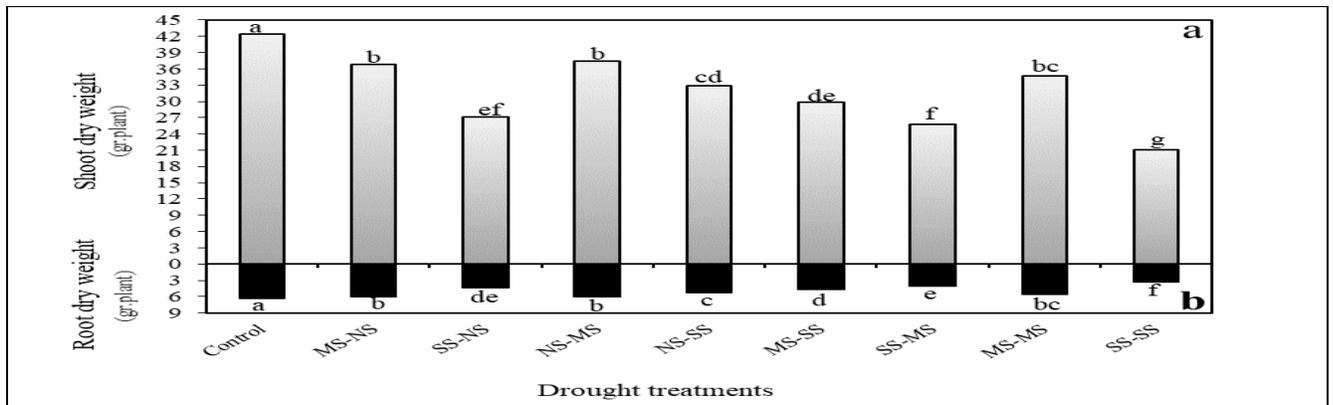


Fig. 2: Shoot (a) and root dry weight (b) of Kochia under drought stress. No Stress (NS=100% field capacity), Moderate stress (MS=70% field capacity), Severe stress (SS=30% of field capacity). The first abbreviation of the bottom is related to the drought stress during the vegetative stage and the second abbreviation after the interval line represents drought stress during the reproductive stage. Means followed by similar letter(s) are not significantly different at $p \leq 0.05$ by using LSD test.

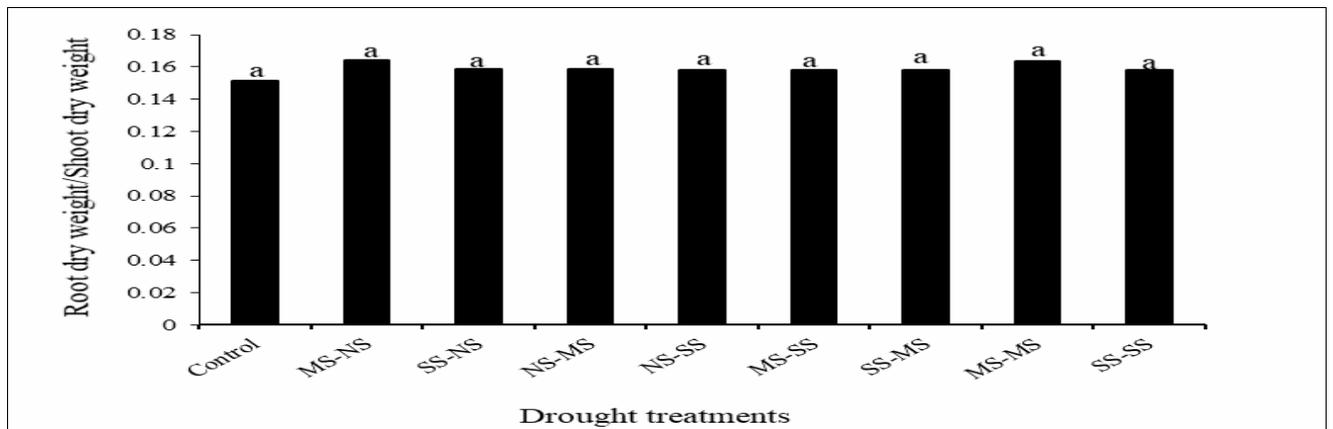


Fig. 3: Root/shoot dry weight of Kochia under drought stress. No Stress (NS=100% field capacity), Moderate stress (MS=70% field capacity), Severe stress (SS=30% of field capacity). The first abbreviation of the bottom is related to the drought stress during the vegetative stage and the second abbreviation after the interval line represents drought stress during the reproductive stage. Means followed by similar letter(s) are not significantly different at $p \leq 0.05$ by using LSD test.

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